ISSN 0378 – 9721

Volume 61 No 3

September / Septembre , 2013

African Union
Inter-African Bureau for Animal Resources

Bulletin of

Animal Health and Production

in Africa



Bulletin de la

Santé et de la Production Animales

en Afrique

Union Africaine
Bureau interafricain des Ressources Animales

ISSN 0378 - 9721

INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES
BUREAU INTERAFRICAN DES RESSOURCES ANIMALES
P.O Box, NAIROBI, KENYA

BULLETIN

September
Septembre
2013 Volume 61 No. 3

AFRICAN UNION
UNION AFRICAINE

IBAR PUBLICATION
PUBLICATION DU BIRA

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA
BULLETIN DE LA SANTE ET DE LA PRODUCTION ANIMALES EN AFRIQUE

A Quarterly journal of Original Article and Abstracts in English and French

Annual subcription: US$ 100.00

ISSN 0378-9721

Revue trimestrielle contenant des articles originaux et des résumés d'études en anglais et en français

Abonnement pour un an : 100$EU

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

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PREVALENCE OF ENDO, ECTO AND HAEMOPARASITES OF AGAMA LIZARDS (Agama agama) IN MAIDUGURI, BORNO STATE

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Abstract

One hundred agama lizards (Agama agama) were investigated for the presence of ecto, endo and haemoparasites in Maiduguri using standard parasitological techniques. Forty four (44) of the lizards were males while the remaining fifty six (56) were females.80 were adults, while 20 were juveniles.The obtained results revealed that all the lizards were positive for one parasite or the other.Endoparasites (100%) were the most abundant as they were found in all the one hundred lizards examined. However only twenty (20) of the males were infected with haemoparasites constituting 55.6% of the haemoparasitic infection, while sixteen (16) of the females were infected with haemoparasites constituting 44.4%.No statistical significant variation in infection was encountered between the sexes (P>0.05).Similarly none of the investigated lizards was positive for ectoparasite.The distribution of the parasites based on the age of the lizards examined shows that the adults were more infected(P<0.05) with 80(80%) and 31(86.1%) infection rates for endoparasites \ and haemoparasites respectively, than the juveniles.Two (2) species of endoparasites consisting of Trichuris spp (70%) and Ascaris spp (30%) were encountered during the study, while haemoparasitic species consisted of Plasmodium (47.2%), Haemoproteus spp (30.6%) and Leucocytozoon spp (22.2%).The findings may be of epidemiological significance in the study area in view of the role of lizards as reservoirs of the identified parasites. Similarly further investigation in the study area using advance techniques such as serology and molecular techniques is needed to ascertain the status of lizards as carriers/reservoirs of diseases such as toxoplasmosis and pentastomosis which are very important in reptiles.

Key words: Agama lizards, Prevalence, Maiduguri, infection rate, Parasites, Examination

PREVALENCE DES ENDO-, ECTO- ET HEMO-PARASITES DES LEZARDS AGAMA (Agama agama) A MAIDUGURI DANS L'ETAT DE BORNO

Resume

Cent lézards agama (Agama agama) ont été étudiés dans le but de rechercher la présence d'ecto-, endo- et hémo-parasites à Maiduguri en utilisant les techniques parasitologiques classiques. De l'ensemble de ces lézards, quarante-quatre (44) étaient mâles tandis que cinquante-six (56) étaient femelles. Quatre-vingt (80) étaient adultes, alors que 20 étaient juvéniles. Les résultats obtenus ont montré que tous les lézards présentaient l'un ou l'autre parasite. Les endo-parasites (100%) étaient les plus abondants car ils ont été détectés chez tous les cent lézards examinés. Cependant, l'infection aux hémo-parasites a été notée seulement chez vingt (20) mâles et seize (16) femelles, représentant respectivement 55,6% et 44,4% de l'infection hémo-parasitaire. Aucune variation statistique significative au niveau de l'infection n'a été détectée entre les sexes (P > 0,05). De même, aucun des lézards étudiés ne présentait des ecto-parasites. La répartition des parasites sur la base de l'âge des lézards étudiés montre que les adultes étaient plus infectés (P<0,05) par rapport aux juvéniles, avec des taux d'infection de 80 (80%) et 31 (86,1%) respectivement pour les endo-parasites et les hémo-parasites. Deux (2) espèces d'endo-parasites, à savoir Trichuris spp (70%) et Ascaris spp (30%), ont été identifiées au cours de l'étude, tandis que les espèces d'hémo-parasites comprenaient Plasmodium (47,2%), Haemoproteusspp (30,6%) et Leucocytozoon spp (22,2%). Les résultats peuvent être d'une importance épidémiologique dans la zone d'étude en raison du rôle des lézards comme réservoirs des parasites identifiés. De même, une enquête plus approfondie dans la zone d'étude, faisant appel à des techniques avancées telles que la sérologie et les techniques moléculaires, est nécessaire pour déterminer le statut des lézards comme porteurs / réservoirs de maladies telles que la toxoplasmose et la pentastomose, qui sont très importantes chez les reptiles.

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Mots-clés : Lézards Agama ; Prévalence ; Maiduguri ; Taux d'infection ; Parasites ; Examen

Introduction

Parasitisms have been investigated among wild and captive bred reptiles particularly lizards, with some having possible zoonotic implications (Ayinmode et al., 2010). The effects of parasitism are mostly confined to captive bred where parasites that are hitherto harmless in wild lizards, become pathogenic under captivity due to several factors such as inadequate transport, overcrowded territories and insufficient feeding, and finally resulting in untoward effects on the health of the reptiles (Ippen and Zwart, 1996). In a review of infectious and parasitic diseases of captive reptiles, Ippen and Zwart, (1996), listed parasites belonging to five different Phyla as parasites likely to infect or infest lizards with the Phyla Protozoa and Nemathelminths containing most of the parasites. In the wild, lizards often harbour a number of parasites, that live in equilibrium with the host, but the system of checks and balances between a host and parasites may be disrupted in captivity, thereby making what had been gentle co-dwellers to be strength-sappers (Downy and Bartlett, 2012). There are several reports in the literatures on the occurrence of parasites in lizards in different parts of the world (Hill, 1953) including Nigeria (Babero and Okpala, 1962). The lizard (Agama agama) has been reported to serve as carrier of the protozoan, Eimeria tenella (Wekhe, 1996). Also, Fernando and Udagama-Randeniya (2009) conducted a study on the prevalence of intestinal and ectoparasites of 19 selected reptilian species consisting of 14 snakes, four chelonians, and one crocodile at the National zoological garden in Sri Lanka. In all, 139 reptiles were examined with the prevalence of 66% and 24% reported for intestinal and ectoparasites respectively. However 10% of the sampled animals harboured both intestinal and external parasites. In a similar study, Rataj et al., (2011) investigated a total of 949 reptiles consisting of 55 snakes, 331 lizards and 563 turtles and belonging to 68 different species for the presence of endoparasites and ectoparasites. A prevalence rate of 76.1% was encountered for endoparasites among the 331 investigated lizards, while only one ectoparasite (Trombiculid) parasite was encoutered. Similarly, the presence of haemoparasites have been demonstrated in the blood of lizards under both natural and experimental conditions and using both conventional parasitological techniques and serology (Schall, 1996; Al Sadoon and El Bahrawy, 1998).

In Nigeria, Avery (1970) working in two different locations in northern Nigeria recovered parasites from 14 reptilian and 2 amphibian species with the recovered parasites distributed across the ectoparasitic, endoparasitic and haemoparasitic groups. From the study, parasite like Thelandros rotundus was reported for the first time in the host Agama agama and the West Africa region. Also, Akinboade (1981) investigated the public health significance of endoparasites and protozoans of Agama agama in Nigeria and reported the presence of endoparasites such as Ascaris cephaloptera, Acanthotaenia agama and Heterakis spimosa, while protozoans including Plasmodium spp, Entamoeba invadens and Tritrichomonas spp were among other species encountered in the study. Similarly, the occurrence of helminth parasites among Agama lizards (Agama agama) was investigated by Adeoye and Ogunbanwo, (2007) in the Lagos area of Nigeria where eight (8) different helminth species belonging to four different classes were encountered in the study, with nematode accounting for 50% of the recovered parasites. Parasitic species encountered during the study include; Strongyluris brevicaudata, Parapharyngodon awokoyai, Capillaria spp. and Oxyuris spp which were all nematodes, while Oochoristica agamae, Mesocoelium monas and Raillietiella spp belong to the Cestoda, Trematoda and Pentastomida classes respectively.

Despite several studies conducted by previous workers, there is still a gap in information on the prevalence of parasites infecting and infesting lizards and the role of lizards as reservoir/transport host of some of these parasites particularly in the northern part of Nigeria. To date, only few documented evidence exist on the prevalence of parasites

of lizards in the north most part of Nigeria, particularly, Borno State. The few previous studies conducted were restricted to ecto and endoparasites, with none on haemoparasites. This study was therefore designed to survey endo, ecto and haemoparsites of lizards in Maiduguri, Borno State .Similarly, this study is also the first documented evidence on the occurrence of haemoparasites in lizards in particular in the study area and northern Nigeria in general. The available information will help in better understanding of the role of lizards as a transport/reservoir host of parasites as previously reported by Wekhe and Olayinka (1999).

Materials and Methods

Study area

Maiduguri, where the study was conducted is the capital of Borno State and the largest urban settlement in the State.Borno State has an area of about 69,436Km2.It lies within latitude 11032 North and 110 42 N and longitude 13020E and 13025E, located between Sudan savannah and Sahel Savannah Zones, with an ambient temperature of 40-450C.It is characterised by short rainy season of about 3-4 months (June-September) followed by prolonged dry season of about more than 8 months (Udoh, 1981)

Sampled Animals

One hundred adult and juvenile, male and female lizards of the specie (Agama agama) were caught using sweep-net or a locally made straw-basket trap (Ameh, 2005) at various feeding and watering points within the Maiduguri Metropolis between October, 2010 and February 2011 and examined for the presence of ecto, endo and haemoparasites at the Parasitology laboratory of the Department of Veterinary Microbiology and Parasitology of the University of Maiduguri. The lizards consisted of forty four (44) males and fifty six (56) females, 80 adults and 20 juveniles.

Experimental Procedure

The experiment was carried out according to International guiding principles for biochemical research involving animals(C.I.O.M.S.1985).Captured (Agama agama) lizards were sedated using (Ketamine hydrochloride;KetalarR) at a dosage of 10mg/Kg given intramuscularly through abdominal muscles (Kumar, 1996) and thoroughly examined from the head to the tip of the tail for ectoparasites by the aid of a magnifying lens as described by (Soulsby,1982). Examination for haemoparasites was carried out by clipping either the claw or the tip of the tail to obtain blood. Thin smear was thus made using the collected blood as described by (Eberhand and Lammie, 1991; Houwen, 2000) and the slides later examined under a light microscope(×100) for the presence of haemoparasites as described by (Soulsby,1982). The presence of endoparasites was determined following humane sacrifice of the lizards used in the experiment. They were eviscerated using thumb forceps, scalpel blades and scissors, thereby exposing the gastrointestinal tracts. The contents of the gastrointestinal tracts were then emptied into tray containing normal saline, while the gastrointestinal mucuosa was thoroughly scraped and washed using normal saline. The wash was sieved into a tray to remove adhering worms. Examination for the presence of the parasite and identification was carried out according to the method of (Suresh, 1977). Identified adult nematodes were later fixed in 10% formalin, before being cleared in xylene and then dehydrated in ascending grades of alcohol, mouted in Canada balsam and examined as described by (Soulsby, 1982; Bhatia et al., 2004).

Statistical Analysis

Descriptive statistics was used to analyse data, tables were used and proportions presented in percentanges.Similarly data were subjected to Chi-Square analysis for test of significance. ($P<0.05$) were considered significant at 95% confidence limit (Maed and Curnow, 1983)

Ethical Consideration

Ethical clearance was obtained from the research and ethics committee of the Faculty of Veterinary Medicine, University of Maiduguri prior to the commencement of this research.

Table 1: Prevalence of Endo, Ecto and Haemoparasites of the Agama lizards (Agama agama) in Maiduguri, Borno State

Parameter		No. Examined	No.(%) infected with *Endoparasites*	*Ectoparasites*	*Haemoparasites*
Sex	Male	44	44(44%)[a]	0(0%)[a]	20(56.6%)[a]
	Female	56	56(56%)[a]	0(0%)[a]	16(44.4%)[a]
	Total	**100**	**100(100%)**	**0(0%)**	**36(36%)**
Age	Young	20	20(20%)[a]	0(0%)[a]	5(13.9%)[a]
	Adult	80	80(80%)[b]	0(0%)[a]	31(86.1%)[b]
	Total	**100**	**100(100%)**	**0(0%)**	**36(36%)**

Different superscripts within columns differed significantly (P<0.05)

Table 2: Species of Endoparasites Encountered Among Agama lizards (*Agama agama*) Examined in Maiduguri, Borno State, Nigeria

Number(%) infected	Endoparasites Encountered
100(100%)	*Trichuris spp* 70(70%)[a]
	Ascaris spp 30(30%)[b]
Total	**100(100%)**

Different superscripts within columns differed significantly (P<0.05)

Table 3: Species of Haemoparasites Encountered Among Agama lizards (*Agama agama*) in Maiduguri, Borno State, Nigeria

Total No. Examined	Total No.(%) infected	Haemoparasites encountered	No.(%) infected
100	36(36%)	*Plasmodium spp*	17(47.2%)[a]
		Leucocytozoon spp	8(22.2%)[b]
		Haemoproteus spp	11(30.6%)[c]
Total			**36(100%)**

Different superscripts within columns differed significantly (P<0.05)

Results

The overall prevalence of ecto, endo and haemoparasites of the examined lizards are presented in Table 1.It showed that, 100 lizards were examined consisting of 44 males and 56 females with an infection rate of 100% recorded for endoparasitism, while 20(55.6%) out of the 44 males and 16(44.4%) of the 56 females examined were positive for haemoparasitism.Similarly, no ectoparasite was encountered during the study .No statistical significant variation(P>0.05) in infection was observed between the sexes. The distribution of the parasites based on the age of the lizards examined showed that 80(80%) of the examined lizards were adults, while 20(20%) were juveniles with all of them infected with endoparasites representing 100% infection rate. However the adults were more infected (P<0.05) having 86.1% infection rate with haemoparasites than their juvenile counterparts.

The various species of endoparasites recovered from the examined lizards are presented in Table 2.Trichuris spp and Ascaris spp,accouting for 70(70%) and 30(30%) respectively, were the endoparasites encountered.Furthermore, Table 3 presents the species of haemoparasites recovered from the sampled lizards with their respective infection rates. Plasmodium spp accounted for the most abundant haemoparasites encountered with 17 (47.2%) out of 36, followed by Haemoproteus spp 11(30.6%), while the least encountered parasite was leucocytozoon spp with 8(22.2%)

Discussion

In the current study, parasites belonging to the haemoparasitic and endoparasitic groups were encoutered, with none belonging to the ectoparasitic group. Among endoparasites recovered, parasites belonging only to the class Nematoda were encountered with none belonging to either of Cestoda, Trematoda and the Pentastomida classes. The results of this findings showed that, the level of intensity of infection for both endo and haemoparasitism did not vary significantly ($P>0.05$) between the sexes. This agrees with the earlier report of Amo et al., (2005) who reported that male and female lizards have similar susceptibility to parasitic infection, but varies with the findings of Uller and Ulsson, (2003) who reported that males are more susceptible to parasitic infections probably due to immune suppressive effects of testosterone and Okoli, (2005) who reported higher worm burdens among females than males infected with the agamid tapeworm (Oochoristica agamae) in Southern part of Nigeria. Based on the age of the investigated lizards, there was no statistical significant variation ($P>0.05$) between the adults and juvenile lizards for endoparasitic infection as they were both similarly infected. However; a statistically significant ($P<0.05$) variation was observed in the level of intensity of infection between adults and juveniles for haemoparasitism.The results obtained for haemoparasitism further agrees with the findings of Adeoye and Ogunbanwo, (2007) who reported that adults were expected to be more infected because they occupy, more frequently, the more favourable places such as basking spots or refuges, and interact more with other adults, thereby exposing them to vectors of diseases.Also,the workers reported that, older lizards supposedly had more time/probability to get in contact with the parasites, compared to young ones while younger lizards are often limited to suboptimal areas by dominant older male lizards, thereby increasing the exposure rate of older lizards. Also, Ribas et al.,(1995) and Amo et al., (2005) both reported that, the prevalence of helminth infection is positively correlated with the adult size of the lizards, as more adults were found to harbour helminths.

The prevalence of 100% reported in this study for endoparasitism in male and female lizards investigated, is in agreement with the findings of Adeoye and Ogunbanwo, (2007) who reported an overall prevalence of 95.5% among 310 Agama lizards examined, with a sex based prevalence of 97.6% and 94.1% for males and females respectively.Variations, however is in the species of helminths encountered in the two studies. In the current study, only two nematodes species namely; Trichuris spp and Ascaris spp were encountered, while Adeoye and Ogunbanwo, (2007) reported four nematode species(Strongyluris brevicaudata, Parapharyngodon awokoyai, Capillaria spp. and Oxyuris spp) different from those encountered in this study, in addition to other parasites belonging to Cestoda, Trematoda and Pentastomida classes. Also, a prevalence rate of 76.1% was reported for endoparasitism by Rataj et al., (2011) in Slovenia among 331 lizards examined.This indicates a high degree of similarity with the current study.The differences however, are in the species of endoparasites encountered which differ from the current study. Unlike the current study where the endoparasites recovered were limited to two species (Trichuris spp and Ascaris spp) from the class Nematoda, the parasites encountered in the former study belonged to six different classes distributed across eighteen different species. Similarly, unlike the current study where no ectoparasite was encountered, the trombiculid mite (Geckobia sp.) was reported by Rataj et al., (2011) accounting for 24% parasitism among the 139 reptiles studied. In the current study, free ranging Agama lizards were used, which differed from the study conducted by Rataj et al., (2011) who used, as his test subjects captive reared animals from a Zoological garden. Another reason for the variation may be the specie of reptiles used in the two studies. Unlike the current study where only lizards were used, three different reptilian species consiting of snakes, chelonians and crocodiles were used in the former. The comparative susceptibility of each of these species along side lizards to ectoparasites need to be investigated. Also the result of the current study varied from the earlier report of Ameh (2005) who investigated ectoparasitism

among seventy (70) wall geckos (also a reptile) in Jos, north central Nigeria and reported a prevalence of 70%.Jos, due to its location on the Plateau is known to have a near temperate climate, thereby making cold blooded animals like reptiles to be inactive and cluster together most of the times.This aids in sharing of vectors of diseases faster and more efficiently, thereby making disease transmission faster, effective and efficient. Furthermore, the zero prevalence of ectoparasitism in this study may be partly due to the high ambient temperature in the area. The long duration of dry season which is a typical phenomenon in Maiduguri, is a known environmental condition which does not favour the development and survival of parasitic stages of parasites (Mbaya et al., 2006) and that could have been partly responsible for relative low prevalence of endoparasitism and the zero prevalence reported for ectoparasitism in comparison to similar studies in other study areas. Most of the protozoan parasites reported by previous workers (Akinboade, 1981; Wekhe and Olayinka, 1999; Fernando and Udagama-Randeniya, 2009; Rataj et al., 2011) were limited to tissue parasites, which are at variance with the haemoparasites exclusively encountered in the current study.

Conclusively, findings from this study have demonstrated that lizards (Agama agama) harbour both ecto and haemoparasites in Maiduguri. The occurrence of haemoparasites among lizards is being reported for the first time in Borno State, and the North eastern part of Nigeria. However further studies need to be done using advance techniques like serology and molecular methods so that the exact roles of lizards in relation to other diseases like Toxoplasmosis and Pentastomosis could be determined, since they have been reported previously in reptiles. Similarly the zoonotic potential of the recovered parasites require further investigation.

Acknowledgements

The Authors are grateful to the technical staff of the Parasitology laboratory, Department of Veterinary Microbiology and Parasitology, University of Maiduguri for their technical help. Similar thanks to University of Maiduguri for making some equipments used during this research available.

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CROSS-SECTIONAL STUDY ON CONTAGIOUS CAPRINE PLEURO PNEUMONIA IN SELECTED DISTRICTS OF KARAMOJA REGION IN UGANDA

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Abstract

Contagious caprine pleuro-pneumonia (CCPP) is an important, devastating disease of goats and sheep, caused by Mycoplasma capricolum subspecies capripneumoniae (Mccp). In Uganda the disease occurs mainly in the Karamoja region and the surrounding districts. It is a devastating disease of goats, caused by Mycoplasma capricolum subspecies capripneumoniae (Mccp). The present cross sectional study was undertaken to determine the prevalence of antibodies against CCPP by Competitive ELISA in goats in Karamoja region, Uganda. A total of 320 sera samples were collected from goats with both vaccination and no vaccination history in four districts of Karamoja region, namely: Kotido, Kabong, Abim and Amudat. Among the unvaccinated goats on Competitive ELISA, the seroprevalence was found to be 33.18% (73/220) and 62% (62/100) among the vaccinated ones. Among the various age groups, higher sero-positivity of (73%) was seen in 1-2 years old goats. Comparing the percentage seroprevalence for the districts; it was highest for Kotido (57%), followed by Kabong (51.25%), Abim (38.75%) and lastly Amudat (32.5%). The results of the study indicate that CCPP is endemic in Karamoja region. Furthermore, even among the vaccinated goats, 38% did not have antibodies against Mccp, indicating that not all the vaccinated goats could be collected. It is therefore recommended that more elaborate and long term studies be undertaken to isolate more Mccp strains from the region for more in-depth epidemiological studies of the disease. It is also important to identify causes of the low sero-conversion among vaccinated goats and come up with corrective measures to improve the protection of goats against CCPP in this region.

Key words: CCPP, Karamoja region, Sero-prevalence, Vaccination, Mccp, Uganda, cELISA,

ETUDE TRANSVERSALE SUR LA PLEUROPNEUMONIE CONTAGIEUSE CAPRINE DANS CERTAINS DISTRICTS DE LA REGION KARAMOJA EN OUGANDA

Résumé

La pleuropneumonie contagieuse caprine (PPCC) est une importante maladie dévastatrice des caprins et des ovins, causée par la sous-espèce capripneumoniae de Mycoplasma capricolum (Mccp). En Ouganda, la maladie est principalement présente dans la région de Karamoja et les districts environnants. C'est une maladie dévastatrice des caprins, causée par la sous-espèce capripneumoniae de Mycoplasma capricolum (Mccp). Cette étude transversale a été réalisée dans le but de déterminer la prévalence des anticorps contre la PPCC par le test ELISA de compétition chez les caprins dans la région de Karamoja en Ouganda. Au total, 320 échantillons de sérum ont été prélevés sur les chèvres avec ou sans antécédents de vaccination dans quatre districts de la région de Karamoja, à savoir : Kotido, Kabong, Abim et Amudat. Parmi les chèvres non vaccinées soumises au test ELISA de compétition, la séroprévalence a été établie à 33,18% (73/220) et à 62% (62/100) respectivement pour les chèvres non vaccinées et les chèvres vaccinées. Parmi les différents groupes d'âge, le taux de séropositivité le plus élevé (73%) a été observé chez les chèvres âgées de 1-2 ans. En comparant les pourcentages de séroprévalence dans les districts, le taux le plus élevé a été noté pour Kotido (57%), suivi de Kabong (51,25%), Abim (38,75%) et enfin Amudat (32,5%). Les résultats de l'étude indiquent que la PPCC est endémique dans la région de Karamoja. En outre, même parmi les chèvres vaccinées, 38% n'avaient pas d'anticorps contre la Mccp, une indication que

les chèvres vaccinées ne pouvaient pas toutes être collected. Il est donc recommandé que des études plus élaborées et à long terme soient menées pour isoler d'autres souches Mccp dans la région pour des études épidémiologiques plus approfondies de la maladie. Il est également important d'identifier les causes de la faible séro-conversion chez les chèvres vaccinées et de proposer des mesures correctives pour améliorer la protection des caprins contre la PPCC dans cette région.

Mots-clés : PPCC ; Région Karamoja ; Séroprévalence ;Vaccination ; Mccp ; Ouganda ; cELISA

Introduction

Contagiouscaprine PleuroPneumonia (CCPP) is a devastating disease of goats, associated with infection by Mycoplasma capricolum subspecies capripneumoniae (Mccp) formerly known as Mycoplasma sp. type F38, was isolated for the first time and characterized several years ago (Leach, Erno, & MacOwan, 1993). The disease is clinically characterized by fever, coughing and respiratory distress, with associated lesions, such as fibrinous pleuropneumonia, unilateral hepatisation of one lung and accumulation of pleural fluid in the thoracic cavity (Thiaucourt & Bolske, 1996a)). The disease is included in the list of notifiable diseases of the World Organization for Animal Health (OIE) because of its high morbidity and mortality, significant economic impact on livestock and the restrictions on trade caused by CCPP once it has been declared present in a Country. The disease threatens a significant number of goat populations throughout the world, with a high impact in Africa and Asia where the total goat population is over 500 million(Rurangirwa et al., 1991). In Uganda, the disease outbreak reports for the past years and ever since the disease was reported (Bolske, Johansson, Heinonen, Panvuga, & Twinamasiko, 1995) have shown CCPP to be tentatively endemic in these Karamoja Zone.

Mycoplasmas are smallest fastidious bacteria which can cause diseases in major species of animals including humans. In small ruminants, they are known for respiratory disease, arthritis, eye lesions, genital disease and mastitis (Nicholas & Churchward, 2012). Most of the members of Mycoplasma mycoides cluster group are the important pathogens for small ruminants. This group comprises six species and subspecies. Some of these Mycolasma species can cause severe and contagious diseases in goats with significant economic impact (Mekuria & Asmare, 2010). The exact distribution of CCPP is not known and there are very few official confirmations of outbreaks. The first reason is that, clinically, CCPP can be confused with a number of diseases inducing similar respiratory signs in goats, such as Peste des Petits Ruminants or pasteurellosis as well as amongst Mycoplasma species that induce various syndromes: Mastitis, Arthritis, Keratitis, Pneumonia and Septicaemia (MAKePS)(Thiaucourt & Bolske, 1996b). The second reason is that Mccp is one of the most fastidious Mycoplasma to be grown in vitro. As a result, isolation trials are often unsuccessful, especially if the conservation of the clinical sample has not been adequate Cross reactions are also very often observed between these two species. when looking for specific monoclonal antibodies (Thiaucourt, Bolske, Libeau, Le Goff, & Lefevre, 1994). Because of these difficulties, the direct detection of Mccp in clinical material may be a very useful alternative for the confirmation of CCPP outbreaks. A higher sensitivity can be achieved with PCR. It is believed that CCPP is very important diseases of small ruminants in Uganda but is widely neglected in routine control programmes yet majority of farmers including population from Northern and North Eastern as well as some other parts of Uganda depend on them for food security, income and employment (MAAIF, 2010). In Uganda, most animals are kept under agro-pastoral systems involving extensive livestock intermingling through communal grazing which favors disease spread (MAAIF, 2006). CCPP was first isolated in Uganda in 1993 during an outbreak (Bolske et al., 1995).Since then, there is little information on the disease epidemiology in Uganda. Currently it is believed to affect an estimated population of about 3 million goats

with severe socio-economic consequences on the human population in districts of Northern and North Eastern Uganda (MAAIF, 2010). It limits productivity levels, decimates stocks and inhibits significantly trade in animals and animal products, heavily affecting food nutrition and household incomes, especially in the pastoral communities.

In the field, the control of CCP is very often based on antibiotic treatment. CCPP vaccines exist and are efficient but largely unavailable. They include Mccp purified and inactivated antigen adjuvant with saponin. The detection of CCPP by serology has been affected by the cross- reactions that occur between the various Mycoplasmas of the mycoides cluster and particularly those that are often found in goats. The complement fixation test does not allow a specific detection of Mccp antibodies as it uses a crude antigen. The cELISA used in this study, provides a specific detection, thanks to the use of a monoclonal antibody "4.52". (Thiaucourt, et al., 1994). The cELISA has the ability to detect antibodies which appears after an infection or after an immunization with a relevant CCPP vaccine. A number of goats had already been reported to be dying due to PPR and other unconfirmed diseases. It was necessary to rule out CCPP since it had already been confirmed in Karamoja region (Bolske et al., 1995), and no more data exists.

Materials and Methods.

The present study was carried out in North Eastern zone, Karamoja region of Uganda that includes; Kabong, Amudat, Kotido and Abim districts. This study was carried out between 30th September- 4th October 2012 to ascertain the prevalence and distribution of the disease among the susceptible goat population in collaboration with OXFAM GB under National Animal Disease Diagnostics and Epidemiology Center (NADDEC) Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). This study was aimed at ascertaining the prevalence and distribution of CCPP.

A number of goats had already been reported to be dying due to PPR and other unconfirmed diseases. It was necessary to rule out CCPP since it had already been confirmed in Karamoja region (Bolske et al., 1995) and no more data exists. Karamoja region lies within an arid agro-ecological zone and is prone to recurrent drought due to erratic rainfall. It borders Sudan to the North and Kenya to the East as shown in figure 1. This region is an area where CCPP was first confirmed in Uganda (Bolske et al., 1995). The region consists of seven districts that include Kotido, Abim, Moroto, Amudat, Napak, Nakapiripirit and Kaabong. Herd population within the region is approximately1.9 million goats (MAAIF, 2009). The sample size was calculated as 380 samples based on the expected prevalence of 50% for simple random sampling method at 95% CI and 5 % absolute precision using the statistical formula by Daniel, (1999). The four districts were selected using systematic sampling technique. The number of samples was selected using probability proportional to population estimate of each species of animals in the area. Within each site, animals were randomly selected using systematic sampling technique. Of the 380 target sera samples, only 320 sera with the corresponding nasal swab were collected from the goats' population in the four districts of the Karamoja region. Out of these 320, a total of 220 sera were from unvaccinated goats and 100 were from vaccinated goats. The samples were collected from animals of different ages.

Blood samples were collected to prepare serum for detection of Mycoplasma



Figure1: Map of Karamoja Region showing the districts where sampling was made

antibodies. About 9 ml of blood was collected in a vacutainer tubes with serum clot activator (Greiner bio-one, Austria) and transported to the laboratory on ice. The separated serum was collected in a screw capped plastic vial and was stored at - 20 °C till further use.

The CCPP antibody detection was carried out using CCPP c-ELISA kit validated through a collaborative project including the National Veterinary Institute (Debre Zeit Ethiopia) and the Kenya Agricultural Research Institute (Muguga Kenya) within the "Vaccines for Negleted Animals diseases in Africa" (VACNADA) project. cELISA contagious caprine Pleuro-Pneumonia is an enzyme immunoassay for the detection of antibodies directed against Mycoplasma capricolum subsp. capripneumoniae (Mccp) in individual caprine serum samples. The kit contained user manual with fact sheets, Mccp Antigen coated microplates, Wash Concentrate (20X), Dilution Buffer 24, Strong Positive, Positive Control (lypholized), Control (lypholized), Negative Control (lypholized), Monoclonal anti-Mccp Antibody (Mab 4.52) (lypholized), Concentrated Anti mouse IgG HRPO Conjugate, TBM-Substrate N. 9 and Stop Solution 3. The c-ELISA test was conducted according to the kit protocol (Thiaucourt, et al., 1994). Sera samples to be tested were premixed with a specific monoclonal antibody Mab 4 52 in a separate plate ("preplate") and content of the "preplate" was transferred into the coated microplate. Any Mccp specific antibodies present in the sample formed an immune complex with Mccp antigen coated on the microplate, competing with Mab 4 52 for the specific epitopes. After washing away unbound material, an anti-mouse antibody enzyme conjugate was added. In presence of immune- complex between Mccp antigen and Antibodies from the sample, Mab 4.52 cannot bind to its specific epitopes and the conjugate is blocked from binding to Mab 4.52. Conversely in the absence of Mccp Antibodies in the test sample, Mab 4.52 can bind to its specific epitopes and the conjugate is free to bind to Mab 4.52. Unbound conjugate was washed away and enzyme substrate (TMB) was then added. In the presence of the enzyme, the substrate is oxidized and develops a blue compound that later becomes yellow after blocking. Subsequent color development is inversely proportional to the amount of anti-Mccp antibodies in the test sample (Thiaucourt et al.,1994). The result for one test sample was expressed in "percentage of inhibition" by comparing the optical density in the test well with the optical densities in the Mab control wells (Thiaucourt et al.,1994).

Results

CCPP antibody Prevalence in Goats with Different Vaccination Status:

A total of 320 sera samples collected from the four districts of Karamoja Region (Kotido, Abim, Kaabong and Amudat) were screened for specific antibodies against Mccp using competitive-ELISA (c-ELISA). Out of 320 sera samples, 220(68.75 %) and 100(31.25%) were from non-vaccinated and vaccinated goats respectively. The findings are as indicated in Fig. 2. Out of the 220 samples from unvaccinated goats, 18% (73/220) showed vaccination sero-positivity among goats' population of the Karamoja region. Among the vaccinated goats, 62% (62/100) were sero-positive.

Mccp Antibody Status by Animal Age

The 320 goat sera sampled were within the ages; below 1 year (n = 32), 1-2 years (n = 145), 2-3 years (n = 87), 3-4 years (n = 46) and above 4 years (n = 10), respectively. The CCPP sero-positivity was; <1year 7 (22 %), 1-2 years 106 (73%), 2-3 years 48(55.44%), 3-4 years 19 (42%) and >4 years 1 (10%).

Mccp Antibody Status by the Studied Districts

At the district level, 74, 73, 93 and 80 sera were collected from Kaabong, Amudat, Kotido and Abim respectively. A total of 38(51.25%), 24 (32.5%), 53 (57%) and 31(38.75%) sera samples were positive for Mccp antibody in Kaabong, Amudat, Kotido and Abim districts, respectively (Table 1). The data indicated that the prevalence was highest for Kotido (57%), followed by Kabong (51.25%), Abim (38.75) and lastly Amudat (32.5%). Furthermore, a high percentage of sero-converted animals was observed in Abim(78.57) district following CCPP vaccination.

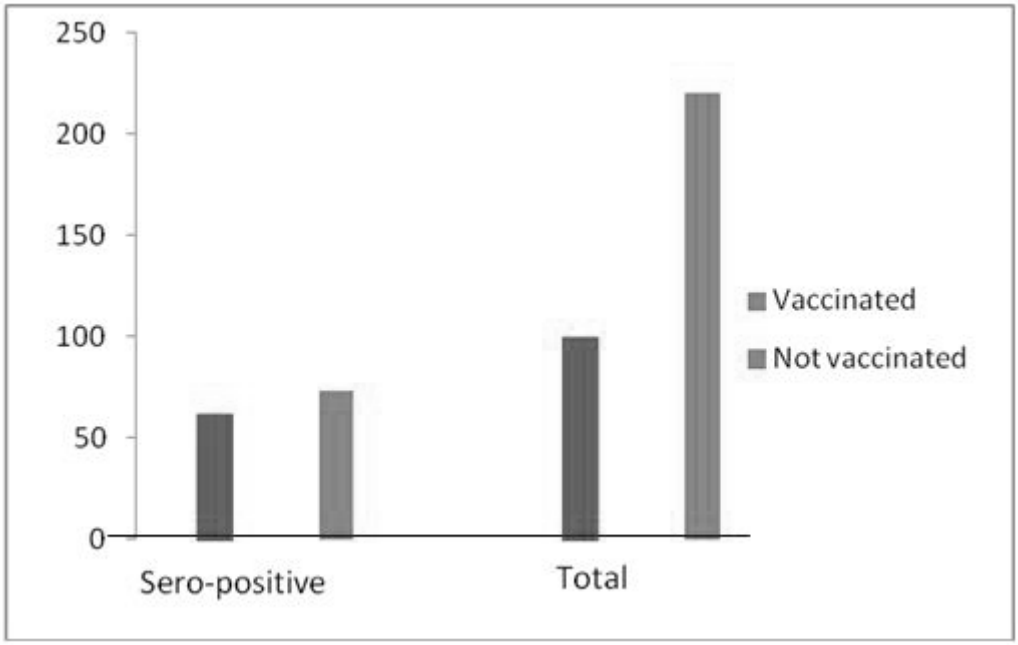


Figure 2: Sero-positivity in vaccinated and no-vaccinated flocks

Table 1: CCPP Sero-prevalence According to the Districts.

District	Total	Not Vaccinated (No. of positive, %)	Vaccinated (No. of positive, %)	Overall % Sero-positivity
Kaabong	74	55(24,43.36)	19(14,73.68)	51.25
Amudat	73	50(9,18)	23(15,65.22)	32.5
Kotido	93	63(40,63.49)	30(13,43.33)	57
Abim	80	52(9,17.3)	28(22,78.57)	38.75

Discussion

This study indicates that among the unvaccinated goats, 18% (73/220) had antibodies to CCPP which may imply that the Mccp had been in circulation. According to Bolske et al., (1995) first report on CCPP in Uganda suggests that CCPP is endemic in these districts of Karamoja region and could have been circulating in Uganda but undetected or misdiagnosed for sometime . As indicated by this study, the sero-positive unvaccinated goats could perpetuate the Mccp dissemination among susceptible goats and sheep. For instance, according to Wesonga, et al., (1998), animals such as goats or sheep that recover from CCPP without becoming sterile are responsible for the perpetuation of the disease in a flock. Therefore, there is necessity of surveillance activities in order to determine the importance of these shedders of CCPP in order to institute more effective control and prevention programmes. Lack of animal quarantine (FAO, 1999) facilities in Karamoja region in the light of nomadic pastoral practices could be responsible for exacerbating the spread and endemicity of Mccp. It was observed that Turkana nomads of north western Kenya occasionally graze their flock in these districts with Mccp infected animals. CCPP transmission involves close contact between infected and naive animals. Overcrowding and confinement favours close contact and circulation of Mccp. Stress factors due to malnutrition and movement over long distances during periods of drought could have predisposed the animals to the disease (Wesonga, Lindberg, Litamoi, & Bolske, 1998). Therefore rampant practice of communal grazing and watering by pastoralists in Karamoja region is one the reasons for high Mccp antibody levels determined in present study showed that Abim district had the lowest

CCPP seroprevalence of (17.3%) as compared to other districts studied. This could be be due to its location is to the interior of Karamoja Sub-region. In this respect there is need to determine the extent of the spread of CCPP in the whole region of Karamoja region, its neighbouring districts and other parts of Uganda, through a nationwide surveillance programme. The proposed study should be more systematic and detailed to determine the role played by sheep other than goats in the epidemiology of the disease in Uganda.

Among the vaccinated goats, the study revealed that, 62% (62/100) were protected against CCPP indicating a 62% level of vaccine administered success or the sero-conversion. As compared to the protection level of 94.1% from death and 65% from lung lesion development (Ayelet et al., 2007), this is relatively low. In normal practice, it is necessary to induce a higher immunologic response, however, this low Mccp sero-positivity level revealed in this study was not according to the expectation because CCPP vaccine has been reported to confer protection for up to 3 years (Ayelet et al., 2007). However, the CCPP vaccine immunogenicity is known to vary. According to Ayelet et al., (2007), antibodies can be detected slightly earlier then take long to decline in regards to live vaccine, and as a result of this, there is longer time of protection as compared to the killed vaccine. Basically, it is known that when certain Mccp antigens' are used antibody levels can decline faster in the absence of further challenge (Thrusfield 2005); hence, necessitating a booster vaccination to sustain protection. Probably, the vaccine employed in Uganda might not have had the suitable antigenicity or lost potency due to poor storage.

This study also suggests that goats 1-2 years old goats had a better sero-positivity to CCPP as compared to other age groups. The results show that sero-positivity rose with age up to 2 years and thereafter declined. The findings indicate that goats older than 2 years and younger than 1 year had lower probability of being sero-positive to CCPP. As indicated in this study, sero-prevalence among age groups revealed a significant difference. The significant difference between the age groups contradicts the established facts. There is also a report that suggestes absence of age factor in CCPP epidemiology (Lefevre et al., 1993). In the area of study, the demographics indicated that most of the tested animals were in 2 years age bracket. There is a likelihood that the findings are associated with the practice of older animals being sold and leaving the young ones as replacement stock (Ian Mc Allister and Ian Robinson, 2009).

At district level, there is a difference in CCPP sero-prevalence among the districts. Higher prevalence was found in Kotido and low in Abim. This may be attributed strongly to the presence of different animal management system, population density, production system and presence of carrier animal in the region (Mekuria, 2010).

Vaccination success at district level indicated that Abim animals were more protected followed by Kaabong, Amudat and Kotido district being with the least sero-positivity. The high seroprevalence in Kaabong might be because of the small sample size in relation to other districts. On the other hand, the difference in Abim could be either as a result of sample size or spread of the disease and animals' movement. Among unvaccinated goats in the different districts, the findings suggested that the disease could be spread by animals' movement and the sero-status indicate different vaccination coverage level in the districts which has implication on the disease control. Prevention and control of CCPP in Karamoja region is a very tedious task due to the mobility of animals under the nomadic pastoral practice and insecurity in the region from rustling that lead to frequent long distance movement, keeping animals in kraals and crisscross animal populations mixing between and within districts and across borders. The disease will continue to spread if these problems are not addressed, hence might pose control challenge.

Conclusion

The cross sectional study undertaken to determine the prevalence of antibodies against CCPP by Competitive ELISA in vaccinated and non-vaccinated goats in four districts of Karamoja, namely: Kotido, Kabong,

Abim and Amudat revealed that among the unvaccinated goats, the sero-prevalence was 33.18% (73/220) and 62% (62/100) among the vaccinated ones. Among the various age groups, higher sero-positivity of (73%) was seen in 1-2 years old Goats. Comparing the percentage seroprevalence for the districts; it was highest for Kotido (57%), followed by Kabong (51.25%), Abim (38.75) and lastly Amudat (32.5%). The study indicates that CCPP is endemic in the Karamoja region.. For instance, in Amudat which had a sero-prevalence of (32.5%). Furthermore, even among the vaccinated goats 38% did not have antibodies against Mccp showing that some of the goats in this region are not fully protected against CCPP. It is therefore recommended that more elaborate and long term studies be undertaken to isolate more Mccp strains from the region for more in-depth epidemiological studies of the disease. It is also important to identify causes of the low seroconversion among vaccinated goats and come up with corrective measures to improve the protection of goats against CCPP in this region.

Public Statement

The study shows that Contagious Caprine pleuropneumonia (CCPP) caused by a mycoplasma organism known by the name Mycoplasma capricolum subspecies capripneumoniae (Mccp), is endemic in the goats of the Karamoja region. This is a very highly contagious disease, therefore, the government of Uganda and the general public are hereby informed accordingly. Movement of goats and sheep in and out of this region should be restricted and vaccination of all goats and sheep should be carried out annually, and yearly epidemio-surveillance performed to determine the status and level of infection and protection against the CCPP. More research should be carried out to understand better the nature of spread and protection against this disease. The disease is not known to infect cattle, camels or humans.

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BOVINE MASTITIS IN SELECTED DISTRICTS OF BORENA ZONE, SOUTHERN ETHIOPIA

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Abstract

A cross-sectional study was conducted on 397 lactating Boran cows in pastoral areas of Borana Zone, Ethiopia to determine the prevalence of mastitis, major causes and associated risk factors of mastitis using California mastitis test and bacteriology. The study showed an overall prevalence of 70.8%; out of which 12.4% were clinical and 58.4% sub-clinical mastitis. Udder quarter level positivity was 49.6% while as much as 13.5% the teats were blind. There was a highly significant difference (p=0.00) between age, parity, and lactation stage groups. The prevalence of mastitis was significantly higher (p=0.00) among animals with tick infestation and those with history of previous exposure to mastitis compared to those without. Similarly, mastitis was also significantly higher (p<0.05) among animals with udder and teat injury. The most frequently isolated bacterial pathogens were Staphylococcus and Streptococcus species accounting for 37.0% (104/281) and 25.9% (73/281), respectively. Absence of hygienic measures during milking and poor environmental conditions has probably contributed to the highest prevalence of the subclinical form. The pastorals are almost exclusively dependent on milk for food. The economic impact could be significant because of the high prevalence of the disease itself and its subclinical presentation which makes identification and treatment very difficult by owners.

Key words: Boran cows, Ethiopia, Mastitis, Pastorals, Prevalence, Risk factor

MAMMITE BOVINE DANS CERTAINS DISTRICTS DE LA ZONE BORENA DANS LE SUD DE L'ETHIOPIE

Résumé

Une étude transversale a été réalisée sur 397 vaches allaitantes Boran dans les aires pastorales de Borana en Ethiopie, dans le but de déterminer la prévalence de la mammite, ses principales causes et les facteurs de risque, en faisant appel au test de mammite de Californie et à la bactériologie. L'étude a montré une prévalence globale de 70,8%, dont 12,4% pour la mammite clinique et 58,4% pour la mammite subclinique. La positivité au niveau des quartiers de la mamelle était de 49,6%, tandis qu'un chiffre égal à 13,5% représentait les trayons bouchés. Une différence très significative (p = 0,00) a été notée entre les groupes d'âge, parité, et stade de lactation. En outre, la prévalence de la mammite était significativement plus élevée (p = 0,00) chez les animaux infestés de tiques et ceux ayant des antécédents d'exposition antérieure à la mammite par rapport aux autres. La présence de blessures à la mamelle et aux trayons a également influencé de façon significative (p <0,05) la prévalence de la mammite. Les bactéries pathogènes les plus fréquemment isolées étaient les espèces Staphylococcus et Streptococcus représentant respectivement 37,0% (104/281) et 25,9% (73/281). L'absence de mesures d'hygiène lors de la traite et les mauvaises conditions environnementales ont probablement contribué à la très forte prévalence de la forme sub-clinique. Les pasteurs dépendent presque exclusivement du lait pour la nourriture. L'impact économique est donc plusieurs fois plus élevé en raison de la prévalence de la maladie elle-même et de sa présentation subclinique qui rend l'identification et le traitement très difficiles pour les propriétaires.

Mots-clés : Bovins Boran ; Ethiopie ; Mammite ; Pasteurs ; Prévalence

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Introduction

At least 137 infectious causes of bovine mastitis are known to date. In large animals the commonest pathogens are Staphylococcus sp Coliform bacteria and Actinomyces pathogens (Du Preeze, 2000; Quinn et al., 2004). Many other organism including Actinomyces pyogenes, Pseudaomonas aeruginose, Nocardia and asteroids, Clostridium perfringens may also be associated with mastitis (Radostits et al., 2007). Mastitis can be manifested as clinical or sub-clinical form. Although the exact reasons why a cow develops clinical or subclinical mastitis are not well understood, different studies suggest that it is likely to be influenced by the pathogen involved as well as the immune status of the cow (Sordillo et al., 1997; Bradley and Green, 2001; Zadoks et al., 2003).

The pastoralists in Borana region almost exclusively depend on their livestock for their living; as a source of their daily food, earnings to meet their house hold expenditures and all other social needs including those for veterinary care. Different reports confirm that mastitis can affect the livelihood of several communities by reducing milk yield (Biffa et al 2005; Getahun et al, 2008) and also have great public health significance (Galal Abdel Hameed et al, 2006; Junaidu et al, 2011). According to Lobago et al., (2001) of the major disease of cross bred cows in Addis Ababa milk shed, mastitis was the second most frequent diseases of intensification next to reproductive health problem in urban and peri-urban dairy production system. However, very little information exists regarding the magnitude of the disease in pastoral areas. Therefore, the main objectives of the present study were determination of the prevalence of mastitis, identification of the bacterial isolates involved and of the risk factors.

Materials and Methods

Study Area

Borana zone is located to southern part of Ethiopia, under Oromia regional state. The Zone consist thirteen districts and borders Kenya in the south and Somali in the southeast. It is generally characterized mainly by lowlands and hills with poor road infrastructure. Selection of the study districts was based on higher livestock population and accessibility on the livestock migratory route. The altitude of the zone ranges between 943 and 2,400 meters above sea level with average annual rain fall of 400 and 1100mm exhibiting a bimodal rainfall (long and short rainy seasons). The long rainy season extends from March to May whereas the short rainy season occurs from mid September to the mid November. The annual temperature varies between 19 – 42 °C. The area is known for being the origin of the Boran cattle breed, a known dual purpose animal. The pastoralists usually follow their animals depending on the availability of forage and water for their animals. Because of the mobility of the pastorals veterinary care is limited to only periods of settlement; even then it is highly influenced by lack of awareness. Animals are mostly free ranging during the day with no proper housing rather occasionally gathered in an unroofed barn during the night. Hand milking is the common practice among the pastorals twice a day (morning and evening) with no specific hygienic measures or management of dry cows. Milk is the main source of food in addition to being the source of income particularly during the rainy season when it is produced in excess.

Sample collection and mastitis test

A cross-sectional type of survey was conducted on 397 lactating Boran breed cows that were randomly selected with an expected prevalence of 50% at 95% confidence interval (CI) and 5% precision. The prevalence of clinical and sub-clinical mastitis was determined using clinical observation, CMT result and microbiological examination from strong positive CMT samples (Santos et al, 2004).

Each quarter was clinically inspected for apparent lesion. Visible abnormalities of udder and teat (injuries, blindness, swelling, asymmetry of the quarters) and abnormal secretions were recorded. Factors associated to prevalence of mastitis such as age, parity number, body condition, stage of lactation, the presence of ticks were also recorded. Age was categorized as young adults (2 - 4 years), adults (5 - 8 years), and old (> 9 years). Parity

number was also categorized into three: 1 - 2, 3 – 4 and > 4. Lactation stage was classified as < 2 months, 3 - 6 months and > 6 months; body condition was determined according to Nicholson and Butherworth (1986).

Milk samples were then collected aseptically before milking time as described by Sears et al. (1991) and Quinn et al. (2004) for subsequent testing using a commercial CMT reagent. The first few streams of milk were discarded and 10 mL of milk was collected into a horizontally held vial. After collection the vials were labelled and placed in Ice box containing ice, the samples were transported as soon as possible to Yabello Regional Veterinary Laboratory for Bacterial Culturing and Isolation. A positive diagnosis was made when at least one quarter was positive either clinically and/or with CMT. Positive cases were further categorized as clinical when visible signs were apparent and subclinical mastitis when animals tested positive only for CMT.

Microbiological tests

Culture of aseptically collected milk samples were performed following a standard microbiological technique (Quinn et al., 2004). The milk was sampled at cow level from all positive quarters within the same vial for bacterial isolation. Both general and selective media were used with further identification of the causative agents on the basis of colony morphology, heamolytic characteristics, Gram's stain, biochemical tests such as coagulase test, oxidase, catalase and growth characteristic on Edward's medium and sugar fermentation.

Data analysis

All the data were organized in Microsoft excel sheet and analysed by using SPSS version 17. Descriptive statistics were used to determine the prevalence of mastitis and other variables. A Chi-square test ($\chi 2$) was used to study differences among variables and the fixed effect of considered risk factors. The level of significance was held at $p<0.05$.

Results

The overall prevalence of mastitis was 70.8% (281/397) of which only 12.3 (49/397) exhibited clinical mastitis with the overwhelming majority (58.4%) showing the sub-clinical form. From a total of 1588 quarters examined, 787 (57.3%) tested positive for CMT while 215 (13.5%) were found to be not functional with blind teats (Table 1). There was no significant difference in the prevalence of mastitis at among the udder quarters

Risk factors

The study result showed different risk factors to have different level of significance in the prevalence of mastitis (Table 2). Age, parity number, lactation stage, tick infestation, udder or teat injury, and previous history of mastitis were significantly associated with mastitis. Relatively older animals with higher number of parity and those at later stages of lactation were most affected. Cows that had tick infestation or had injury of the udder or teat were also more prone to having mastitis compared to those without. Previous exposure to mastitis also predisposed animals to a second infection particularly when animals went through chronic or subclinical course of the disease.

Bacterial isolates

From both clinical and sub-clinical cases 281 randomly selected milk samples were collected for bacterial culture. Staphylococcus and streptococcus species were the most frequently isolated bacteria accounting for 37.0% (104/281) and 25.9% (73/281), respectively. List of bacteria isolated from the milk samples are given in table 3. About 3.6% (10) of the culture didn't show any growth of bacteria while 6.8% (19) samples showed mixed growth.

Discussion

The present study revealed that mastitis is highly prevalent in pastoralist area. The higher prevalence of the subclinical form generally agrees with earlier reports from different parts of Ethiopia (Workineh et al., 2002; Kerro and Tareke, 2003; Mungube et al., 2004; Biffa et al., 2005; Hunderra et al., 2005; Getahun et al., 2008; Almaw et al., 2008; Lakew et al., 2009; Mekibib et al., 2010). The limited awareness to sub-clinical forms of the

Table 1: Quarter level prevalence of mastitis in Boran cows

Udder quarter	Positive (%)	Blind teat (%)
Right front	14.9 (204/1373)	29.8 (64/215)
Right hind	14.1 (194/1373)	28.4 (61/215)
Left front	13.8 (190/1373)	21.4 (46/215)
Left hind	14.5 (199/1373)	20.5 (44/215)
(%)	57.3 (787/1373)	13.5 (215/1588)

Table 2: The prevalence of mastitis in association to different risk factors in Boran cows

Factors	N	Positive diagnosis (%)	$\chi 2$	P-value
Age				
Young adult	135	50.4 (68)	45.59	0.00
Adult	114	75.4 (86)		
Old	148	85.8 (127)		
Body condition				
Good	46	58.7 (27)	5.16	0.076
Medium	220	74.5 (164)		
Poor	131	68.7 (90)		
Parity number				
1 - 2	210	61.4 (129)	31.88	0.00
3 - 4	106	70.8 (75)		
> 4	81	95.1 (77)		
Lactation stage				
< 2 months	138	70.3 (97)	19.20	0.00
3 - 6 months	173	62.4 (108)		
> 6 months	86	88.4 (76)		
Tick infestation				
Present	336	75.9 (255)	29.08	0.00
Absent	61	42.6 (26)		
Udder or teat injury				
Present	82	82.9 (68)	7.56	0.006
Absent	315	67.6 (213)		
Previous history of mastitis				
Positive	91	87.9 (80)	17.03	0.00
Negative	306	65.7 (201)		

diseases is known to contribute for such high occurrence.

The level of quarter prevalence found in this study compares with previous studies both in Ethiopia and elsewhere (Shirmeko, 1996; Hunderra et al., 2005). Higher incidence of blind teat means a great economic significance for the pastoralists who depend on milk for living. Blind teats often arise as a result of chronic untreated mastitis or tick infestation. Different studies also confirm that the risk of clinical and subclinical mastitis increased significantly with advancing age, lactation, and parity of the cow (Abera et al., 2012; Biffa et al., 2005, Mungube et al., 2004; Kerro and Tareke, 2003; Molalegne et al., (2010). Absence of dry period therapy,

Table 3: Bacterial isolates identified in milk samples from animals that had clinical and subclinical forms of mastitis (n=281)

Species of bacteria identified	Clinical (%)	Sub-clinical (%)	Total (%)
Staphylococcus aureas	12 (24.5)	64 (27.6)	76 (27)
Coagulase negative Staphylococcus	5 (10.2)	23 (9.9)	28 (9.9)
Micrococcus species	2 (4.0)	10 (4.3)	12 (4.3)
Streptococcus agalactiae	9 (18.4)	50 (21.5)	59 (20.9)
Streptococcus dysgalactia	1 (2.0)	9(3.9)	10 (3.6)
Streptococcus Uberis	1 (2.0)	3 (1.3)	4 (1.4)
Actinomyces pyogens	0	3 (1.3)	3(1.1)
Corynebacterium bovis	2 (4.1)	2 (0.9)	4 (1.4)
Bacillus spp	0	4 (1.7)	4 (1.4)
Escherichia coli	7 (14.3)	45 (19.4)	52 (18.5)

presence of udder/or teat injury and pressure of milk on teat orifice makes easy entrance of bacteria to teat canal in early lactation. Further, the susceptibility of the mammary gland to new infection during both early and late dry period also increase prevalence of bovine mastitis (Radostits et al., 2000; Molalegne et al., 2010).

Similar bacterial isolates were also reported from other studies in Ethiopia in which Staphylococci and Streptococci species accounted for 50-89% of the total isolates of bacteria from mastitic milk (Abera et al., 2012; Kerro and Tareke, 2003; Almaw et al., 2008). However, higher occurrence of Staphylococcus species were found in this study compared to 23.1-31.1% reported from different localities within Ethiopia (Tolossa, 1987; Zerihun, 1996; Addisalem and Mersha, 2012). Staph. aureus infected cows are known to be the main source of infection for other cows in the herd mainly through the milker's hand .Apart from this, the isolation of Staph. aureus and Streprococcus spp are of public health significance since they are the commonly recovered pathogen in outbreaks of food poising due to milk and milk products, also causing various gastrointestinal upset ranging from abdominal pain to diarrhea (Ankalo and Sterneojo, 2006; Galal Abdel Hameed et al, 2006; Junaidu et al, 2011)

Changing patterns of control methods have apparently also changed the importance of some organisms relative to others, such that the classic causes of contagious mastitis (e.g. Streptococcus agalactiae) are now considerably less important than they were, while the incidence of mastitis caused by the so-called 'environmental bacteria', such as Streptococcus uberis, has increased. It has also been reported that production loss is not greatly affected by the type of causative organism (DeGraves and Fetrow, 1993) making isolation of bacterial agents equally economically significant. The presence of these agents on the skin and mucous membranes; and adaptation of some of them to survive in the udder and become contagious gives them a relatively higher chance to be shed into the milk that also serves as source of infection for healthy cows (Quinn et al., 2004; Carter and Wise, 2004; Radostits et al., 2007). The occurrence of environmental pathogens like coliforms is mostly associated with poor quality management of housing (Radostits et al., 2007).

Conclusion

This study showed that a high prevalence of bovine mastitis which is a major health problem of dairy cows in the study area. The vast majority being subclinical in occurrence imply a higher economic loss from its adverse effect on productivity. This is more significant as the pastoralists are highly dependent on milk for their living. There were a number of identified risk factors affecting the prevalence of mastitis considerably. The fact that the most

important bacteria isolated in this study were both contagious and environmental pathogens indicates the existence of poor management and hygienic conditions like absence of good housing management, lack of effective teat preparation and disinfection before and after milking, and absence of prompt identification and treatment/control of clinical mastitis. Further study should aim at creating awareness about the importance of subclinical mastitis among the pastoralist and evaluation of its economic and public health impact.

Acknowledgements

The authors would like to acknowledge Yabello Regional Veterinary Laboratory for all the field and laboratory material support.

Impact

Mastitis is one of the most economically important diseases of dairy animals. Particularly in the pastoral areas, milk is the primary source of food. It is also source of income hence loss of milk production has a direct economic implication. The fact that many animals acquire subclinical form of the disease mean the loss is mostly unrecognized. The condition is further complicated with different management problems such poor milking hygiene due to lack of awareness. This study is the first step towards identification of the magnitude of the problem, risk factors involved and the most commonly implicated bacterial agents.

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AMELIORATION OF ANTI-NUTRITIVE EFFECTS OF CASTOR OIL SEED (*RICINUS COMMUNIS*) MEAL IN BROILERS' RATION USING NATURAL FERMENTATION AND DL-METHIONINE SUPPLEMENTATION

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Abstract

Three hundred and twenty (320) day old male broilers were used to investigate the amelioration of anti-nutritive effects of castor oil seed (Ricinus communis) meal in broilers' ration using natural fermentation and DL-Methionine supplementation. The experimental designed was a 4 × 2 factorial arrangement of dietary treatments consisting of 4 inclusion levels of fermented castor seed meal (FCASM; 0, 50, 100, and 150 g/kg) supplemented with or without 5 g/kg DL-Methionine. Growth performance, coefficient of total tract apparent digestibility, serum metabolites, dressing percentage, and retail cuts were determined at the end of the study, which lasted for 42 days. There existed a linear reduction in weight gain and poorer feed to gain ratio with increasing dietary inclusion levels of FCASM. However, broilers fed control diet (without 5g/kg DL-methionine supplement) showed similar daily weight gain, feed to gain ratio and protein efficiency ratio with birds fed diet containing 50 g/kg FCASM supplemented with 5g/kg DL-Methionine. In a similar manner, low dressing percentages recorded with broilers fed diets containing 100 and 150 g/kg FCASM without supplemental DL-Methionine were slightly improved following dietary supplementation of the diets each with 5 g/kg DL-Methionine. Relative weight of gastro-intestinal tract increased linearly ($P < 0.05$) while total tract apparent digestibility of crude fibre reduced quadratically ($P < 0.05$) with increasing dietary inclusion levels of FCASM. Total serum protein and serum uric acid concentration reduced ($P < 0.05$) with increasing dietary inclusion of FCASM with or without 5 g/kg DL-Met supplementation. In conclusion, supplementation of diets containing 50g/kg fermented castor seed meal with 5g/kg DL-Methionine resulted in improved growth response with no symptoms of castor toxicity. Dietary supplementation of DL-methionine thus showed prospects in masking the anti-nutritive effect of fermented castor seed meal thus improving its utilization as potential oil seed meal in broilers ration.

Key words: Castor oil seed meal; DL-methionine; Broiler chickens; Serum metabolites

AMELIORATION DES EFFETS ANTI-NUTRITIFS DE LA FARINE DES GRAINES DE RICIN (RICINUS COMMUNIS) DANS LA RATION DES POULETS DE CHAIR PAR FERMENTATION NATURELLE ET SUPPLEMENT DE DL -METHIONINE

Résumé

Trois cent vingt (320) poulets de chair mâles âgés d'un jour ont été utilisés pour étudier l'amélioration des effets anti-nutritifs de la farine des graines de ricin (Ricinus communis) dans la ration des poulets de chair par fermentation naturelle et supplémentation de DL -Méthionine. Le dispositif expérimental était un arrangement factoriel 4 × 2 de régimes alimentaires comportant 4 niveaux d'inclusion de farine de graines de ricin fermentée (FCASM : 0, 50, 100 et 150 g / kg) complétée ou non avec 5 g / kg de DL -Méthionine. La performance de croissance, le coefficient de digestibilité apparente totale dans le tractus, les métabolites sériques, le rendement carcasse, et les découpes au détail ont été déterminés à la fin de l'étude qui a duré 42 jours. Il a été noté une réduction linéaire du gain pondéral et un ratio aliments/gain faible à la suite de l'augmentation des niveaux d'inclusion alimentaires de FCASM. Cependant, les poulets soumis au régime témoin (sans supplément de DL -méthionine à 5g/kg) ont montré un gain pondéral quotidien, un ratio aliments/gain et un ratio protéines/efficacité similaires, les oiseaux recevant un régime contenant 50 g / kg de FCASM avec supplément de 5g/kg de DL -Méthionine. De la même manière,

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les faibles rendements en carcasse enregistrés chez les poulets de chair aux régimes contenant 100 et 150 g / kg FCASM sans supplément de DL -méthionine ont été légèrement améliorés suite à la supplémentation alimentaire des régimes chacun avec 5 g / kg de DL -Méthionine. Le poids relatif du tractus gastro-intestinal a augmenté de façon linéaire (P <0,05), tandis que la digestibilité totale apparente dans le tractus des fibres brutes a diminué de quatre fois (P <0,05) suite à l'augmentation des niveaux d'inclusion alimentaires de FCASM. La protéine sérique totale et la concentration d'acide urique sérique ont diminué (P <0,05) suite à l'augmentation de l'inclusion alimentaire de FCASM avec ou sans supplément de 5 g / kg de DL -Met. En conclusion, la supplémentation des régimes alimentaires contenant 50g/kg de farine de graines de ricin fermentée avec 5g/kg DL -Méthionine a permis d'améliorer la réponse de croissance sans aucun symptôme de toxicité du ricin. La supplémentation alimentaire avec la DL- méthionine a donc montré le potentiel de masquer l'effet anti-nutritif de la farine de graines de ricin fermentée, améliorant ainsi son utilisation comme farine de graines oléagineuse potentielle dans la ration des poulets de chair.

Mots-clés : Farine des graines de ricin ; DL-méthionine ; Poulets de chair ; Métabolites sérique

Introduction

Castor oil seed (Ricinus communis) is popularly grown in the tropical countries for its oil (Dacosta and Laun, 1961), which is used for industrial purposes. The seed is commonly used as food condiments in local dishes in West African countries. In Nigeria, it serves as a flavouring agents used to add value to local soups and dishes (Okorie et al., 1985). Castor oil seed is rich in protein, making it a potential protein supplement for use in poultry (Darby et al., 2001), rabbit (Adedeji et al., 2006) and other livestock feeds. However, castor seed contains a group of toxic glycoproteins (ricin group), ricinoleic acid (1, 2 hydroxyoleic acid) and alkaloid ricinin, which limits its utilization in poultry diets (Anandan et al., 2005). Ricin was reported as the most lethal of the toxins (Hinkson et al., 1972). Incidence of ricin toxicity has been reported in poultry birds (Jensen and Allen, 1981). Limited success has been achieved in the past to obtain detoxified and de-allerginised meal for use in livestock nutrition (Ani and Okorie, 2002; Anandan et al., 2005). No report however has been made on possible detoxification of the seed through fermentation and its subsequent utilization in feeds for broiler chickens.

Ricin which is the most lethal of the toxins is known structurally to be a pyrimidine-based compound. Previous studies reported that when a labile methyl group is combined with pyrimidine-based compound, it leads to further detoxification of the compound (Rodwell, 1979). It was therefore hypothesized in the present study that dietary supplementation of DL-Methionine (a methyl group) might assist in further detoxification of poisonous castor ricin, ameliorate its anti-nutritive effect and improve its utilization in broilers ration. Vilhjalmsdottir and Fischer (1971) reported that methionine is one of the limiting amino acid in castor seed meal. Increased net protein utilization (NPU) of castor bean protein was also reported following DL-methionine supplementation in rats (Pablo et al., 1974). Dietary inclusion of DL-Methionine has been reported to improve poultry performance (Han and Baker, 1993) and ameliorate poor growth response obtained in poultry fed with some oil seeds (Cheeson, 1993). The present study, therefore, was conducted to investigate the possible amelioration of anti-nutritive effects of castor oil seed (Ricinus communis) meal in broilers' ration using natural fermentation and DL-Methionine supplementation..

Materials and methods.

Test ingredient and chemical composition

The castor seeds used in the study were obtained from the Raw Material Research and Development Council Kaduna, Nigeria, West Africa. The seeds were steeped in water (maintained at ambient temperature of 31oC) at a ratio of 1 kg seed to 4 L of water (kg/L) in an air tight environment (sealed container) for 5 d. At the expiration of this period, the sealed lid was opened up and the supernatant liquid was decanted. The fermented seeds were washed 3 to 4 times in clean water, drained and sun dried to a moisture content of 100 to 120 g/kg. The seed were later hammer milled (using

2 mm sieve) to obtain the meal. Resultant meal was screw-pressed overnight for 12 hours using the manual hand screw press. The screw-pressed meal was later used along with other feed ingredients to compound the experimental diets.

Milled samples of the raw seed (n = 4) and representative samples (n = 4) of the fermented meal were analyzed for proximate composition (AOAC, 1990) as presented in Table 1. The ash solutions of the samples were prepared with wet digestion and analyzed for mineral contents using atomic absorption spectrophotometer (Perkin Elmer Optima 4300DV ICP spectrophotometer, UK). Trypsin inhibitor (expressed as amount (in milligrams) of pure trypsin inhibited per gram sample) and haemagglutinin content (expressed as reciprocal of minimum quantity (milligrams) of sample per millilitre of the assay medium which produced haemagglutination) were determined as described by Kakade et al. (1973) and modified by Liu and Markakis (1989). Total polyphenol content was estimated using Folin-Ciocalteu (FC) assay as described by Wright et al. (2000). The flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). The extractable tannin was determined according to the method of Hoff and Singleton (1977) while saponin content determined as described by Edeoga et al. (2005).

Experimental birds and management

Three hundred and twenty (320) day-old, male Anak 2000 broiler chickens obtained from a commercial hatchery were weighed and distributed on weight equalization into 32 identical pens with each pen containing 10 birds. Brooding was done for 21 d. During brooding, temperature was controlled at 36oC for the first 0 to 2 d and then gradually reduced by 2oC per week to a final temperature of 32oC at the last week of brooding. Temperature was maintained at a stable ambient condition (31.5oC) throughout the study period. Feed and water were supplied ad libitum. The birds were reared intensively on floored pen in a deep litter housing system with wood shavings used as beddings.

Dietary treatments

The experiment was designed as a 4 × 2 factorial arrangement of treatments having four dietary inclusion levels of fermented castor seed meal (FCASM) (ie 0, 50, 100 and 150 g/kg) supplemented with or without 5 g/kg of DL-Methionine. The experimental diets are presented in Table 2. Birds contained in each pen were fed with 1 of 8 experimental diets such that there were 4 pens allocated to each dietary treatment. A single diet was used throughout the duration of the experiment which lasted for 42 days. The crude protein and metabolizable energy contents of the feeds were balanced within the recommended range (NRC, 1994). The birds were fed without restriction and managed intensively for 42 days. The conduct of the study agreed with the ethical policy and guideline of the Poultry Management Technical Committee of the Federal University of Agriculture Abeokuta Nigeria.

Parameters measured

Feed intake was computed as the difference between the feed offered and leftovers. Gain in weights and feed intake were measured at weekly intervals. Feed to gain ratio was computed as the ratio of feed consumed to weight gain. A record of mortality was kept as it occurred. At the end of the 42-d study, 2 broilers were selected at random from each pen and housed individually in clean wired floor metabolic cages (60 cm long × 45 cm wide × 60 cm high). Three days of acclimatization were allowed before the commencement of the digestibility study. A known weight of feed (slightly above the daily requirement) was given to the birds contained in the metabolic cage daily. Leftovers were measured and discarded on a daily basis. Excreta collection was done daily for a period of three days according to the procedure outlined by Onifade et al. (1999). The daily excreta voided for each bird was dried overnight (at 60oC for 12 h), while the total collection per bird at the expiration of 3 d metabolic trial was pooled. Excreta samples were used to determine the proximate compositions according to the method of AOAC (1990). Dried ground excreta samples (n = 8 per dietary treatment) and experimental

diets (n = 4 per dietary treatment) were analyzed for proximate constituent (AOAC, 1990). For mineral analysis of experimental diets (Ca and P), samples were dried in a hot air oven (at 105 °C for 8 h) and ground to pass through 0.5 mm sieve. Samples were ignited at 400 °C for 4 h in a muffle furnace. The ash was treated with HNO_3 under mild heat and digested. Analysis of constituent minerals was estimated using the Flame Atomic Absorption Spectrophotometer (Analyst 100, US). Gross energy of samples was carried out using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA).

At the end of 42d, blood samples were collected into heparinised tubes from 2 birds selected per pen by puncturing of the wing vein (brachial vein) using sterilized syringes. The blood samples collected (about 2.5 mL per bird) were centrifuged at 2,000 × g for 15 min and the serum harvested (Hayat et al., 1999). Serum samples were analyzed for total protein, albumin, creatinine and uric acid concentration. The measurement of serum metabolites were carried out using routine standard clinical chemistry procedures as described by Olorede et al. (1996).

For carcass analysis, 2 birds per pen whose weights are representative of the average weight (or close to the average weight) of birds contained in each pen were selected out and weighed. Bird selected for carcass analysis were fasted overnight, slaughtered by cervical dislocation following exsanguinations, defeathered after scalding in warm water according to the procedure of Oluyemi and Robert (2000). The defeathered carcasses were eviscerated manually following standard commercial procedures (Jensen, 1984). Individual carcasses were drained, weighed and dissected into retail cuts following the standard description of the working method of dissection of poultry carcasses into its parts (Hahn and Spindler, 2002). The dressed weight were expressed as percentage (%) of final live weight while, weights of retail cuts (breast, back, drumstick, and thigh) and gastrointestinal tract expressed in relative terms as gram per kg live weight.

Statistical analysis

Determined chemical composition of the raw and fermented castor seed meal was analysed using SAS (1996). Significant means were separated using Duncan's multiple range test (Duncan, 1955). Data obtained from the feeding trial were analysed by the general linear model of SPSS (1997). Analysis was done to determine the main effects (fermented castor seed meal inclusion levels, DL-Methionine supplementation) and their interaction (fermented castor seed meal inclusion levels × DL-Methionine supplementation). Polynomial contrast (linear and quadratic) was applied to determine the main effects for inclusion levels (0, 50, 100 and 150 g/kg) of fermented castor seed meal used. A probability of $P < 0.05$ was considered to be statistically significant.

Results

Chemical composition of castor oil seed meal

The chemical composition of raw and fermented castor seed meal is as presented in Table 1. Fermentation reduced ($P<0.05$) the ash, organic matter, ether extract content while it increased ($P<0.05$) the P and Na content of the resultant fermented castor seed meal. The assayed proximate composition of fermented castor seed meal revealed that it has a moderately high crude protein, ether extract and ash contents of 291, 51 and 105 g/kg values, respectively. It also contained a high crude fibre (140 g/kg) and gross energy values (10.2 MJ/kg). The mineral profile showed high assayed values for Ca (45 mg/100 g), Mg (11.2 mg/100 g), P (132 mg/100 g), K (114.1 mg/100 g) and Na (103.51 mg/100 g). However, it contained a low content of Mn (0.01 mg/100 g), Fe (0.91 mg/100 g) and Cu (0.02 mg/100 g). Fermentation of the raw meal showed significant reduction ($P<0.05$) in the trypsin inhibitor, tannin and total polyphenol content from 0.60mg/g, 0.20% and 2.20% to 0.10mg/g, 0.05% and 1.30 %, respectively. Processing of the raw meal showed no effect ($P>0.05$) on the haemagluttinin, flavonoid and saponin content of the resultant meal

Growth response

The growth performance of broiler chickens fed with varying inclusion levels of

Table 1: Determined chemical composition of castor seed meal

Item	Raw castor meal	Fermented castor seed meal
Composition (g/kg)		
Dry matter	890.7+11.22	899.0+9.82
Ash	112.1+8.55[a]	105.0+7.20[b]
Organic matter	887.9+9.40[a]	709.0+7.99[b]
Crude Protein	293.9+7.02	291.0+6.44
Ether extract	65.3+1.42[a]	51.0+1.95[b]
Crude fibre	143.2+4.55	140.0+3.95
Gross energy (MJ/kg)	10.44+0.66	10.20+0.50
Metabolisable energy (MJ/kg)[1]	8.63+0.16	8.69+0.01
Mineral profile (mg/100 g)		
Ca	43+1.20	45+1.10
Mg	11.10+0.90	11.22+0.62
Mn	0.01+0.0004	0.01+0.0001
P	129+5.05[b]	132+7.20[a]
K	114+6.02	114.1+2.20
Na	96.7+3.02[b]	103.5+7.11[a]
Fe	0.90+0.007	0.91+0.001
Cu	0.02+0.001	0.02+0.001
Anti-nutrients		
[2]Trypsin inhibitor (mg/g)	0.60+0.01[a]	0.10+0.005[b]
[3]Haemaglutinnin (mg/ml)-1	0.40+0.002	0.30+0.001
Total polyphenol (%)	2.20+0.04[a]	1.30+0.003[b]
Tannin (%)	0.20+0.01[a]	0.05+0.001[b]
Flavonoid (%)	0.20+0.001	0.20+0.003
Saponin (%)	0.30+0.02	0.20+0.01

[a,b] Means on the same row having different superscripts are different significantly (P<0.05)
[1]Metabolisable energy estimated using the formula of NRC (1994): ME = 26.7(DM) + 77(EE) - 51.22(CF).
[2]Milligrams of pure trypsin inhibited per gram of sample
[3]Reciprocals of minimum quantity (milligrams) of sample per milliliter of the assay medium which produced haemagglutination

fermented castor seed meal (FCASM) with or without 5 g/kg of DL Methionine is as presented in Table 3. The weight gain and feed intake reduced (P < 0.001), while the feed to gain ratio became worsened (P < 0.01) with increasing dietary inclusion levels of FCASM. There existed significant interaction effect (P<0.05) of FCASM inclusion level and 5 g/kg DL-Methionine supplementation on all the growth performance indices and mortality rate, which caused differences in the magnitude of response obtained. Broilers fed diet containing 150 g/kg FCASM without 5 g/kg DL-Methionine had the least (P < 0.05) daily weight gain, worst (P < 0.05) feed to gain ratio, protein efficiency ratio and highest (P < 0.05) mortality percentage. Supplementation of diets containing 50g/kg FCASM with 5 g/kg DL-Methionine showed increased (P < 0.001) daily weight gain, improved (P < 0.01) feed to gain ratio, increased (P < 0.05) protein efficiency ratio with no mortality. For instance, broilers fed with control diet (without DL-methionine supplement) showed similar daily weight gain, feed to gain ratio and protein efficiency ratio with birds fed with diet containing 50 g/kg

Table 2: Composition of the experimental diets

	0 g DL-Met/kg				5 g DL-Met/kg			
Item FCASMa (g/kg):	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**
Ingredient (g/kg)								
Maize	550.0	550.0	550.0	550.0	550.0	550.0	550.0	550.0
Soyabean meal	250.0	200.0	150.0	100.0	250.0	200.0	150.0	100.0
FCASMa	-	50.0	100.0	150.0	-	50.0	100.0	150.0
Blood meal	6.5	11.50	16.5	21.5	6.5	11.50	16.5	21.5
Brewer's dried grain	132.5	127.5	122.5	112.5	127.5	122.5	117.5	107.5
DL-Met	-	-	-	-	5.0	5.0	5.0	5.0
Other ingredients b	61.0	61.0	61.0	61.0	61.0	61.0	61.0	61.0
Determined composition								
ME (MJ/kg) c	12.1	12.1	12.0	12.0	12.1	12.1	12.0	12.0
Crude protein (g/kg)	220.4	219.2	217.5	216.6	220.0	218.4	217.0	216.0
Crude fibre (g/kg)	36.70	41.10	44.27	46.60	35.40	40.00	43.02	45.40
Ether extract (g/kg)	43.7	43.3	43.1	42.7	43.9	43.1	43.0	42.2
Calcium (g/kg)	15.6	15.8	16.1	16.4	13.5	13.7	13.5	16.2
Phosphorus (g/kg)	7.3	8.3	8.0	7.0	7.2	8.2	7.9	7.0

[a] FCASM = fermented castor seed meal.

[b] Contained: 30.0 g/kg of fish meal (72 % crude protein), 22.50 g/kg of bone meal, 15.0 g/kg of oyster shell, 2.0 g/kg of Lys≅HCl, 2.50 g/kg of salt (NaCl), and 2.50 g/kg of vitamin/mineral premix (provided the following per kilogram of premix: 233 g Ca; 1,200,000 IU vitamin A; 300,000 vitamin D3; 4,200 mg vitamin E; 4,2 00 mg vitamin E; 200 mg vitamin K3; 200 mg vitamin B1; 664 mg vitamin B3; 500 mg vitamin B6; 2,000 µg vitamin B_{12}; 100 mg folic acid; 15 mg bitoine; 1,500; 1,500 mg Ca panthothenate, 70 g choline chloride; 12 mg antioxidant (BHT); 500 mg Cu; 5,135 mg Zn; 6 g Fe; 7,100 mg Mn; 62 mg I; and 20 mg Se).

[c] Calculated from standard tables [estimated ME of FCASM using NRC (1994) was used in computing the ME of diets].

fermented castor seed meal supplemented with 5g/kg DL-Methionine. Highest overall daily weight gain ($P < 0.001$), protein efficiency ratio ($P < 0.05$) and the best ($P < 0.01$) feed to gain ratio was obtained with broilers fed diet containing 0 g/kg fermented castor seed meal (control) but supplemented with 5 g/kg DL-Methionine.

Dressing percentage and retail cuts

The dressing percentage and retail cuts of broiler chickens fed with diets containing varying inclusion levels of fermented castor seed meal with or without 5 g/kg of DL-Methionine is as shown in Table 4. Main effect of inclusion level of FCASM showed increased ($P<0.05$) relative weight of gastro-intestinal tract with increasing inclusion levels of fermented castor seed meal. Significant interaction effect ($P < 0.05$) of inclusion levels of fermented castor seed meal and dietary supplementation of 5 g/kg of DL-Methionine existed for slaughter weights, dressing percentages and breast meat yield. The least ($P < 0.05$) dressing percentage was obtained with broilers fed diet containing 150 g/kg FCASM but not supplemented with 5 g/kg of DL-Methionine. However, the low dressing percentages obtained for broilers fed with diets containing 100 and 150 g/kg FCASM without supplemental DL-Methionine was drastically improved ($P < 0.05$) following dietary supplementation of the diets each with 5 g/kg DL-Methionine. Broilers fed diet containing 0 g/kg fermented castor seed meal supplemented with 5 g/kg DL-Methionine recorded the highest overall slaughter weight ($P < 0.001$), dressing percentage ($P < 0.05$) and breast meat yield ($P < 0.05$).

Table 3: Growth performance of broiler chickens fed with varying levels of fermented castor seed meal (FCASM) with or without DL-Met supplementationa

Item	FCASM (g/kg):	0 g DL-Met/kg				5 g DL-Met/kg				SEM	P-value		
		0	50	100	150	0	50	100	150		FCASM		DL-Met
											Linear	Quadratic	
Initial weight gain (g)		38.0	39.0	43.0	38.0	40.0	39.0	41.0	40.0	0.20	0.905	0.990	0.780
Daily weight gain (g)		24.7[b]	21.7[c]	18.5[d]	10.6[f]	30.8[a]	23.7[b]	17.1[e]	16.3[e]	6.1	<0.001	0.003	<0.001
Daily feed intake (g)		93.6[a]	92.3[a]	78.2[d]	74.9[e]	86.0[b]	84.8[b]	80.2[c]	70.0[f]	2.7	<0.001	0.010	0.061
Feed to gain ratio		3.79[b]	4.25[c]	4.23[c]	7.05[e]	2.79[a]	3.58[b]	4.69[d]	4.30[c]	0.51	<0.001	0.030	0.055
Daily protein intake (g)		19.5[a]	19.7[a]	17.5[b]	16.4[c]	18.3[b]	18.1[b]	16.2[c]	15.1[d]	0.60	0.040	0.020	0.055
Protein efficiency ratio		1.26[b]	1.10[b]	1.06[b]	0.65[c]	1.68[a]	1.26[b]	1.05[b]	1.08[b]	0.08	0.061	0.072	0.042
Mortality (%)		1.07[b]	0.00[c]	0.00[c]	2.41[a]	0.00[c]	0.00[c]	1.11[b]	0.00[c]	0.01	0.022	0.031	0.041

[a, b, c, d, e, f] Means on the same row having different superscripts are different significantly (P<0.05)
[a] SEM = standard error of the mean.

Table 4: Dressing percentage and retail cuts of broiler chickens fed with varying levels of fermented castor seed meal (FCASM) with or without DL-Met supplementiona

Item	FCASM (g/kg):	0 g DL-Met/kg				5 g DL-Met/kg				SEM	P-value		
		0	50	100	150	0	50	100	150		FCASM		DL-Met
											Linear	Quadratic	
Slaughter weight (g)		1422[b]	1256[d]	1078[e]	635h	1763a	1311c	1000f	950[g]	113	<0.001	0.002	<0.001
Dressing percentage (%)		80.0[b]	83.4a	80.5[b]	77.5[c]	84.2[a]	80.1[b]	83.3[a]	80.1[b]	3.4	0.030	0.061	0.031
Breast (g/kg liveweight)		112.1[c]	117.2[b]	98.9[e]	117.6[b]	163.9[a]	100.1d	115.9[b]	98.8[e]	7.2	0.047	0.071	<0.001
Back (g/kg liveweight)		133.4	108.3	88.7	98.3	90.6	93.8	93.3	93.4	5.1	0.077	0.069	0.072
Drumstick (g/kg		19.5[a]	19.7[a]	17.5b	16.4[c]	18.3[b]	18.1[b]	16.2[c]	15.1[d]	0.60	0.040	0.020	0.055
liveweight)		96.5	108.3	112.9	94.3	115.8	111.7	117.1	108.1	1.2	0.080	0.071	0.079
Thigh (g/kg liveweight)		82.2	85.2	81.3	73.8	83.8	73.3	76.1	74.7	1.7	0.061	0.066	0.059
GIT (g/kg liveweight)		79.9	89.9	92.9	91.2	83.6	86.3	95.8	97.2	2.1	0.041	0.074	0.062

[a, b, c, d, e, f] Means on the same row having different superscripts are different significantly (P<0.05)
[a] SEM = standard error of the mean; GIT = gastro-intestinal tract.

Table 5: Coefficient of total tract apparent nutrient digestibility and serum metabolites of broiler chickens fed varying levels of fermented castor seed meal (FCASM) with or without DL-Met supplemention a

Item	FCASM (g/kg):	0 g DL-Met/kg				5 g DL-Met/kg				SEM	P-value		
		0	50	100	150	0	50	100	150		FCASM		DL-Met
											Linear	Quadratic	
Coefficient of total tract apparent digestibility													
Crude protein		0.66[ab]	0.68[a]	0.60[b]	0.60[b]	0.65[ab]	0.63[b]	0.68[a]	0.61[b]	0.11	0.044	0.061	0.031
Ether extract		0.80	0.75	0.77	0.72	0.80	0.78	0.77	0.75	0.08	0.256	0.221	0.126
Crude fibre		0.62	0.61	0.57	0.50	0.66	0.67	0.61	0.51	0.21	0.059	0.039	0.212
Organic matter		0.84	0.81	0.80	0.82	0.84	0.81	0.84	0.82	0.07	0.441	0.112	0.065
Serum metabolites													
Total serum protein (g/L)		88.2[a]	82.5[b]	75.1[e]	60.5[h]	80.4[c]	77.2[d]	72.7[f]	65.2[g]	3.0	0.042	0.059	0.040
Serum albumin (g/L)		52.9	53.5	43.9	39.3	50.6	42.3	40.9	35.6	2.2	0.056	0.060	0.039
Serum globulin (g/L)		35.3	29.1	31.2	21.2	29.8	34.9	31.8	29.6	1.5	0.100	0.140	0.111
Serum uric acid (mg/dL)		44.5[a]	39.3[b]	35.2[c]	32.4[d]	43.0[a]	39.8[b]	35.8[c]	32.8[d]	1.5	0.039	0.061	0.200
Serum creatinine (mg/dL)		1.30	1.80	1.80	1.40	1.90	2.00	1.80	1.20	0.11	0.100	0.190	0.110

[a, b, c, d, e, f] Means on the same row having different superscripts are different significantly (P<0.05)

[a] SEM = standard error of the mean.

Total tract apparent digestibility and serum metabolites

The coefficient of total tract apparent nutrient digestibility and serum metabolites of broiler chickens fed diets containing fermented castor seed meal with or without 5 g/kg of DL-Methionine is as shown in Table 5. Main effect of FCASM inclusion showed linear reduction in total serum protein concentration and quadratic reduction in total tract apparent digestibility coefficient of crude fibre with increasing dietary inclusion levels of fermented castor seed meal (Table not shown). Dietary supplementation with 5 g/kg of DL-Methionine showed no effect ($P > 0.05$) on serum albumin, globulin, creatinine, total tract apparent digestibility coefficient of ether extract and organic matter. There were significant FCASM × DL-Methionine interactions ($P < 0.05$) on total tract apparent digestibility coefficient of crude protein, total serum protein and serum uric acid concentration. The least ($P < 0.05$) total tract apparent digestibility coefficient of crude protein obtained with broilers fed diet containing 100 and 150 g/kg FCASM without supplemental DL-Methionine was slightly improved ($P < 0.05$) when the diets were supplemented each with 5 g/kg of DL-Methionine. Irrespective of dietary supplementation or not with 5 g/kg DL-Methionine, total serum protein and serum uric acid concentration reduced ($P < 0.05$) with increasing dietary inclusion of fermented castor seed meal.

Discussion

The reduced ash and ether extract content obtained with fermented castor seed meal following fermentation of the raw meal could be due to increased hydrolysis reaction during fermentation which led to gradual leaching of the nutrients and mineral in fermenting water. Screw pressing of the meal following fermentation also resulted in further loss of oil and mineral content of the meal. The crude protein value obtained for fermented castor seed meal in this study compared favorably with other oil seed meal (Aduku, 1993) and confirmed it as a potential plant protein ingredient. The high crude fibre content obtained in the current study for fermented castor seed meal showed its limitation as potential protein supplements in monogastrics (especially poultry) feeding. The relatively high P, K, Ca and Mg content obtained for fermented castor seed meal implied that the meal could be a major source of macro minerals. However, castor seed meal was reported to contain low levels of Lysine, Methionine, Tryptophan, and cystine content, which limited its inclusion levels as oil seeds in poultry feeds (Vilhjalmsdottir and Fischer, 1971).

Values of trypsin inhibitor and haemaglutinnin obtained for both raw and processed castor seed meal are very low and constituted no hazard for poultry nutrition. Reduction in trypsin inhibitor, total polyphenol and tannin obtained following fermentation agreed with earlier report that anti-nutrients contained in most oil seeds could be reduced following water soaking and fermentation (Mubarak, 2005). The presence of phenolic compounds and tannin in castor meal are responsible for the bitter taste following its consumption. Although phenolic compound have been shown to have antimicrobial potential (Okwu, 2001) while tannin have been reported to have astringent properties, hastens the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The flavonoid and saponin content of the meal are very low and constitute no hazard in poultry nutrition. Saponin are known to show cytotoxic effect while flavonoids are plant phenolic compounds which exhibited antioxidant and anticancer properties (Salah et al., 1995).

The reduced feed intake and weight gain obtained following increased dietary inclusion of fermented castor seed meal with or without 5 g/kg of DL-Met supplementation resulted from increased resultant dietary fibre and reduced apparent crude protein digestibility obtained with increased inclusion of castor seed meal. The growth performance of poultry has been shown to reduce following dietary dilution with fibre leading to increased bulkiness of the diets, reduced feed intake, nutrient intake and nutrient digestibility (Longe and Ogedengbe, 1989). Protein intake and digestibility also reduced drastically with increased dietary inclusion of fermented castor

seed meal. This will eventually lead to reduced weight gain. Poultry birds are generally known to be poor digester of fibre. The low total tract apparent digestibility coefficient of crude fibre and protein obtained with birds fed diet containing 100 and 150 g/kg FCASM implied poor digestibility and utilization of castor protein. Castor bean meal have been implicated to reduce nutrient utilization as low net protein utilization (NPU) were recorded for rats fed with water extracted castor bean meal (Vilhjalmsdottir and Fischer, 1971). Cambell and Lastly (1979) had earlier reported that percentage nitrogen retention is an indirect measurement of efficiency of utilization of dietary protein.

The poor feed to gain ratio, reduced weight gain and high mortality obtained with increased dietary inclusion of fermented castor seed meal could also be linked with increased dietary concentration of ricin. Dietary ricin content has been reported to adversely affect the feed consumption and nutrient utilization in chicks (Vilhjalmsdottir and Fischer, 1971; Okorie and Anugwa, 1987). Highest mortality, low protein efficiency ratio, least daily weight gain and worst feed to gain ratio obtained with broilers fed with diets containing 150 g/kg of fermented castor seed meal but not supplemented with 5 g/kg of DL-Met might be linked with high ricin content of the diet. Incidence of increased mortality of livestock animals resulting from castor bean poisoning (due to ricin) have been reported (Rao et al., 1984; Purushotham et al., 1985; Aslani et al., 2007).

The reduced slaughter weights of broilers obtained in this study with increasing dietary inclusion levels of FCASM corroborated the trend observed with growth performance. Resultant slaughter weight of bird is a function of the final live weight. The low dressing percentages recorded for broilers fed with diets containing 100 or 150 g/kg fermented castor seed meal is implicative of poor nutritional plane of the diets created as dietary inclusion of fermented castor seed meal increased. Low nutritional based diets will result in poor protein utilization which, will eventually translate into lower carcass yield and dressed weight. Skinner et al. (1992) confirmed that the dressing percentage increased as the nutrient plane of the diet increased.

The increased gastro-intestinal tract weight obtained with increasing dietary inclusion of fermented castor seed meal could be due to the high fibre content of the castor meal resulting in bulkier feed and digesta. In previous related studies, increased gastrointestinal tract weight, gizzards weight and small intestinal weight were reported with dietary inclusion of fibrous feed stuffs in broiler ration (Smith et al., 1997; Hetland et al., 2003). The economic importance of carcass yield and retail cuts of poultry (like breast meat yield, drumstick and thigh) has been highlighted since it provides consumers with information on the greatest edible portion of meat (Fanimo et al., 1996).

Supplementation of diets containing 50 g/kg of fermented castor seed meal diets with 5 g/kg of DL-Methionine showed significant improvement in daily weight gain, feed to gain ratio and protein efficiency ratio comparable to birds fed with control diet (without methionine supplement). This is suggestive of the masking effect of castor toxicity by methionine and showed prospects in improving the utilization of fermented castor seed meal when supplemented with DL-Methionine. The low dressing percentages obtained for broilers fed with diets containing 100 and 150 g/kg FCASM without DL-Methionine supplementation were slightly improved in the current study following dietary supplementation of the respective diet with 5 g/kg DL-Methionine. The improvement in growth performance, dressing percentage and reduced mortality obtained especially with broilers fed with diets containing castor seed meal supplemented with 5 g/kg of DL-Methionine agreed with Han and Baker (1993) and Cheeson (1993) who reported that addition of synthetic amino acids have been shown to counter the effect of lowered dietary crude protein level in broiler nutrition. Hesselman (1989) also reported that the addition of synthetic amino acids like Lysine and Methionine significantly improve the digestibility of nutrients, body weight gains and feed conversion ratio in broilers. Aletor et al. (1998) already showed that the growth of broiler chicken was not influenced

by decreasing dietary crude protein when such diets were supplemented with essential amino acids that met the minimum specifications.

The mechanism through which the supplementation of DL-Methionine in diets containing castor meal lead to reduced mortality, improved performance and amelioration of the castor ricin toxicity is not yet clear. One reason could be due to the presence of labile methyl groups provided by DL-Methionine which could be used to detoxify the pyrimidine compounds (ricin) contained in castor bean meal (Rayudu et al., 1970; Rodwell, 1979). The importance of DL- methionine in rations containing castor meal were first pointed out by Vilhjalmsdottir and Fischer (1971) who observed higher weight gained and feed utilization of chicks fed castor seed meal diets supplemented with L-Lysine + DL-Methionine + Tryptophan than those fed with similar diets but supplemented only with L-Lysine + Tryptophan. The findings of this study agreed with Okorie et al. (1987) who suggested inclusion of synthetic amino acids in diets containing castor seed meal for broiler chickens.

The values obtained for serum parameters in this study lied within the normal range for poultry birds as reported by Sturkie (1986). The reduction in serum uric acid concentration obtained with increasing inclusion of fermented castor seed meal not withstanding dietary supplementation or not with 5 g/kg DL-Methionine showed prospects for improved nitrogen utilization (Sturkie, 1986). High values of serum uric acid concentration have been reported as a consequence of increased deamination resulting from poor renal function of poultry birds (Oduguwa and Ogunmodede, 1995). Reduced total serum protein concentration recorded with increasing inclusion of fermented castor seed meal could be indications of hypoproteinemia (Altman, 1979) created as a result of increased inclusion of castor meal.

Conclusion

Dietary inclusion of fermented castor seed meal without supplementation with 5g/kg DL-methionine showed poor growth response, serum biochemistry and reduced carcass yield of broilers. However, supplementation of diets containing 50g/kg fermented castor seed meal with 5g/kg DL-Methionine showed prospects in ameliorating the anti-nutritive effect of castor toxicity resulting in improved growth performance and nutrient digestibility comparable to birds fed control diet

Acknowledgement

The provision of the castor seed by the Raw Material Research and Development Council (Kaduna, Nigeria) and the support of the Poultry Management Technical Committee of the Federal University of Agriculture (Abeokuta, Nigeria) are gratefully acknowledged.

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CARACTÉRISATION PHÉNOLOGIQUES DE LA POULE BARRÉE DE L'OUEST CAMEROUN

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Resume

Entre mai et Juin 2011, les performances de croissance et les caractéristiques phénologiques de la poule barrée des hautes terres de l'Ouest Cameroun ont été évaluées à la Ferme d'Application et de Recherche de l'Université de Dschang. Les données sur le poids vif, les mensurations corporelles et les caractéristiques de la carcasse ont été enregistrées sur 120 poules. Les matrices de corrélation et les courbes de régression du poids vif sur les mensurations corporelles ont été établies. Les résultats ont révélé une grande variabilité dans les caractères morphologiques et biométriques de la poule barrée. A 20 semaines, le poids vif moyen (1204,09g et 1566,87g respectivement chez les femelles et les mâles) et les valeurs des différentes mensurations chez les mâles étaient significativement supérieure (P<0,05) à celles des femelles. Par ailleurs, les coefficients de corrélation du poids vif sur les mensurations corporelles ont été moyens et positifs chez les femelles (0,53 à 0,67). Chez les mâles, ils ont été positifs et faibles (0,28 à 0,50) par rapport à ceux des femelles. Dans l'ordre d'importance croissant, les caractères pouvant servir à la prédiction du poids chez les mâles ont été le pourtour thoracique, la longueur du tarse et du corps mais avec une précision plutôt faible. A l'exception du rendement carcasse, du poids du gésier, du gras abdominal et du bréchet qui étaient comparables entre mâles et femelles, tous les autres paramètres ont été significativement (P<0,05) plus élevés chez les mâles comparés à ceux des femelles.

Mots clés : Performances de croissance, mensurations corporelles, poule locale

PHENOLOGICAL CHARACTERISTICS OFTHE BARRED CHICKEN INWESTERN CAMEROON

Resume

Between May and June 2011, the growth performance and phenological characteristics of local barred chicken of the Western Highland Cameroon was carried out in the Teaching and Research Farm of the University of Dschang. The data on body weight, body measurements and carcass characteristics were collected on 120 chickens. Matrices correlation and regression curves of body weight on body measurements were established.

The results revealed high variability in morphological and biometric characters of barred chicken. At 20 weeks, the average body weight was 1204.09 and 1566.87 g respectively for female and male. The values of the different measurements were significantly (P < 0.05) higher in males as compared to the females. Otherwise, the correlation coefficients of body weight on body measurements were positive in females (0.52 to 0.67). In males, they were positive and weak (0.28 to 0.50) as compared to the females. The characters that can be used to predict weight in males were thoraxic perimeter, tarsus and body length but with a rather low accuracy. Appart from the carcass yield, weight of gizzard, abdominal fat and breast muscle which were comparables between males and females, all other parameters studied including carcass characteristics and various visceral organs were significantly (P < 0.05) higher in males as compared to the females.

Key words: Growth performance, Phenological characteristics, Local barred chicken

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Introduction

En Afrique sub-saharienne, l'aviculture villageoise est pratiquée dans un système extensif en milieux rural et peri-urbain et répond mieux aux méthodes culinaires et aux goûts des populations africaines (Kperegbeyi et al., 2009). Elle joue un rôle capital dans la couverture des besoins des populations en protéines animales jusqu'alors déficitaires. Elle a une très faible productivité et sa caractérisation préalable en vue de son amélioration génétique reste un problème majeur (Kéambou et al., 2007a). Cette activité revêt une importance très significative comme source de revenus particulièrement chez les femmes (Zaman et al., 2004) elle constitue une caisse d'épargne facilement mobilisable en cas de besoins urgents (maladie, rentrée scolaire…). Outre sa valeur économique, la poule locale joue un rôle important dans la vie socioculturelle des africains. Elle est utilisée dans les cérémonie
recherche en aviculture a été pendant très longtemps orientée vers le développement de la production des produits de croisement des poules de races standards, négligeant la production de la poule villageoise dont l'intérêt s'accroit de façon remarquable au fil du temps. Malgré toute l'attention portée sur les poules sélectionnées, la demande en viande et en œufs de table en Afrique et surtout au Cameroun reste encore très élevée par rapport à l'offre. Les produits issus de l'aviculture villageoise représente environ 50% des produits avicoles du pays (Poné, 1998; Teleu et Ngatchou, 2006). Il est par conséquent nécessaire d'envisager une intensification de sa production qui passe par une meilleure connaissance du matériel animal. La poule locale camerounaise n'est pas suffisamment connus et le défaut de connaissance des différents types génétiques et des contraintes liées à leur production rend extrêmement difficile leur exploitation rationnelle et la mise sur pied d'un programme de développement qui puisse bénéficier aux populations locales (Kéambou et al., 2007a).

L'objectif de cette étude est de contribuer à l'amélioration des connaissances sur la croissance de la poule villageoise en vue d'initier un programme de sélection visant à l'intensification de la production de viande et d'œufs de table.

Matériel et Méthodes

Présentation de la zone de l'étude :

Cette étude a été menée à la Ferme d'Application et de Recherche de la Faculté d'Agronomie et de Sciences Agricoles (FASA) de l'Université de Dschang (Cameroun) entre mai 2010 et juin 2011. Dschang est situé à environ 1420 m d'altitude (LN 5-7°, LE 8-12°). Le climat est de type Soudano-guinéen avec environ 2000 mm de pluie par an, répartie sur une seule saison allant de Mars à Novembre. La température moyenne est de 20°C et l'humidité relative est généralement supérieure à 60%.

Matériel animal

Au total 120 poussins d'un jour pesant en moyenne 33g obtenus par incubation artificiel des œufs de parentaux présents à la ferme ont été utilisés dans cet essai. Les poussins ont reçu une couverture sanitaire en vigueur dans la zone; il s'agit des traitements préventifs contre la coccidiose et des vaccinations contre les maladies virales telles que la pseudo-peste aviaire, la bronchite infectieuse et la maladie de Gumboro. Les poussins ont été élevés en groupes de 30 sujets en phase démarrage sur litière faite de copeaux de bois à une densité de 8 animaux /m2 pendant 12 semaines. Au terme de cette période, les males au nombre de 50 ont été élevés séparément des femelles, sur caillebotis à une densité de 4/m² pendant huit semaines correspondant à la phase croissance. Les femelles (70) sont restées dans le bâtiment de départ a une densité de 5/m2 jusqu'à l'âge de 20 semaines (phase croissance). Chaque loge était équipée d'une mangeoire conique en plastique et d'un abreuvoir de dix litres. Les animaux ont été identifiés à l'aide des bagues métalliques placées au niveau du tarse.

Rations expérimentales

A chaque phase de l'étude, une ration faite à base de maïs, de son de blé, de tourteau de soja, de coton, de coquillage a été distribuée ad libitum aux animaux. Ces rations ont été formulé sur la base des besoins nutritionnelles des pondeuses d'œufs de table avec des

caractéristiques qui variaient en fonction de l'âge des animaux et de la phase d'élevage (Tableau 1) soit ; démarrage (1-12 semaines) et croissance (13-20 semaines).

Collecte des données

La consommation alimentaire hebdomadaire a été calculée en faisant la différence entre la quantité d'aliment distribuée au courant de la semaine et les restes à la fin de la même semaine. Les animaux ont été pesés à l'éclosion et tous les sept jours par la suite, en même temps que les aliments. Pendant les deux premières semaines, les poussins ont été pesés en groupe. Par la suite, des bagues d'identification ont permis d'effectuer les pesées individuelles. Le gain de poids hebdomadaire a été obtenu en faisant la différence entre deux poids hebdomadaires consécutifs.

A l'âge de vingt semaines, 16 mâles et 16 femelles ont été choisi au hasard, mis en diète alimentaire pendant 24 heures puis pesés, saignés, plumés et éviscérés tel que préconisé par Jourdain (1980). Le poids vif, Le cœur, le foie, le pancréas, le gésier et la graisse abdominale, la carcasse, la tête, les pattes, les cuisses, le bréchet, les ailes et l'intestin ont été pesés à l'aide d'une balance électronique de précision 1g.

Le poids relatif de chaque organe a été calculé en faisant le rapport du poids de l'organe sur le poids vif.

La longueur de l'intestin a été mesurée de la loupe duodénale jusqu'au cæcum à l'aide d'un mètre ruban. La densité de l'intestin a été calculée à partir de la formule suivante :

Densité intestin (g/cm) = (Poids de l^' intestin (g))/(Longueurde l^' intestin (cm))

Au cours de l'essai les mensurations corporelles ont été prises sur les sujets toutes les deux semaines à l'aide d'un pied à coulisse et d'un mètre ruban :

- Le pourtour thoracique (périmètre thoracique) qui est la circonférence de la poitrine prise en dessous des ailes et au niveau de la région saillante du bréchet ;
- La longueur du bec, égale à la distance entre le bout de la mandibule supérieure et la commissure des deux mandibules ;
- La longueur du corps mesurée entre la fin de la nuque (trou occipital) et le croupillon,
- Longueur de la patte mesurée entre l'articulation du bassin et la cheville ;
- Longueur du tarse: distance entre le calcanéum articulation du genou et la cheville ;
- Diamètre du tarse : mesuré entre le calcanéum et la cheville un peu au-dessus de l'ergot ;
- Longueur de l'aile étendue depuis la jonction de l'humérus à la colonne vertébrale jusqu'au bout de l'aile (sans plume).

Analyse statistique

Les résultats ont été exprimés en moyennes ± écart types et soumis à la statistique descriptive. Les coefficients de corrélation et de régression ont été utilisés pour évaluer les relations entre poids vif et mensurations corporelles des poules (Steel et Torrie, 1980). Le logiciel SPSS 14.0 a été utilisé pour ces analyses.

Resultats

Performances zootechniques

La consommation d'aliment a varié avec l'âge et le sexe. La quantité d'aliment cumulée consommée par une poule barrée entre 1 et 12 semaines et entre 13 et 20 semaines a été respectivement de 3169,75g et de 5221,90g. Par ailleurs, entre 13 et 20 semaines, la consommation moyenne cumulée des mâles 5632,23g a été plus élevée par rapport à celle des femelles 4735,95g. En effet, les femelles ont consommée 11,86% moins d'aliment que les mâles.

A l'éclosion le poussin tout sexe confondu a pesé en moyenne 35,20g. Entre 13 et 20 semaines, le poids vif des mâles a été significativement ($P<0,05$) plus élevé comparé à celui des femelles (Tableau 2).

L'indice de consommation entre 1 et 12 semaines et entre 13 et 20 semaines a été respectivement de 4,14 et de 8,89. Par ailleurs, entre 13 et 20 semaines l'indice de consommation des femelles (6,94) a été inférieur à celui des males (9,70) même comme la différence n'a pas été n'a pas été significative ($P>0,05$).

Tableau 1: Valeurs nutritives et coût de production des rations expérimentales

Composition (kg)	Démarrage (0 à 12 semaines)	Croissance (13 à 20 semaines)
Composition chimique calculée		
Protéine brute (%)	23,2	20,7
Energie métabolisable (kcal/kg)	2913	3013
Calcium (%)	1,48	1,51
Phosphore (%)	0,69	0,73
Lysine (%)	1,29	1,10
Méthionine (%)	0,43	0,40
EM/PB	125	145

Tableau2: Performances zootechniques de la poule barrée des hautes terres de l'ouest Cameroun.

Période (semaine)		Paramètres			
		Consommation alimentaire cumulé (g)	Poids vif (g)	Gain de poids cumulé (g)	Indice deconsommation
1-12	mâle, femelle	3169±306	781±14,8	747±34,1	4,14±1,97
	mâle	5632±513	1566±15,9	652±52,6	6,94±2,88
13-20	femelle	4735±864	1204±59,9	446±75,1	9,70±2,48
	mâle, femelle	5221±875	1401±194	549±125	8,89±5,29
1-20	mâle, femelle	8391±1182		1361±12,9	5,89±4,15

PV= poids vif ; LA= longueur de l'aile; LC= longueur du corps ; LP= longueur de la patte ; PT= pourtour thoracique ;LT= longueur du tarse ; DT= diamètre du tarse ; LTe= longueur de la tête ; LB=longueur du bec ; N=effectif ; ET = Ecart Type.

Caractéristiques phénologiques

Aussi bien chez les mâles (1186g à 1846g) que chez les femelles (950g à 1321g) à l'âge de 20 semaines, le poids vif a été très variable en fonction du sexe. Une variation de 660g et de 371g ont été enregistrée respectivement chez les mâles et les femelles. Le taux de variation des mâles (42% du poids vif) a été plus fort que celui des femelles (33% du poids vif), suggérant que ce paramètre peut être exploité en vue d'une sélection des males pour la production de la chair. Quelles que soient les mensurations prises, les valeurs des différents paramètres chez les mâles ont été significativement (P<0,05) plus élevées comparé à celles des femelles (Tableau 3).

Entre 1 et 20 semaines, les corrélations entre poids vif et les mensurations corporelles ont été très fortes et significatives (Tableau 4). Le coefficient de corrélation a été de 0,88 avec la longueur de l'aile et de 0,93 avec la longueur du tarse et du corps. Par ailleurs, les coefficients de détermination entre le poids vifs et la longueur de l'aile ont été très faibles chez les mâles (0,13) et les femelles (0,29) par rapport à tous les autres paramètres morphométriques étudiés.

Caractéristiques de la carcasse

A l'exception du rendement carcasse, du poids relatif du gésier, du gras abdominal et du bréchet pour lesquels les mâles et les femelles étaient comparables (P>0,05), tous les autres paramètres étudiés ont été significativement (P<0,05) supérieures chez les mâles comparés aux femelles (Tableau 5).

Discussion

La consommation cumulée d'aliment de la poule barrée pendant la période de démarrage (3169,75g) est légèrement inférieure au 3515,92g rapportée par Kreman et al. (2012) chez cette poule dans la même

zone. Par contre, pendant la période de croissance la consommation enregistrée dans cette étude (5221,90 g) a été plus élevé que celle rapportée par cet auteur (3615,55g). En effet, les sujets utilisés dans la présente étude sont les descendants (F2) des sujets de Kreman et al. (2012). Ceci suggère que la consommation s'améliore d'une génération à l'autre pendant la phase croissance.

Le poids vif de la poule barrée à 12 semaines (781,84g) enregistré dans cette étude est inférieur à celui de rapporté par Kreman et al. (2012) chez le même phénotype (886,62g). Par contre, Fosta (2008), a enregistré un poids à 12 semaines de 511,40g chez la poule locale de la Région du centre Cameroun qui est très inférieur à celui de la présente étude. A vingt semaines, le poids moyen des mâles (1566,87g) est comparable à celui rapporté par Kreman et al. (2012). Par contre, le poids vif des femelles (1204,09g) enregistré dans cette étude est supérieur au 1088g rapporté par ces auteurs.Au Congo, Akouango et al. (2010), ont enregistré des poids vifs à 5 mois plus faibles (1239g et 897g respectivement pour les mâles et les femelles) comparés aux résultats enregistrés

Tableau 3 : Caractéristiques morphologiques de la poule barrée à l'âge de 20 semaines (mensurations corporelles en centimètre et poids vif en gramme)t

	Sexe	**N**	**Moyenne ± ET**	**Minimum**	**Maximum**	**P**
PV	mâle	16	1565±160[b]	1186	1846	0,000
	femelle	16	1104±114[a]	950	1321	
	mâle, femelle	32	1334±117	1103	1518	
LA	mâle	16	23,5±1,73 [b]	20	26	0,000
	femelle	16	20,6±1,14 [a]	18	22	
	mâle, femelle	32	22,0±1,02	20	24	
LC	mâle	16	38,1±2,93 [b]	32	41	0,002
	femelle	16	33,5±3,17 [a]	27	39	
	mâle, femelle	32	35,8±2,27	32	40	
LP	mâle	16	33,4±3,16 [b]	28,5	39,7	0,000
	femelle	16	27,7±3,42 [a]	21,5	34,0	
	mâle, femelle	32	30,6±2,88	25,0	36,9	
L T	mâle	16	9,32± 0,58 [b]	8,3	10,3	0,000
	femelle	16	7,31± 0,59 [a]	6,7	8,5	
	mâle, femelle	32	8,32± 0,45	7,8	9,3	
L Te	mâle	16	4,70± 0,31[b]	4,2	5,0	0,019
	femelle	16	4,21± 0,19 [a]	4,0	4,5	
	mâle, femelle	32	4,46± 0,19	4,3	4,8	
L B	mâle	16	3,72± 0,24 [b]	3	4	0,000
	femelle	16	3,23± 0,22 [a]	3	4	
	mâle, femelle	32	3,48± 0,16	3	4	
PT	mâle	16	31,9±2,22 [b]	30	36	0,000
	femelle	16	26,5±1,47 [a]	25	28	
	mâle, femelle	32	28,9±1,60	25	31	
DT	mâle	16	1,33 ± 0,12 [b]	1,2	1,5	0,000
	femelle	16	1,08 ± 0,08 [a]	1,0	1,2	
	mâle, femelle	32	1,20 ± 0,09	1,1	1,4	

[a ,b]: Dans la même colonne, les valeurs affectées de la même lettre ne sont pas significativement différents (p>0,05) pour le même caractère.

Tableau 4 : Régression entre le poids vif et quelques paramètres morpho métriques

Sexe	Paramètres	Equation de régression	r2	R	N
mâle	PV- PT	PV= 71,3PT-789	0,46	0,50	52
femelle		PV = 45,8PT - 270	0.56	0,60	68
mâle, femelle		PV = 67,8PT - 813	0,87	0,93**	120
mâle	PV – LA	PV=23,2LA+713	0,13	0,36	52
femelle		PV=23,1LA+450	0,29	0,53	68
mâle, femelle		PV= 69,8LA - 525	0,78	0,88**	120
mâle	PV- LT	PV= 228LT - 734	0,55	0,49	52
femelle		PV= 207LT - 610	0,42	0,65	68
mâle, femelle		PV= 194LT - 524	0,88	0,93**	120
mâle	PV-DT	PV = 1187DT - 171	0,54	0,37	52
femelle		PV = 886DT +39,6	0,36	0,65	68
mâle, femelle		PV = 148DT - 605	0,59	0,91**	120
mâle	PV- LC	PV = 37,3LC - 46,0	0,35	0,48	52
femelle		PV = 46,1LC - 521	0,52	0,67	68
mâle, femelle		PV = 49,7LC - 606	0,87	0,93**	120
mâle	PV- LP	PV = 37,3LP - 46,0	0,35	0,28	52
femelle		PV = 46,1LP - 521	0,52	0,53	68
mâle, femelle		PV = 52,0LP - 363	0,88	0,90**	120

** corrélation significative ($p < 0,01$), N = nombre de poule, r 2 = coefficient de détermination, r = coefficient de corrélation, PV= poids vif, PT = pourtour thoracique, LA= longueur de l'aile, LT= longueur du tarse, DT= diamètre du tarse, LC=longueur du corps et LP= longueur de la patte

Tableau 5: Les caractéristiques de la carcasse, des organes viscéraux et des différentes parties de la poule barrée de 20 semaines d'âge

Variables	Femelles	Mâles	Mâles/Femelles	P
Nombre	16	16	32	
Poids à l'abattage (g)	1104±86,9[a]	1583±68,9[b]	1343±58,9	0,00
Rendement carcasse A(%)	71,4±5,15[a]	66,6±4,60[a]	70,2±1,66	0,28
Densité intestin (g/cm)	0,32±0,03[a]	0,42±0,06[b]	0,37±0,30	0,00
Surface Intestin (cm2)	217±15,2[a]	268±36,0[a]	243±15,2	0,04
Poids de la tête (g)	33,8±4,98[a]	58,5±3,70[b]	46,1±4,28	0,00
Poids des pattes (g)	32,8±5,30[a]	56,9±3,27[b]	44,8±3,76	0,00
Poids gésier (g)	23,9±3,35[a]	29,9±5,50[a]	26,9±4,30	0,22
Poids du cœur (g)	5,21±1,07[a]	10,2±1,10[b]	7,73±0,98	0,00
Poids du gras abdominal (g)	16,3±10[a]	10,9±4,29[a]	13,6±4,68	0,56
Poids du foie (g)	18,5±2,11[a]	28,5±4,49[b]	23,5±2,17	0,00
Poids du bréchet (g)	208±13,12[a]	242±23,9[a]	197±58,1	0,26
Poids cuisse et du pilon(g)	234±21,1[a]	356±10,7[b]	295±13,7	0,00
Poids des ailes (g)	109±10,6[a]	142±18,9[b]	125±11,6	0,00

[a,b,c] : Les moyennes portant la même lettre sur la même ligne ne sont pas significativement différentes ($p>0,05$).

ET = Ecart Type, carcasse A= carcasse prête à cuir conventionnelle,

dans la présente étude. Par ailleurs, Tike et Ronny, (2006) ont enregistré chez la poule locale en Indonésie des poids à vingt semaines de l'ordre de 1507 et 2290g respectivement pour les femelles et les males qui sont largement supérieurs à ceux de la présente étude. Cette grande variabilité de poids pourrait être due à la diversité génétique qui caractérise les poules locales et aux conditions d'élevage.

Dans cette étude, l'expression du dimorphisme sexuel pour le poids en faveur des mâles s'est établie dès la 6ème semaine. Ce résultat est en accord avec ceux rapportés par Keambou et al. (2007b), Fosta et Manjeli, (2010) et Kreman et al. (2012) chez les poules locales du Cameroun, et par Momoh et al. (2010), au Nigeria. Ceci suggère par ailleurs qu'un programme de sélection des coqs pour la production de viande serait plus avantageux qu'avec les femelles. Le dimorphisme en faveur des mâles s'est exprimé plus tardivement dans notre étude comparée aux résultats de Hako Touko et al. (2009) et Yapi-gnaoré et al. (2009) qui ont rapporté que le dimorphisme s'établie dès la quatrième semaine chez la poule locale africaine. Cet écart pourrait être dû au matériel génétique, à l'environnement et à l'alimentation et surtout au type génétique.

Les différences observées entre les mâles et les femelles pour les mensurations corporelles sont en accord avec les travaux de Pérez et al. (2004). Cette différence lié au dimorphisme sexuel qui apparait dès l'âge de 6 semaines et s'accentue avec l'âge, a également été observée chez la poule villageoise Sénégalaise par Guéye et al. (1998) et par Keambou et al. (2007b) chez les poules locales du Cameroun.

Durant la période allant de 1 à 20 semaines, les coefficients de corrélation du poids vif sur les mensurations corporelles ont été moyens et positifs chez les femelles (0,53 à 0,67). Les coefficients de corrélation les plus élevés ont été obtenu avec la longueur du corps, de la tête, du tarse et le diamètre du tarse. Ceci suggère que ces caractères peuvent être utilisés pour la prédiction du poids vif chez la femelle entre 13 et 20 semaines. Chez les mâles, ils ont été positifs et faibles par rapport à ceux des femelles (inférieur à 0,5). De même, pendant cette période, on a observé des coefficients de détermination plus faibles chez les mâles par rapport aux femelles. Ceci pourrait signifier que dans cette souche il est plus facile de prédire le poids vif avec les mensurations corporelles chez les femelles que chez les mâles. Comme déjà observé Guéye et al. (1998) et par Keambou (2007b) chez la poule villageoise, dans l'ordre d'importance croissant, les caractères pouvant servir à la prédiction du poids chez les mâles ont été le pourtour thoraxique, la longueur du tarse et du corps mais avec une précision plutôt faible. Comme observé par Gawande et al. (2007), les caractéristiques de la carcasse et les organes viscéraux de la poule villageoise varient avec le sexe, celles du mâle étant en général supérieures à celle de la femelle. Abstraction faite du sexe, le rendement carcasse prêt à cuire (69,04%) enregistré dans cette étude est inférieur aux données obtenues par Kreman et al. (2012) au Cameroun sur même la poule âgée de 20 semaines (71,45%) et par Akouango et al. (2010) au Congo pour le coq local à 6 mois d'âge (78%). Par contre, ce résultat est supérieur à celui rapporté par Gawandé et al (2007) sur la poule villageoise indienne âgée de 5 mois (65,78%) et à celui rapporté par Koko et al (2006) à Madagascar sur les rendements carcasses des poules villageoises de Madasgacar âgées de 5 mois (64 à 66%). En effet, ce rendement s'élève avec l'âge car le volume sanguin, les plumes et les viscères augmentent moins vite que le poids vif (Leclercq 1990).

Conclusion

La poule barrée présente une grande variabilité dans ses caractères morphologiques et biométriques. Le dimorphisme sexuel apparait dès l'âge de 6 semaines pour le poids vif et les mensurations corporelles et s'accentuent avec l'âge. Les caractères pouvant servir à la prédiction du poids chez les mâles sont le pourtour thoraxique, la longueur du tarse et du corps, mais avec une précision plus faible.

La grande diversité pour le poids vif, les caractéristiques de la carcasse et des différents organes viscéraux enregistrées dans la présente étude fait penser qu'une sélection bien organisée pourrait permettre à terme de

produire des animaux plus lourds à 20 semaines d'âge.

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PREVALENCE AND RISK FACTORS ASSOCIATED WITH CHICKEN ANAEMIA VIRUS IN KHARTOUM STATE-SUDAN

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Abstract

A total of 450 serum samples were collected from commercial chicken of 25 farms in different areas in Khartoum State (210 layers and 240 broilers) in 2012. the samples were analysed for chicken anaemia virus antibodies using indirect enzyme linked immuno-sorbant assay The overall prevalence rate of CAV antibodies was 73.3% and that of layers and broiler flocks were 95.7% and 53.8% respectively. The highest value (85.1) was recorded in the Omdurman Governate and Ombada locality (100%) while the lowest (43.2) recorded in Khartoum governate in Jabelawlia. The governate, locality, type of production, breeds, age, poultry population, husbandry system, body condition, source of poultry, floor type and vaccinations were the risk factors that significantly associated with CAV antibodies. These factors were analysed using logistic regression. It is found that, the increased risk of odds of being CAV positive was associated with governate, type of production, poultry population, husbandry system, source of poultry and vaccination ($p \leq 0.05$). Urgent need therefore exists to initiate a realistic programe for surveillance, control and eradication of this disease in Sudan especially the use of suitable vaccines for safeguarding poultry health and production in the country.

Keywords: chicken anaemia virus, prevalence, risk factors,Khartoum-Sudan

PREVALENCE ET FACTEURS DE RISQUE ASSOCIES AU VIRUS DE L'ANEMIE DU POULET DANS L'ETAT DE KHARTOUM AU SOUDAN

Résumé

Quatre-cent-cinquante (450) échantillons de sérum ont été prélevés sur des poulets de 25 fermes commerciales dans différentes régions de l'État de Khartoum (210 pondeuses et 240 poulets de chair) en 2012. Les échantillons ont été analysés pour rechercher la présence des anticorps du virus de l'anémie aviaire en utilisant le dosage immuno-enzymatique sorbant indirect. Le taux de prévalence globale des anticorps (du virus de l'anémie aviaire –CAV) était de 73,3 % et celui des pondeuses et des poulets de chair étaient respectivement de 95,7% et 53,8%. La valeur la plus élevée (85,1) a été enregistrée dans le Gouvernorat d'Omdurman et la localité d'Ombada (100%) tandis que la plus faible (43,2) a été notée dans le Gouvernorat de Khartoum à Jabelawlia. Le gouvernorat, la localité, le système de production, les races, l'âge, la population de volailles, le système d'élevage, l'état physique, la source de la volaille, le type de sol et les vaccinations étaient les facteurs de risque significativement associés aux anticorps anti CAV. Ces facteurs ont été analysés par régression logistique. On a constaté que l'augmentation du risque de probabilités d'être positif pour le CAV était associé au gouvernorat, au type de production, à la population de volailles, au système d'élevage, à la source de volailles et à la vaccination ($p \leq 0,05$). Il est donc urgent de mettre en place un programme réaliste de surveillance, de contrôle et d'éradication de cette maladie au Soudan, en particulier l'utilisation de vaccins appropriés pour protéger la santé et la production de volailles dans le pays.

Mots-clés : Virus de l'anémie du poulet ; Prévalence ; Facteurs de risque ; Etat de Khartoum

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Introduction

Chicken anemia virus (CAV) disease is an immunosuppressive disease of young chickens but also infects chickens of all age groups, caused by a virus of the family ciroviriade, genus Gryovirus (Pringle, 1999). Clinically, CAV disease is characterized by severe anaemia, aplasia of bone marrow and generalized lymphoid atrophy with concomitant immunosuppression. (CAV) was first described in 1979 by Yuasa et al. (1979) in commercially produced chickens. Since that time, the virus has been detected by isolation or serology in most other countries in both laying and broiler chickens (von Bulow and Schat, 1997). Infections with CAV are considered to be economically significant because of the clinical disease associated with vertical transmission and its potential for inducing immune dysfunction alone on in combination with other pathogens (Rosenberger and Cloud, 1998).

A serological study of CAV disease in Sudan was previously conducted (Ballal et al., 2005, Izdihar, 2009). The knowledge of epizootology of the diseases is still fragmentary and far from being complete. Therefore this study was conducted to estimate the seroprevalence rate of CAV antibodies in chicken flocks and to investigate the potential risk factors associated with occurrence of CAV in Khartoum State in Sudan.

Materials and Methods

Study Design

This study used a cross-sectional survey design and a multistage sampling method to determine the prevalence of chicken anaemia virus and to investigate the risk factors associated with the disease among commercial poultry flocks in Khartoum State. A single method was made to collect samples and filling out the questionnaire. Three localities (Khartoum, Bahry and Omdurman) were selected from the seven localities of Khartoum State. Then from each locality two administrative units were selected and farms were selected from each unit within the selected poultry, a convenience sampling method as done. Twenty five flocks were visited, in each unit and just 10-15 chicken from each pen were examined. At the flock level stratified sampling was performed

Study area and sample collection

A total of 802 Serum samples (based on availability of ELISA kits, only 450 were tested) were collected from 25 chickens flocks (6 from Khartoum, 13 from Bahry and 6 from Omdurman). These were from different breed types (14 layers and 11 broilers) and from different ages. The sample collection was carried out during the period of March-April 2012.

Sample Size

The sample size was calculated based on 95% confidence level, ±5level of precision and the prevalence rate which calculated as described by Martin et al., (1987).

Prevalence rate =

$$\frac{\text{No. of bird with CAV} * 100}{\text{Total no. of bird at a particular point in time}}$$

The sample size will be calculated by the formula:-

$$N = \frac{4*P*Q}{L2}$$

N= sample size
P= expected prevalence
L= desired absolute precision
Q= (1-P). (Martin, et al., 1987)

According to the study on prevalence of CAVD on farms in the Khartoum state the prevalence was estimated about 62%,(Balal et,al 2005) then the sample size were calculated

$$N = \frac{4*(0.62)*(1-0.62)}{(0.0025)} = 744 \text{ samples}$$

Questionnaire survey

A pre-tested structured questionnaire with the primary objective of elucidating the multifactorial background of CAV disease was conducted, the filling out of the questionnaire

was done by asking the owners to determine the risk factors associated with the disease, the flock attributes includes type of production, breed, age, body condition. The farms attributes includes flock size, source of poultry and type of husbandry system. The general management factors include floor type, and CAV vaccination history.

Serologic Assays

Indirect ELISA

Evaluation for antibodies to chicken anemia virus in sera was carried out using a commercial ELISA kit (Synbiotics Corporation, ProFlock, USA). The test was carried out according to manufacturer instructions. The optical density of each test plate was read using ELISA Reader (Multiskan EX) set at 405 nm wave length. Positive serum was determined based on the serum sample to positive control ratio, Serum samples with S/P ratios of less than or equal to 0.5 were considered negative and samples with S/P ratios greater than 0.5 were considered positive.

Data Management and analysis

All collected data during sampling and that collected from owners and results were entered, coded and stored electronically in a Microsoft® Excel for Windows® 2007 data base. The Statistical Package for Social Sciences (SPSS) for Windows® version 16.0 was used for all appropriate statistical analysis. 2-tailed Chi-square test was used to test the association between the disease and potential risk factors (univariate analysis) at the significant level of P≤0.20. A logistic regression model was used and deem significant if P≤0.05.

Results

Prevalence of CAV antibodies

Out of 450 chicken sera tested, 330 (73.3%) were positive for CAV antibodies. The highest value (85.1) was recorded in the Omdurman Governate followed by Khartoum North (79.7) and Khartoum (43.2). At the locality level, the highest prevalence rate (100%) was observed in Ombada while the lowest (43.2) in Jabelawlia (table 1).

Univariate analysis of risk factor with CAV antibodies

As illustrated in table 1. There was significant variations of CAV antibodies between Governates (p-value=0.000), localities (p-value=0.000), type of production (p-value=0.000),poultry population (p-value=0.000), husbandry system (p-value=0.000), body condition (p-value=0.000), source of poultry (p-value=0.000), floor type (p-value=0.000) , vaccinations, breeds (p-value=0.000) and age (p-value=0.000).

Multivariate analysis of association of risk factors with CAVD antibodies

Table 2. showed the results of multivariate analysis using logistic regression with a confidence interval of 95% and a p-value of ≤0.05 which used to assess the associations between identified risk factors in the univariate analysis (Governate, locality, type of production, poultry population, ,husbandry system, body condition, source of poultry, floor type, vaccinations, breed and age)with the positive CAV antibodies. The regression coefficients (Exp(B)) express the "odd ratios" (OR) of occurrence in comparison to reference (OR=1). The risk factors found significantly associated with increased odds of being ELISA positive were governate, Khartoum north(Exp(B)=4.6),Omdurman (Exp(B)=11.06), type of production (Exp(B)=7.2), poultry population (Exp(B)=0.816), husbandry system(Exp(B)=3.8), source of poultry (Exp(B)=3.4),and vaccination (Exp(B)=4.5).

The factors not significantly associated with increased odds of being ELISA positive were age (Exp(B)=0.33), floor type (Exp(B)=0.582) (P>0.05).

Discussion

The data of the present study along with the previous studies confirmed that CAV antibodies were prevalent in Khartoum State and it is indicative of natural infection of chickens as most of studied farms were not vaccinated against CAV. the overall prevalence rate of CAV antibodies detected in this investigation was higher when compared to previous similar

Table 1: Univariate analysis of potential risk factors with CAVD antibodies in 450 tested chickens in Khartoum State using the Chi-square (X^2) test.

Risk Factors	No. tested	No. +ve	%	Chi square	DF	P-value
Governate				57.2	2	0.000
Khartoum	95	41	43.2			
Khartoum North	241	192	79.7			
Omdurman	114	97	85.1			
Localities				75.7	4	0.000
Ombada	24	24	100			
Bahry	156	137	87.8			
Shargelnil	85	55	64.7			
Karary	90	73	81.1			
Jabelawlia	95	41	43.2			
Type of production				1.009	1	0.000
Layer	210	201	95.7			
Broiler	240	129	53.8			
Poultry population				7.02	1	0.000
>7500	100	63	63			
<7500	350	267	76.3			
Age (days)				81.1	1	0000
< 60	291	173	59.5			
> 60	159	157	98.7			
Breed				1.009	1	0.000
Cobb	240	129	53.8			
Hisex	210	201	95.7			
Husbandry system				22.8	1	0.000
Close	130	75	57.7			
Open	320	255	79.7			
Body condition				33.7	1	0.000
Bad	195	170	87.2			
Good	255	160	62.7			
Source of poultry				48.8	1	0.000
Ommat	245	147	60			
Other companies	205	183	89.3			
Floor type				33.9	1	0.000
Cement	239	148	61.9			
Cement &saw dust	211	182	86.3			
Vaccination				8.8	1	0.003
Yes	15	6	40			
No	435	324	74.5			

DF=degree of freedom

Table 2: multivariate analysis of association of risk factors with CAVD antibodies in chickens in Khartoum State

Risk Factors	No. tested	No. +ve (%)	Exp (B)	P-value	95% CL for Exp(B) Lower- Upper
Governate*					
Khartoum	95	41(43.2)	Ref		
Khartoum North	241	192(79.7)	4.6	0.011	1.427-15.216
Omdurman	114	97(85.1)	11.06	0.012	1.693-721.263
Type of production*					
Layer	210	201(95.7)	Ref		
Broiler	240	129(53.8)	8.08	0.002	2.128-30.730
Poultry population*					
>7500	100	63(63)	Ref		
<7500	350	267(76.3)	7.2	0.008	1.664-31.973
Age group(days)					
< 60	291	173(59.5)	Ref		
> 60	159	157(98.7)	0.33	0.244	0.057-1.961
Breed			1.009	0.000	
Cobb	240	129			
Hisex	210	201			
Husbandry system*					
Close	130	75(57.7)	Ref		
Open	320	255(79.7)	3.8	0.016	1.280-11.301
Body condition					
Good	255	160(62.7)	Ref		
Bad	195	170(87.2)	0.816	0.791	0.183-3.646
Source of poultry*					
Ommat	245	147(60)	Ref		
Other companies	205	183(89.3)	3.4	0.003	1.505-7.907
Floor type					
Cement &saw dust	239	148(61.9)	Ref		
Cement	211	182(86.3)	0.582	0.143	0.282-1.201
Vaccination					
Yes	15	6(40)	Ref		
No	435	324(74.5)	4.5	0.05	0.962-21.575

*Indicate significant risk factor

studies conducted in Khartoum State, A rate of (62.7%) CAV antibodies was detected by Ballal et al., (2005) and lower than the findings of Izdihar (2009) who reported (88.2%) CAV antibodies. These differences could be probably attributed dissimilarities in the size of tested samples in each study. The variation of investigated area may be another point of difference, considering the fact that each area has its specific and unique indigenous components and risk factors. There is a statistically significant difference in CAV antibodies prevalence rate between localities, the highest rate was detected in Ombada locality (100%); this could be due to the very small sample size which did not reflect the epidemiological status of the virus in this locality.

The current study revealed that the production type has significant effect on the sero-prevalence of CAV antibodies that may attributed to the difference in the life span of poultry used. Same results were also reported by other workers (Hegazy et al., 2010). Regarding the age factor, the older chickens (> 60 days) recorded higher prevalence rate which may be to the long exposure of these ages to the field virus, this observation was supported by Izdihar, (2009). It is pointed that, the sero-prevalence was higher in flocks kept in cement and sawdust than cement floor only, this findings could be linked with the ability of the virus to live longer in this type of floor. Also population density positively affects the prevalence and transmission of the virus. On the other hand, open system was more suitable for horizontal transmission of the virus than closed system.

It was observed from the results of the present study, that the body condition of the surveyed chickens has a positive association with the ser-prevalence of CAV. The percent of CAV antibodies was significantly higher among bad body conditions birds than those of good body conditions. This could be attributed to the fact that bad body conditions chickens are usually under bad management conditions which in turn might be reflects on high prevalence of the virus. The source of chickens used in this study was significantly associated with CAV prevalence. This could be due to the facts that most of breeder farms did not adopt any programs for CAV vaccinations. Also the number of vaccinated birds in the surveyed farms is very small. This may be to the educational status of the owners and their un awareness of the benefits of vaccination. A high CIAV prevalence rate recorded in the present investigation, along with earlier virus detection reports, indicates the widespread distribution of the virus and that CIAV should be considered an economically important poultry pathogen affecting poultry industry of Sudan.

This is the first study evaluating the risk factors associated with the seroprevalence of CAV in Sudan.

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CURRENT SURVEY OF TRYPANOSOMOSIS AMONG LIVESTOCK AND WILDLIFE IN THE ARID REGION OF NORTHEASTERN, NIGERIA

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Abstract

Examination of blood from sedentary and nomadic livestock, captive carnivores and Artodactyla/ Proboscidae for trypanosome infections in the arid region of northeastern, Nigeria, was conducted from 2009 – 2012 using thin, thick and buffy coat smears. Among the sedentary livestock examined, 14.3% were infected with Trypanosoma vivax, 0.3% with T. brucei and 2.3% with T. congolense out of 700 cattle; 52% and 26.2% were infected with T. evansi out of 125 camels and 65 horses examined, respectively; 2.0% were infected with T. vivax and T. evansi and 1.0% with T. congolense out of 100 sheep; and 1.3% were infected with T. vivax while 2.6% had T. brucei out of 78 goats. Among the nomadic livestock, 1,324 cattle were infected with T. vivax, T. brucei and T. congolense, respectively, while 39.2% of the 655 camels had T. evansi and the horses had no infection. Among the 450 nomadic sheep, 1.6%, 0.4%, 0.2% had T. vivax, T. brucei, T. congolense and T. evansi infections respectively, while the infection rates were 0.6%, 0.3% and 0.6% for T. vivax, T. brucei T. congolense and T. evansi respectively among the 365 nomadic goats examined. Out of the 10 lions examined, 20% and 30% had T. vivax and T. brucei infections, respectively. The 9 stripped hyeanas examined showed infections rates of 22.2%, 44.4% and 11.1% for T. vivax, T. brucei and T. congolense, respectively. Out of the 6 spotted hyeanas examined, 66.7% and 33.3% had T. vivax, T. brucei and T. congolense infections, respectively; whereas 2(50%) of 4 jackals examined had both T. vivax and T. brucei infections. One caracal examined (100%) was infected with T. brucei. Infection with T. vivax was 100% in 2 African elephants, 1 cape eland, 4 western kob and 2 Senegal hartebeest. There were infections with T. vivax, T. brucei and T. congolense in 30%, 20% and 15% out of 20 dorcas gazelles, 25% and 12.5% out of 8 sitatungas and 38.9%, 16.7% and 22.2% out of 18 red fronted gazelles respectively. Among the 10 Grimm's duicker, 20%, 10%, 30% and 10% were infected with T. vivax, T. brucei, T. congolense and T. evansi respectively. The probable vectors involved were Tabanus, Stomoxys and Hippobosca. However, oral transmission among the carnivores was another possibility. Therefore, trypanosomosis is endemic in the region with implication that, sustained surveillance and control measures are required to reduce risks to wildlife conservation and losses in livestock production.

Key words: survey, trypanosomosis, livestock, wildlife, arid region, Nigeria

ENQUETE SUR L'ETAT DE LA TRYPANOSOMOSE PARMI LE BETAIL ET LA FAUNE DANS LA REGION ARIDE DU NORD, NIGERIA.

Résumé

L'examen de sang à l'aide de l'élevage sédentaire et nomade, carnivores en captivité et Artodactyla / proboscidiens pour les infections trypanosomes dans la région aride du nord-est, au Nigeria, a été menée de 2009 - 2012 à l'aide de frottis mince couche, épaisse et Buffy. Parmi les animaux d'élevage sédentaire examiné, 14,3% étaient infectés avec Trypanosoma vivax, 0,3% avec T. brucei et de 2,3% avec T. congolense sur 700 bovins, 52% et 26,2% étaient infectés par T. evansi sur 125 chameaux et 65 chevaux a examiné, respectivement, 2,0% étaient infectés avec T. vivax et T. evansi et de 1,0% avec T. congolense sur 100 moutons et 1,3% étaient infectés par T. vivax tandis que 2,6% ont eu T. brucei sur 78 chèvres. Parmi les animaux

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d'élevage nomade, 1.324 bovins ont été infectés avec T. vivax, T. brucei et T. congolense, respectivement, tandis que 39,2% des 655 chameaux eu T. evansi et les chevaux eu aucune infection. Parmi les brebis nomade 450, 1,6%, 0,4%, 0,2% avaient T. vivax, T. brucei, T. congolense et infections à T. evansi respectivement, tandis que les taux d'infection était de 0,6%, 0,3% et 0,6% pour T. vivax , T. brucei T. congolense et T. evansi respectivement parmi les chèvres nomades 365 examinés. Sur les 10 lions examinés, 20% et 30% avaient T. vivax et T. brucei, respectivement. Les 9 hyeanas dépouillés examinés ont montré des taux d'infections de 22,2%, 44,4% et 11,1% pour T. vivax, T. brucei et T. congolense, respectivement. Sur les 6 tacheté hyeanas examinés, 66,7% et 33,3% avaient T. vivax, T. brucei et les infections à T. congolense, respectivement, tandis que 2(50%) de 4 chacals examinés présentaient à la fois T. vivax et T. brucei. Un caracal examinés (100%) a été infecté par T. brucei. L'infection à T. vivax était de 100% en 2 éléphants d'Afrique, 1 Eland du Cap, 4 occidentale kob et 2 bubales Sénégal. Il y avait des infections à T. vivax, T. brucei et T. congolense à 30%, 20% et 15% sur 20 gazelles dorcas, 25% et 12,5% sur 8 sitatungas et 38,9%, 16,7% et 22,2% sur 18 gazelles à front rouge respectivement. Parmi les duicker du 10 Grimm, 20%, 10%, 30% et 10% ont été infectés avec T. vivax, T. brucei, T. congolense et T. evansi respectivement. Les vecteurs probables impliqués étaient Tabanus, Stomoxys et Hippobosca. Cependant, la transmission orale chez les carnivores était une autre possibilité. Par conséquent, la trypanosomose est endémique dans la région, avec l'implication que les mesures de surveillance et de contrôle soutenus sont nécessaires pour réduire les risques pour la conservation et les pertes dans la production de bétail faune.

Mots-clés: enquête, trypanosomose, l'élevage, la faune, région aride, Nigeria

Introduction

Trypanosomosis in livestock constitutes a major threat to food security in several parts of Sub-Saharan Africa despite decades of chemotherapeutic control (Swallow, 2000). In endemic parts of Africa, the cyclical vector (Glossina) is responsible for transmitting Trypanosoma vivax, T. congolense and T. brucei. However, haematophagus vectors such as Tabanus, Hippobosca, Lyperosia species and Stomoxys calcitrans have been incriminated in the mechanical (non-cyclical) transmission of T. vivax and T. evansi in Northeastern Nigeria (Mbaya, 1988; Nawathe et al., 1990; Nawathe et al., 1994) and else where in the world (Mahmoud and Gray, 1980; Njiru et al., 2001).

Trypanosomosis has been extensively studied among wild animals in other parts of the world (Kaguraka, 1992; Marie, 1998; Reichard, 2002; Parija and Bhattacharya, 2005) and rarely in Nigeria. Most of the wild ungulates in West, East, Central and South Africa serve as reservoirs of human and animal trypanosomes (Kaguraka, 1992; Radomski et al., 1995). The Gambian (chronic) and Rhodesian (acute) forms of human African trypanosomosis (sleeping sickness) occur with severe consequences in West and East Africa, respectively (Solano et al., 2003). In as much as T. vivax was exclusively reported with a low prevalence rate among cattle in the tsetse free arid region of northeastern Nigeria almost two decades ago (Mbaya, 1988; Nawathe et al., 1990; Nawathe et al., 1994), a sustained surveillance is required to ascertain the current species of trypanosomes and their prevalence among livestock and captive wildlife in the tsetse free arid region of northeastern Nigeria and its vectors. This is with the view to proffering control measures to reduce the risks to wildlife conservation and losses in livestock production.

Materials and Methods

Study site

The study was conducted between January 2009 and December 2012. The area where the study was conducted is located in Maiduguri and Yobe State which lies between latitude 110 05'N and 110 40'N and longitude 130 05'E and 130 25'E. It is located between the Sudan Savannah and Sahel vegetation Zones. A total of 4 sedentary herds located at the University of Maiduguri Livestock Farm, Faculty of Veterinary Medicine, University of Maiduguri, Field Station, Munna Livestock Investigation and Breeding Centre (LIBC), Gujba Cattle Ranch and Gombole Cattle Ranch were selected for the study. Similarly, four nomadic herds located around Sambisa Game Reserve were also selected. Both sedentary and nomadic livestock were comprised of cattle, camels, sheep and goats. The captive wild animals used in the

study were from Maiduguri Zoological Garden located in Maiduguri, the capital and largest urban centre in Borno State, Nigeria. The Zoo was first established in 1975 with a few donated wild animal species. The zoo presently has 37 different species representing over 320 captive wild animals. The coordinates of each collection, was recorded using the Global Positioning System (GPS) hand-held receiver. The animals were not subjected to any form of distress during the study. Animal welfare was strictly observed according to international guidelines.

Restraint, Sample collection and examination

The study involved 1,068 sedentary animals, 3,002 nomadic animals, 30 captive carnivores and 65 Artiodactyla/Probocidae. The desired sample size was calculated at 95% confidence interval with 5% absolute precision using the formula of Thrusfield, (1995). The larger Artiodactyla/Probocidae were immobilized using the Dan Inject® capture gun with varied doses of etorphine hydrochloride (M99) (Immobilon®, Reckitt and Coleman) and revived with its analogue diprinorphine hydrochloride (M98) (Revivon®). The smaller Artiodactyla were captured using drop nets. The carnivores were trapped in a squeeze cage and tranquilized by intramuscular injections of varied doses of ketamine hydrochloride (Ketalar, Parke-Davies®). Blood samples were aseptically taken from the jugular vein in the livestock and Artiodactyla, the lateral ear vein in the Proboscidae and the sapheneous vein in the carnivores into sterile vacutainers containing EDTA as anti-coagulant. Initial detection of parasitaemia was by wet mount and buffy coat microscopy (BCM) according to standard criteria (Murray et al., 1983). Buffy coat smears stained with 10% Giemsa stain was used for the morphological identification of trypanosomes using standard keys (Soulsby, 1982). Blue and black/grey biconical and NITSE traps baited with octenol (1-octen-3-ol), phenol (4-methyl phenol) and ox-urine were used as colour and odour attractants respectively to trap arthropod vectors at the various sampling sites. The 89 traps used in the study were placed at a spacing of 25 meters according to the methods of Siziya, (1994). The morphological identification of the vectors was carried out using standard keys (Soulsby, 1982).

Statistical analysis

Data collected were subjected to t-test in pair wise comparison of prevalence rates where $p < 0.05$ were considered significant. A 2 x 2 contingency table was used to analyse the relative risks. Chi-square was used for independence and trend which gave the measure of strength of association between the variables (Maed and Curnow, 1983).

Results

Table 1 presents the trypanosomosis prevalence among the sedentary livestock in the arid region of Northeastern Nigeria. Among these animals examined, 14.3% were infected with Trypanosoma vivax, 0.3% with T. brucei and 2.3% with T. congolense out of 700 cattle; 52% and 26.2% were infected with T. evansi out of 125 camels (C. dromedarius) and 65 horses examined, respectively; 2.0% were infected with T. vivax and T. evansi and 1.0% with T. congolense out of 100 sheep; and 1.3% were infected with T. vivax while 2.6% had T. brucei out of 78 goats.

Table 2 presents the trypanosomosis prevalence among the nomadic livestock in the arid region of Northeastern Nigeria. Among these animals 1,324 cattle were infected with T. vivax, T. brucei and T. congolense, respectively, while 39.2% of the 655 camels had T. evansi infection and the horses had no infection. Among the 450 nomadic sheep, 1.6%, 0.4%, 0.2% had T. vivax, T. brucei, T. congolense and T. evansi infections, respectively, while the infection rates were 0.6%, 0.3% and 0.6% for T. vivax, T. brucei T. congolense and T. evansi respectively among the 365 nomadic goats examined.

Table 3 presents the trypanosomosis prevalence among the captive carnivores in the arid region of Northeastern Nigeria. Out of the 30 carnivores examined, 20% and 30% of the 10 lions (Panthera leo) examined had T. vivax and T. brucei infections, respectively. The 9 stripped hyenas (Hyena hyena) examined showed infections rates of 22.2%, 44.4% and 11.1% for T. vivax, T. brucei and T. congolense, respectively. Out of the 6 spotted hyenas

(Crocuta crocuta) examined, 66.7% and 33.3% had T. vivax, T. brucei and T. congolense infections, respectively; whereas 2(50%) of 4 the jackals (Canis aureaus) examined had both T. vivax and T. brucei infections. One caracal (Felis lynx) examined (100%) had only T. brucei. Table 4 presents the trypanosomosis prevalence among captive Artidactyla/Prboscidae in the arid region of Northeastern Nigeria. Out of the 65 Artidactyla/Prboscidae examined, 100% infection rate with T. vivax occurred in 2 African elephants (Loxodonta africana), 1 cape eland (Tauratragus derbesensis), 4 western kobs (Kobus kob) and 2 Senegal hartebeest (Damaliscus korrigum). They were infections with T. vivax, T. brucei and T. congolense, respectively, in 30%, 20% and 15% out of 20 dorcas gazelles (Gazella dorcas), 25%. 12.5% out of 8 sitatungas (Tragelaphus speikei) and 38.9%, 16.7% and 22.2% out of 18 red fronted gazelles (Gazella rufifrons). Among the 10 Grimm's duicker (Sylicaprea grimmia), 20%, 10% 30% and 10% were infected with T. vivax, T. brucei, T. congolense and T. evansi.

Table 5 presents the files caught using fly traps in the vicinity of the sapling sites. In the vicinity of the sedentary herds, 2,400 flies were caught. This comprised of Stomoxys 600(25.0%), Tabanus 700(29.2%), Lyperosia 500(20.8%), Hippobosca 42(17.5%) and unclassified flies 200(8.3%). In the vicinity of the nomadic herds, 1,970 flies wee caught which comprised of Stomoxys 760(38.6%), Tabanus 430(2.59%), Lyperosia 400(20.3%), Hippobosca 300(15.2%) and Musca species 80(4.1%). In the vicinity of the various captive wildlife enclosures/cages 1,830 flies were caught which comprised of Stomoxys 620(33.9%), Tabanus 350(19.1%), Lyperosia 200(10.9%), Hippobosca 320(17.5%), unclassified flies 153(8.36%) and Musca species 187(10.2%).

Table 1: The trypanosomosis prevalence among sedentary livestock in the arid region of Northeastern Nigeria

Animals	**No. Examined**	**No. Infected with (%):**			
		T. vivax	***T. brucei***	***T. congolense***	***T. evansi***
Cattle	700	100(14.3)[a]	2(0.3)[a]	16(2.29)[a]	0(0)[a]
Camels	125	0(0)[b]	0(0)[b]	0(0)[b]	65(52.0)[b]
Horses	65	0(0)[b]	0(0)[b]	0(0)[b]	17(6.15)[c]
Sheep	100	2(2.0)[c]	0(0)[b]	1(1.0)[c]	2(2.0)[d]
Goats	78	1(1.28)[c]	2(2.56)[c]	0(0)[b]	0(0)[e]
Total	**1,068**	**103(9.6)**	**4(0.4)**	**17(1.6)**	**84(7.9)**

Different superscripts in columns differed significantly ($p<0.05$)

Table 2: The trypanosomosis prevalence among nomadic livestock in the arid region of Northeastern Nigeria

Animals	**No. Examined**	**No. Infected with (%):**			
		T. vivax	***T. brucei***	***T. congolense***	***T. evansi***
Cattle	1, 324	200(15.1)[a]	16(1.2) [a]	70(5.3)[a]	0(0)a
Camels	655	0(0) b	0(0)b	0(0)b	245(39.2)b
Horses	208	0(0)b	0(0)b	0(0)b	0(0)c
Sheep	450	7(1.56)c	2(0.44)c	1(0.2)c	1(0.2)d
Goats	365	2(0.55)c	1(0.3)c	2(0.6)c	2(0.6)d
Total	**v3,002**	**209(6.96)**	**19(0.63)**	**73(2.43)**	**245(8.16)**

Different superscripts in columns differed significantly ($p<0.05$)

Table 3: The trypanosomosis prevalence among captive carnivores in the arid region of Northeastern Nigeria

Animals	No. Examined	No. Infected with (%):			
		T. vivax	*T. brucei*	*T. congolense*	*T. evansi*
Lions (Pantherea leo)	10	2(20.0)[a]	3(30.0)[a]	0(0)[a]	0(0)[a]
Stripped hyena (Hyena hyena)	9	2(22.2)[a]	4(44.4)[b]	1(11.1)[b]	0(0)[a]
Spotted hyena(Crocuta crocuta)	6	4(66.7)[b]	1(16.7)[c]	2(33.3)[c]	0(0)[a]
Jackals(Canis aureaus)	4	2(50)[c]	2(50)[c]	0(0)[a]	0(0)[a]
Caracal (Felis lynx)	1	0(0)[d]	1(100)[c]	0(0)[a]	0(0)[a]
Total	**30**	**10(33.3)**	**11(36.7)**	**3(10.0)**	**0(0)**

Different superscripts in columns differed significantly ($p<0.05$)

Table 4: The trypanosomosis prevalence among captive Artiodactyla/Proboscida in the arid region of Northeastern Nigeria

Animals	No. Examined	No. Infected with (%):			
		T. vivax	*T. brucei*	*T. congolense*	*T. evansi*
African elephants (Loxodonta africana)	2	2(100)[a]	0(0)[a]	0(0)[a]	0(0)[a]
Cape eland (Tauratragus derbisiensis)	1	1(100)[a]	0(0)[a]	0(0)[a]	0(0)[a]
Grimm's duicker (Sylvicaprea grimmia)	10	2(20.0)[b]	1(10)[b]	3(30)[b]	1(10)[b]
Western kob (Kobus kob)	4	4(100)[b]	0(0)[a]	0(0)[a]	0(0)[a]
Dorcas gazelle (Gazella dorcas)	20	6(30)[c]	4(20)[c]	3(15)[c]	0(0)[a]
Sitatunga (Tragelaphus speikei)	8	2(25)[c]	1(12.5)[d]	1(12.5)[c]	0(0)[a]
Senegal hartebeest (Damaliscus korrigum)	2	2(100)[a]	0(0)[a]	0(0)[a]	0(0)[a]
Red fronted gazelles (Gazella rufifrons)	18	7(38.9)[c]	3(16.7)[e]	14(22.2)[b]	0(0)[a]
Total	**65**	**26(40)**	**9(13.8)**	**11(16.9)**	**0(0)**

Different superscripts in columns differed significantly ($p<0.05$)

Discussion

The high prevalence rates of single or mixed trypanosome infections encountered among the livestock and wildlife shows that trypanosomosis is endemic in the area. This is in contrast to previous studies where cattle in the area harboured mainly T. vivax (Mbaya, 1988; Nawathe et al., 1994). In as much as trypanosomes have been encountered among wild animals in the same park (Mbaya et al., 2008), the occurrence of T. vivax among the elephants, T. brucei in the caracal (F. lynx) and a variety of mixed infections in both carnivores and Artidactyla/Proboscidae are being reported for the first time in the arid region in particular and Nigera in general. This also showed that, new infections were acquired by the animals over time. During the study it was observed that all the wild animal species that harboured either single or mixed infections of pathogenic

Table 5: Flies caught using fly traps in the vicinity of sampling sites in the arid region of Northeastern Nigeria

Status/Locations	Total catch	Types/Number of flies (%)	
Sedentary herds / Borno/Yobe State	2,400	(i) Stomoxys	600(25.0)[a]
		(ii) Tabanus	700(29.2)[a]
		(iii) Lyperosia	500(20.8)[a]
		(iv) Hippobosca	420(17.5)[a]
		(v) Musca	200(8.3)[b]
Nomadic herds / Sambisa Game Reserve	1, 970	(i) Stomoxys	760(38.6)[a]
		(ii) Tabanus	430(2.6)[b]
		(iii) Lyperosia	400(20.3)[a]
		(iv) Hippobosca	300(15.2)[a]
		(v) Musca	80(4.1)[c]
Captive animalEnclosures/Maiduguri Zoo	1, 830	(i) Stomoxys	620(33.9)[a]
		(ii) Tabanus	350(19.1)[b]
		(iii) Lyperosia	200(10.9)[c]
		(iv) Hippobosca	320(17.5)[b]
		(v) Unclassified	153(8.4)[c]
		(vi) Musca	187(10.2)[c]

Different superscripts in column differed significantly ($p<0.05$)

trypanosomes of livestock did not show any clinical symptoms which suggest their possible role as reservoir hosts in the area (Mbaya, 2012). However, stress associated with captivity has been reported as the possible reason for an outbreak of T. brucei infection among a herd of red fronted gazelles (G. rufifrons) in the same zoo (Mbaya, 2007). The presence of cyclically and mechanically transmitted trypanosomes among the various captive wildlife species coupled with the presence of haematophagus arthropod vectors in the area, might be of potential hazard to livestock or vice versa. The possibility of mechanical transmission through Tabanus, Stomoxys, Hippobosca and Lyperosia, whose painful bites irritates the hosts, which immediately ward them off thereby favouring interrupted feeding, have been reported as the mode of spread of T. vivax among livestock in the tsetse free-arid region of northeastern Nigeria (Mbaya, 1988; Nawathe et al., 1990; 1994). On one hand, this might likely be the mode of transmission of the T. vivax or T. evansi from one animal to another in the area. While on the other hand, the high prevalence of mixed or single infections with T. brucei, T. congolense or T. vivax among the carnivores in that order might be associated with either biological transmission or oral transmission. Of great concern is the fact that some of the animals in the zoo were captured from Sambisa Game Reserve in the arid region of northeastern Nigeria and were found to have low parasitaemia due to biologically transmitted trypanosomes. This might point towards the direction that perhaps, mechanical transmission in the Game Reserve might be accompanied by biological transmission. Therefore, if this hypothesis is true, then a national survey is required to re-asses the tsetse boundaries. This is because, areas such as the Obudu, Jos and Mambilla plateaux which were ones considered to be tsetse free have now been infested with Glossina species (Leeflang, 1978). In view of this it is therefore necessary to know if the Guinea Savannah tsetse fly; (G. morsitans) has crossed its natural boundary. Or perhaps seasonal migration might have caused the free-living wild animals in Sambisa Game Reserve to wander into the nearest tsetse belt (Guinea Savannah) where they might have acquired the infection before

there were subjected to captivity in Maiduguri Zoo. Oral transmission is possibly another way in which the trypanosomes might have been transmitted among the carnivores examined. This is because the usual feeding practice in the zoo for the past 18 years involves feeding of the carcases of Artiodactyla or Proboscidae that died in the zoo to the captive carnivores (Mbaya et al., 2008). This practice might have been responsible for the high prevalence of trypanosomes among the captive carnivores. However, for oral transmission to be successful, feeding of a dead infected animal to a non infected animal must be done immediately after death for trypanosomes to be infective because "carrion" feeding does not favour mechanical transmission of trypanosomes (Sasaki et al., 1995). Oral transmission through abrasions caused by bone splinters in the oral mucosae in predatory carnivores preying on infected Artiodactyla has been associated with high prevalence of trypanosomosis among wild carnivores (Sasaki et al., 1995; Mbaya et al., 2008). It is therefore recommended that chemoprophylaxis and chemotherapy be undertaken among livestock and the use of fly traps in the area to reduce risks to wildlife conservation and losses in livestock production.

Impact

This manuscript finding shows how various trypanosomes encountered among the sedentary and nomadic livestock, captive carnivores and Artodactyla/Proboscidae in the tsetse free arid region of Northeastern, Nigeria had high prevalence rates. On one hand, this can affect the in-situ conservation and productivity of these endangered species. On the other hand, this could lead to losses in livestock production. Haematophagus arthropod vectors caught in the area are probably responsible for mechanical transmission of trypanosomes between the various animal groups.

Acknowledgements

The authors would like to thank the management and staff of Sanda Kyarimi Park and Sambisa Game Reserve for assistance rendered in the course of the study. The work was funded solely by the authors and there are no conflicts of interests whatsoever.

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ENZOOTIC NASAL ADENOCARCINOMA: CYTOLOGICAL AND CLINICOPATHOLOGICAL OBSERVATIONS IN A WEST AFRICAN DWARF GOAT.

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Abstract

Enzootic nasal adenocarcinoma (ENA), a contagious retroviral disease of sheep and goats, characterized by neoplastic growth of the ethmoidal mucosa in the nasal cavity is described in a West African Dwarf goat (WAD). A two-year old WAD goat, weighing approximately 20kg was observed in the Teaching and Research Farm of the University of Agriculture Abeokuta, Nigeria. The WAD goat was presented with a clinical history of 31/2 months seromucoid to mucopurulent nasal discharge, dyspnoea with audible rales, stertorous breathing with open mouth, coughing and sneezing. Cytological evaluation revealed anaplastic features such as hyperchromasia and binucleation with 2 or more nucleoli. Haematological profile revealed leukocytosis, due to lymphocytosis initially, which later changed to neutrophilia with left shift, however, the was absence of anaemia. Grossly, there was unilateral, left facial swelling, bilateral, separate and discrete nodular and papillary tumourous masses in each nasal cavity with concomitant inflammation, necrosis and distortion of the nasal bones, turbinates and the median septum. Microscopically, two forms of the tumour mass were observed, which include; polyploidy and papillary forms and characterized by well-differentiated tubulo-acinar and cystic glands with their content Periodic Acid Schiff positive for glycoprotein. This report, to the best of our knowledge, is the first reported case of ENA in WAD goats in Nigeria.

Keywords: Enzootic nasal adenocarcinoma, West African Dwarf goat, Nigeria.

ADENOCARCINOME NASAL ENZOOTIQUE : OBSERVATIONS CYTOLOGIQUES ET CLINICO-PATHOLOGIQUES CHEZ UNE CHEVRE NAINE D'AFRIQUE DE L'OUEST

Résumé

L'adénocarcinome nasal enzootique (ENA), une maladie rétrovirale contagieuse des ovins et caprins caractérisée par une croissance néoplasique de la muqueuse ethmoïdale dans la cavité nasale, est décrit chez une chèvre naine d'Afrique de l'Ouest (WAD). Une chèvre naine âgée de deux ans, pesant environ 20 kg, a fait l'objet d'une observation dans la Ferme d'application et de recherche de l'Université d'Agriculture d'Abeokuta au Nigeria. La chèvre naine avait des antécédents cliniques d'écoulement nasal séromucoide - mucopurulent de 31/2 mois, une dyspnée avec des râles audibles, une respiration ronflante avec le museau ouvert, une toux et des éternuements. L'évaluation cytologique a révélé des caractéristiques anaplasiques telles que l'hyperchromasie et la binucléation avec 2 ou plusieurs nucléoles. Le profil hématologique a révélé une hyperleucocytose, en raison d'une lymphocytose initialement, qui s'est transformée plus tard en neutrophilie avec déviation à gauche ; cependant, il n'y avait pas d'anémie. L'examen macroscopique a révélé un gonflement unilatéral gauche du visage, des masses tumorales nodulaires et papillaires bilatérales séparées et discrètes dans chaque cavité nasale avec une inflammation concomitante, une nécrose et une déformation des os du nez, des cornets et de la cloison médiane. L'examen au microscope a révélé deux formes de masse tumorale, qui comprennent des formes polyploïdies et papillaires et caractérisées par des glandes tubulo-acineuses et kystiques bien différenciées, leur contenu étant PAS (acide périodique Schiff) positif pour la glycoprotéine. Ce rapport, à notre connaissance, est le premier cas rapporté d'ENA chez les chèvres naines ouest-africaines au Nigeria.

Mots-clés : Adénocarcinome nasal enzootique ; Chèvre naine d'Afrique de l'Ouest ; Nigeria.

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Introduction

Enzootic nasal adenocarcinoma (ENA) is a contagious upper respiratory, viral, neoplastic disease of sheep and goats, characterized by profuse initial seromucoid and then purulent nasal exudate, associated with clinical signs of respiratory distress. Various synonyms have been given to the condition in the literature such as enzootic nasal tumor, infectious adenopapillomatous, infectious adenopapilloma and infectious nasal adenocarcinoma (Duncan et al., 1967; Njoku et al 1978; De las Heras et al., 1995, 2003).

A viral agent has been incriminated as the aetiology of the disease in small ruminants (Yonemichi et al., 1978: Cousens et al., 1996; 1999). Based on complete molecular sequencing, the virus has been classified as a type D retrovirus (Cousens et al., 1999; Ortın et al., 2003). Its synergistic relationship with ovine enzootic nasal tumour virus that causes ovine pulmonary adenomatosis has been demonstrated (Ortın et al., 2003).

Few reported cases of ENA in goats have been documented from different parts of the world such as Italy, Spain, France, Greece, India and Canada (Rajan et al., 1980; Vitellozzi et al., 1993; Scocco et al., 2001). In Africa, the condition has only been reported in sheep and pig from Ghana, Nigeria and South Africa (Vohradsky, 1974; Njoku et al., 1978; Mc Connell, 1970).

In the subtropical region, tumors of the respiratory tracts are uncommon findings in WAD goats. To the best of our knowledge, ENA has not been reported in goats in Africa. Therefore, this report described cytological and clinico-pathological observations in a WAD goat in Nigeria.

Case History

Signalments

A two-year old West African Dwarf goat, weighing approximately 20kg, was observed in the Teaching and Research Farm of the University of Agriculture Abeokuta, Nigeria with a history of 31/2 months seromucoid nasal discharge.

On clinical examination, the temperature ranged between 37.5^{0}C to 39.7^{0}C. The respiratory and heart rate were 17/mins and 90beats/mins respectively over the entire period. There was persistent and free flowing seromucoid nasal discharge which later became mucopurulent with intermittent epistaxis. Clinical signs such as dyspnea with audible rales, stertorous breathing with open mouth, coughing and sneezing were observed. The bacteria culture of the seromucoid nasal discharge yielded no result and a presumptive diagnosis of pneumonia was made. Tylosin was administered for 5 days, but there was no improvement. Toward the end of the clinical course, there was copious mucopurulent nasal discharge with facial asymmetry, due to a swelling of about 4cm in diameter extending over the nasal bone with indistinct border (Fig. 1).

Cytology and Haematology

Fine needle aspiration through the facial swelling was performed. The aspirate showed a cloudy, greyish white and slightly blood-tinged fluid with offensive odour. Cytological examination of the aspirate revealed numerous polymorphonuclear cells. Cuboidal epithelial cells were also observed interspersed between the polymorphonuclear cells. The morphology of the cuboidal cells revealed some anaplastic features such as marked hyperchromatic nuclei, high nuclei-cytoplasmic ratio, binucleation, with two or more nucleoli and deeply basophilic cytoplasm (Fig. 2). Numerous bacteria with round to coccoid appearance were also observed.

Initial haematological profile revealed absence of anaemia but there was leukocytosis [Total WBC=17.5x103µl, Normal value = 16 x103µl], due to lymphocytosis [Lymphocytes = 57.4 x 103 µl, Normal value= 46 x103 µl], which later changed to neutrophilia with left shift [Segmenters=70 x103µl, Normal value=48 x103 µl, Band Neutrophils=5 x 103 µl, Normal value = 0.5 x 103 µl (Oduye, 1976)]. Results of serum biochemical analysis were unremarkable.

Microbiological findings

Bacterial culture of the mucopurulent exudate yielded heavy growth of both Pseudomonas aurigenosa and Staphylococcus

aureus. The P. aurigenosa was sensitive to norfloxacin (10µg) and ciprofloxacin (10µg). Also the S. aureus was sensitive to norfloxacin (10µg), ciprofloxacin (10µg), gentamycin (10µg), enrofloxacin (5µg) and streptomycin (10µg). After 4 months of the clinical manifestations of the condition and before the initiation of treatment, the goat was found dead.

Pathology

At necropsy, the carcass was in good body condition. There was mucopurulent nasal discharge with unilateral left facial swelling on the nasal bone which pitted upon pressure. When incised, the left nasal bone was soft and distorted. A mass was observed on the surface of the ethmoid cartilage which occupied the caudal nasal cavities. It was bilateral, separate and discrete in each nasal cavity with accompanied inflammation, necrosis and distortion of the nasal bones, turbinates and the median septum. The mass showed both nodular and papillary appearance. The nodular growths were soft to firm in consistency with grayish-white colour and showed less mucoid exudation on the surface. The papillary form was extremely soft and fragile with grayish-tan, red and dark colouration and abundant mucoid exudate (Fig. 3). Tissues from different parts of the mass were fixed in 10% neutral buffered formalin, then dehydrated, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin (H & E). Parts of the sections were also stained with Periodic Acid Schiff (PAS).

Microscopically, two forms of the tumor mass were observed, which include; polyploidy and papillary forms (Fig. 4). Both were characterized by well- differentiated, tubulo-acinar (Fig.5) and cystic glands. Multifocal areas of fibrous connective tissues and myxoid areas were also observed. Cystic tubular structures were present within the stroma. Numerous inflammatory cells, (mostly neutrophils and macrophages), were observed in the necrotic areas. The neoplastic cells were either columnar or non-ciliated cuboidal in appearance. Nuclei were uniform round to ovoid-shaped, vesicular and hyperchromatic with indistinct nucleoli. They were basally displaced and the cytoplasm showed apical vacuolations which were positive for glycoprotein with PAS stain reaction (Fig. 6). The mucinous secretions with the cystic tubular structures in the stroma were also PAS positive. Mitotic figures were scanty.

The polyploidy form showed well-differentiated, pseudostratified columnar epithelial cells, forming a continuous convoluted layer, overlying a pedunculated stalk of loose myxoid, fibrovascular connective tissue (Fig. 7). The subepithelial layer showed moderately diffuse mononuclear cells infiltration, comprising of lymphocytes, plasma cells and macrophages. The nuclei were round to oval in shape and basally displaced with marginated chromatin and indistinct nucleoli.

The apical portion of the cytoplasm contained vacuoles which were positive for glycoprotein with PAS reaction. There were foci of mineralization at the base of the stalk.

Discussion

West African dwarf goats are found in the humid forest zone of the subtropical region of African. They thrive in this environment because of their high tolerance to excessive humidity and trypanosomiasis (Upton, 1985). Besides poultry, WAD goats are the most common domestic animals in this region, due to the fact that nearly every home has WAD goats as pets and source of income in many families (Osuagwuh, 1984).

The most common respiratory disease of WAD goats is a peste des petits ruminant caused by morbillivirus (Obi et al., 1983). The occurrence of tumour in this breed of goat is uncommon. Most of the reported cases of ENA have been documented in sheep in Nigeria (Njoku et al., 1978) and few cases in goats in different parts of the world (Rajan et al., 1980; Vitellozzi et al., 1993; Scocco et al., 2001). Among the few cases observed in goats, to the best of the authors' knowledge, no single report has been documented in WAD goats.

The clinical manifestations of this condition in WAD goat is similar to those reported by other workers in sheep and goats elsewhere (De las Heras et al., 1991; Vitellozzi et al., 1993). However, clinical manifestations such as emaciation, bloating and exophthalmia reported by these workers were not observed



Figure 1: Photograph of the West African Dwarf goat showing facial swelling and mucopurulent nasal discharge.

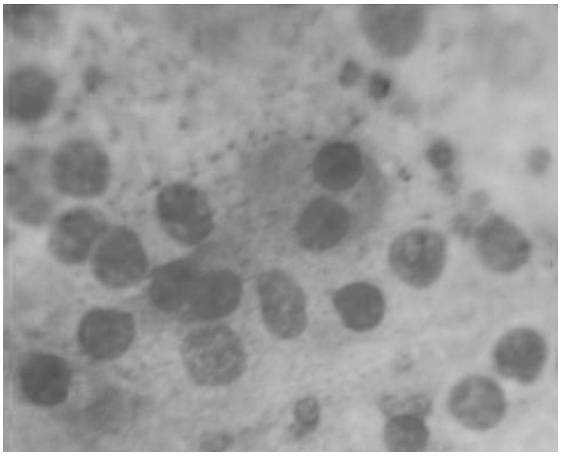


Figure 2: Fine needle aspirate of the mass showing hyperchromatic nuclei, binucleation (arrow) and two or more nucleoli with deeply basophilic cytoplasm. Giemsa Stain. X400.

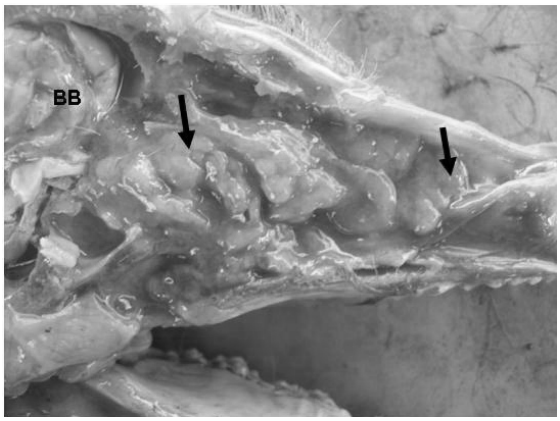


Figure 3: Head saggittal section, showing the brain (BB), grayish tan nodular and papillary masses (arrows) in the nasal cavity.

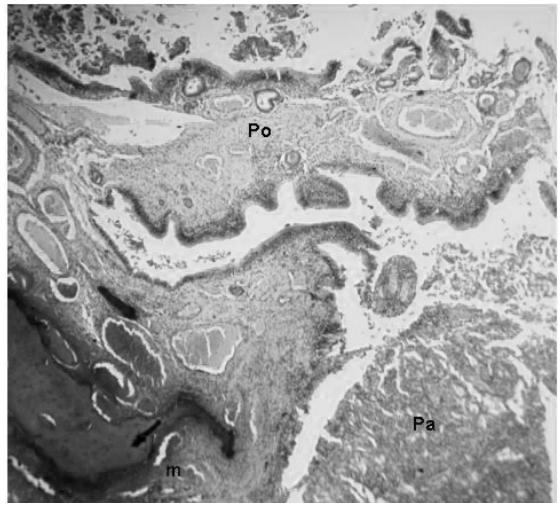


Figure 4: Photomicrograph of the tumour mass showing both papillary (Pa) and polyploidy (Po) form with areas of mineralization (m) at the root of the stock. X 250. H & E stain

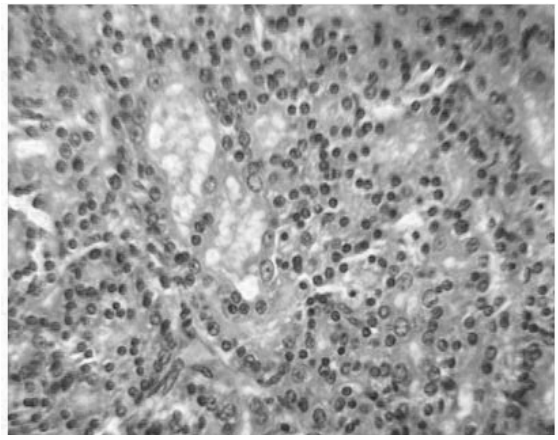


Figure 5: Photomicrograph from the papillary form of the mass showing tubulo-acinar pattern with engorged blood vessels. X 350. H & E stain.

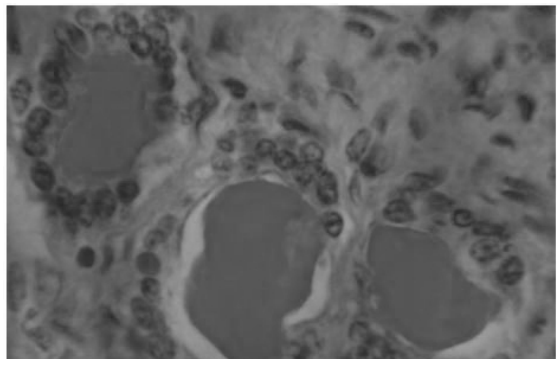


Figure 6: Photomicrograph from the polyploidy form of the mass showing cystic tubular pattern within the loose myxoid stroma with PAS positive glycoprotein material. X 400. PAS stain.

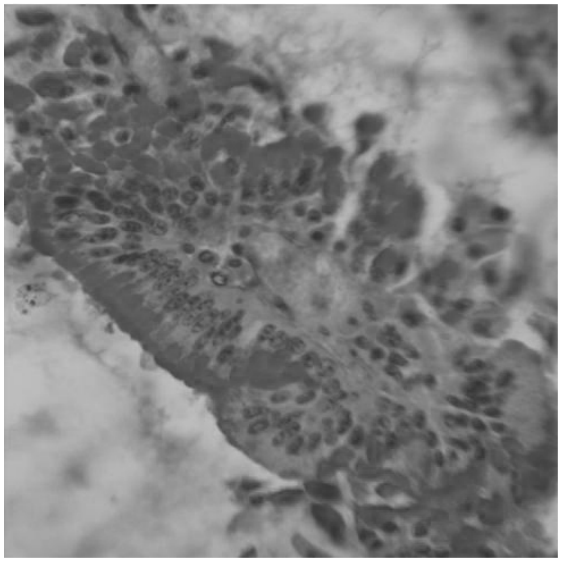


Figure 7: Photomicrograph of the pseudostratified neoplastic cells with basally displaced vesicular nuclei showing two or three nucleoli and PAS positive glycoprotein on the apical part. X 300. PAS stain.

in this case. Although, Mckinnon et al., (1982) affirmed that sheep succumbed to the disease after 90 days of clinical signs, but in this report the WAD goat died at 127 days after the onset of clinical manifestations. The reason for this prolonged clinical course is unknown. It is possible to speculate that WAD goat is very tolerance and hardy to infectious disease (Upton, 1985)

A retrovirus type–D has been incriminated as the aetiology of this condition (De las Heras et al., 1991). This probably might have been responsible for the initial lymphocytosis observed in this report. Fine needle aspiration of the mass has been difficult to examine due to the bony structures surrounding the nasal cavity (McKinnon et al., 1982). In this report, the necrosis of the nasal bone allowed for fine needle aspiration and consequently, the cytology of the tumor. The scanty numbers of mitotic figures attested to the fact that the tumor mass was growing at a very slow pace.

The gross and histopathological changes of the tumor were similar to those documented by other workers in different breeds of sheep and goats (De las Heras et al., 1991, 1998; Švara et al., 2006).

ENA seemed to have been a very vrare condition in WAD goats, but this report underscores the possibility of underreporting of ENA in WAD goats in Nigeria. This may be due to the fact that people consider goat heads as a local delicacy. Thus, the possibility of the disease diagnosis by veterinarians might be obscured due to high demand for goat head during slaughter.

Conflicting reports abound in the literature on the histogenesis of this tumour (De las Heras et al., 1991; Vitellozzi et al., 1993). Scocco et al., (2001) have demonstrated histochemically the two recognizable growth pattern of the tumour; papillary pattern which originate from the respiratory glands and the polyploidy portion which originate from the olfactory glands. They affirmed that the two growths pattern are not only different morphologically but also reflect different histochemical behaviour that could confirm two-fold histological origin. In this report, although histochemical behavioral pattern was not performed, the tubulo-cystic and cytoplasmic staining of glycoprotein by PAS might likely suggest two possible origins of the tumour in this WAD goat.

In conclusion, the incidence and prevalence of ENA in WAD goats in the subtropical region remain to be determined. This report, to the best of our knowledge, appears to be the first reported case of ENA in West African Dwarf goats in the region.

Acknowledgement

The authors are very grateful to Mr. Anise for his technical assistance.

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AN EVALUATION OF PINHOLE CASTRATION AS AN ALTERNATIVE TECHNIQUE FOR DOG POPULATION CONTROL IN RESOURCE-POOR COMMUNITIES

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Summary

We evaluated pinhole castration as an alternative technique for dog population control in resource poor rural communities of Gulu, Northern Uganda. Through a campaign dubbed 'Big Fix Gulu', households in selected communities were mobilized using radio announcements, posters and school visits, to present dogs for mass sterilization during community mobile spay/neuter clinics. All male dogs presented were sterilized using the pinhole method under xylazine-thiopental anesthesia. Castrated dogs were closely monitored for up to five days after castration. In 12 days, 278 dogs were castrated. The mean duration for pinhole procedure was 11.4 minutes. Duration of pinhole castration reduced significantly ($p<0.05$) with increase in dog weight. Each pinhole procedure cost approximately 2.12 United States dollars. The cost significantly ($p<0.05$) increased with increase in dog weight. Post-operative complications were reported in 23 (8.30%) dogs 2-3 days after castration. These included painful swelling of the testes and dullness of castrated dogs. There was unilateral testicular atrophy in two (0.72%) cases three (3) months after castration. We concluded that pinhole castration is indeed a simple, cheap and effective alternative for mass dog castration; the technique could be adopted for dog population in developing countries.

Keywords: Dogs, mass sterilization, poor communities, pinhole castration

EVALUATION DE LA CASTRATION STÉNOPÉE COMME TECHNIQUE ALTERNATIVE DE CONTRÔLE DE LA POPULATION CANINE DANS LES COMMUNAUTÉS PAUVRES EN RESSOURCES

Résumé

Nous avons évalué la castration sténopée comme technique alternative pour le contrôle de la population canine dans les communautés rurales pauvres en ressources de Gulu, dans le nord de l'Ouganda. Dans le cadre d'une campagne baptisée Big Fix Gulu, les ménages des communautés sélectionnées ont été mobilisés au moyen des annonces radio, des affiches et des visites scolaires, pour présenter les chiens à la stérilisation de masse au cours des cliniques mobiles communautaires de stérilisation animale. Tous les chiens mâles présentés ont été stérilisés à l'aide de la méthode sténopée sous anesthésie avec xylazine-thiopental. Les chiens castrés ont été étroitement suivis jusqu'à cinq jours après la castration. En 12 jours, 278 chiens ont subi l'opération de castration. La durée moyenne de la castration sténopée était de 11,4 minutes. La durée de la castration sténopée a baissé de manière significative ($p <0,05$) avec l'augmentation du poids du chien. Chaque opération sténopée a coûté environ 2,12 USD. Le coût a augmenté de manière significative ($p <0,05$) avec l'augmentation du poids du chien. Des complications post-opératoires ont été signalées chez 23 (8,30%) chiens, 2-3 jours après la castration. Il s'agissait notamment d'un gonflement douloureux des testicules et la torpeur des chiens castrés. Une atrophie testiculaire unilatérale a été observée chez deux (0,72%) cas, trois (3) mois après la castration. Nous avons conclu que la castration sténopée est effectivement une alternative simple, bon marché et efficace pour la castration de chiens en masse ; la technique pourrait être adoptée pour le contrôle de la population canine dans les pays en développement.

Mots-clés : Chiens ; Stérilisation de masse ; Communautés pauvres ; Castration sténopée

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Introduction

Pet overpopulation and its associate stray dog phenomenon is a common menace in many developing countries. Stray dogs play a key role in sustenance and spread of diseases; especially rabies in human and animal populations1. Thus reducing stray dog populations is important in rabies control2. To achieve this, authorities; especially in developing countries have traditionally carried out mass killing of free roaming dogs by use of poisoned baits or even shooting3. Mass killing is however, considered grossly inhumane1.

A noble approach to dog population control is to have a sizable proportion of the dogs in a given population sterilized3. This approach is often championed by charitable welfare organizations in poor communities. These campaigns still largely employ conventional surgical means for mass dog sterilization. Surgical sterilization is however considered stressful, costly, laborious and prone to post-operative complications1, 4. Additionally, because it is a lengthy process, too few animals can be sterilized within a given time by surgical sterilization. Furthermore, surgery requires the services of skilled and experienced practitioners who unfortunately are few in rural communities. Thus, there is need to develop alternative animal sterilization methods that are simple, affordable and less stressful5.

To date, the most promising alternative to conventional surgical sterilization is the in situ spermatic cord ligation (pinhole) technique. This novel technique has been variously described as simple, safe, effective, minimally invasive and a quicker approach to castration1,5,6. So far, the technique has been evaluated only in controlled experiments1,4,5. Its suitability for application in mass animal sterilization has not yet been evaluated. It is hence, not widely known amongst animal health practitioners and in the communities. The current study, evaluated the effectiveness, material costs and complications associated with this novel technique during a mobile mass spay/neuter clinic in resource-poor communities of Gulu District, Northern Uganda.

Materials and methods

Ethical considerations

The higher degrees committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University in its capacity as the Institutional Review Board (IRB), received and approved this study. During the clinics, dog owners were informed about the process and the likely complications pinhole castration. a dog was castrated only if the owner was willing through a verbal consent.

The study area

The study was undertaken in Gulu District, northern Uganda (02°45'N 32°00'E) (Figure 1). Gulu is largely inhabited by subsistence farmers; many of whom are still recovering from post-war trauma caused by displacement into concentration camps for over 20 years by Uganda's recent civil war. In Gulu, there is extreme poverty and life is difficult for both humans and animals. Consequently, people cannot afford to pay for veterinary care much as they are compassionate about animals. There are overwhelming number of stray dogs and cats in the district and rabies is a major scare7.

Community spay/neuter clinics

Through a campaign dubbed 'Big Fix Gulu', locals were mobilized to present dogs for sterilization during community mobile spay/neuter clinics. Radio announcements, posters and fliers were used in community mobilization. Visits were also made to schools, and other public places such as churches and community markets for purpose of mobilization. On such visits, communities were educated about animal welfare, rabies control and the need for dog population control. Specific clinic days were announced in villages within the communities. During each clinic day, all dogs presented were provided free veterinary care including clinical examination and treatment if necessary, rabies vaccination, deworming and spot-on insecticide application (Advantix®, Bayer Animal Health, Germany). Depending on the owner's willingness, the pet was spayed (ovariectomy) or neutered (castrated) free of

charge.

The pinhole procedure

Prior to castration, each dog was sedated using Xylazine (Bomazine®, BOMAC Laboratories, New Zealand) at 1mg/kg body weight intramuscularly. After xylazine administration, the dog was observed for up to 10 minutes to allow for emesis just in case it was not starved prior to presentation. General anesthesia was then induced through an intravenous injection of freshly reconstituted sodium thiopental (Crion Drugs and Pharmaceuticals Pvt., Ltd, India) at 20mg/kg body weight.

All male dogs presented for sterilization were subjected to pinhole castration5. Spermatic cords were ligated using 3.5 metric polygalactin 910 (Vicryl®, Johnson and Johnson, UK) threaded through a hypodermic needle (18G, 1.5in; TERUMO®, USA). The total volume of xylaxine and thiopental used in each case was noted. Length of suture material used was also estimated in centimeters. The cost of each material used was computed from the current market rates in Uganda. Using a stop clock, the duration of each pinhole procedure was estimated as time (minutes) elapsed after induction of anesthesia to completion of the last knot. An independent person timed the operations while those performing the procedure were completely oblivious of the timing.

Monitoring castrated dogs

After each pinhole procedure, the dog was laid on a blanket to recover from anesthesia. It was then handed to the owner/attendant soon after recovery. The owner was advised to closely watch his pet daily for at least 5 days after treatment and report any change in appetite, demeanor, gait/posture and scrotal/testicular appearance. Reported cases were visited and treated for pain/inflammation using parenteral Ketoprofen at 2 mg/kg body weight.

Statistical analysis

All statistical tests were performed using R, Version 3.0.18. Linear mixed models were fitted to examine the effect of age and weight of dogs on duration and cost of castration9.

Results

Characteristics of dog owners

Up to 227 owners or attendants presented 278 male mongrel dogs for pinhole castration over a period of 12 days. Up to 95% of the pet owners were male (boys/men), only 5% were women. Mean age of attendants ranged from 11 to 62 years with a mean age of 18 years. Majority (65%) of attendants were however under 18 years (Figure 2).

Characteristics of dogs presented for castration

The dogs ranged in age from 0.30 to 14 years, and averaged approximately 2.24 years. Most (73%) of the dogs presented were under 3 years. The modal weight of the dogs was 10kg (Figure 3).

Duration of pinhole castration

The mean duration for pinhole procedure was 11.4 minutes. The shortest time taken on a case was 8 minutes while the longest duration was 17 minutes (Figure 4). Duration of pinhole castration reduced significantly ($t = -5.16$, $p<0.05$) with increase in dog weight. For every kilogram increase in dog weight, the pinhole procedure was 0.15 minutes shorter in duration (Table 1). Age was not an important variable in predicting the duration of pinhole operation.

Material costs of pinhole castration

On average, each pinhole procedure required 0.84mls of 2% xylazine, 1.27mls of 5% sodium thiopental and 10.83cm of Vicryl® (Table 2). The total mean cost of these materials was 2.12 US dollars. From the mixed model predictions, the cost of pinhole castration significantly ($t = 18.46$; $p<0.05$) increased with every kilogram increase in dog weight. The cost of pinhole castration increased by about 0.1 dollars for every 1kg increase in dog weight (Table 3). Age and other animal attributes though important had no significant effect on costs.

Table 1: Effects of weight on duration of pinhole castration

Fixed effects	β	SE	d.f.	t-value	P-value
Intercept	13.00	0.35	271	36.82	<0.001
Weight Dog	-0.15	0.03	271	-5.16	<0.001

Table 2: Material requirements and cost of pinhole castration in community neuter clinics

Materials	Quantity (± SD)	Mean Cost (USD)
Xylazine (mls)	0.84 ±0.24	1.35 ± 0.39*
Thiopentol (mls)	1.27 ± 0.55	0.25 ± 0.11
Vicryl® (cm)	10.83± 2.03	0.52 ± 0.10
Total mean cost (USD)		**2.12 ± 0.47**

*Cost of xylazine was more than double the combined cost of thiopental and Vicryl®

Table 3: Effect of weight of dog on cost of pinhole castration

Fixed effects	β	SE	D.F.	t-statistic	P-value
Intercept	1.08	0.08	271	13.97	<0.001
Weight of Dog	0.09	0.01	271	18.46	<0.001*

*Cost of castration significantly increased with weight of dog

Table 4: Complications of mass pinhole castration in dogs

Age category (years)	Number of dogs	Number of dogs with complications (%)
<01	55	1 (01.8)
01-02	147	5 (03.4)
03-04	45	8 (17.8)
05-06	13	3 (23.1)
07-08	15	4 (26.7)
09-10	2	1 (50.0)
11-12	0	0 (0.00)
13-14	1	1 (100)
Total	**278**	**23 (8.30)**

Complications of mass pinhole castration

Of the 278 dogs castrated, owners of 23 (8.30%) called 2-3 days later to report complications observed (Table 4). The common complications reported were painful swelling of the testes and dullness of the treated animals. These were treated for pain and inflammation using Ketoprofen at 2 mg/kg body weight intramuscularly. Two dogs (0.72%) reportedly continued to mate 3 months after castration. In both cases, there was unilateral testicular atrophy (Figure 5). On histopathology, the non-atrophic testes had tissue architecture typical of a viable testis (Figure 6). Generally, the frequency of complications increased with the age of the dog at the time of castration. Dogs



Figure1: Map of Uganda showing Gulu district (highlighted red). Adopted form Wikipedia.com

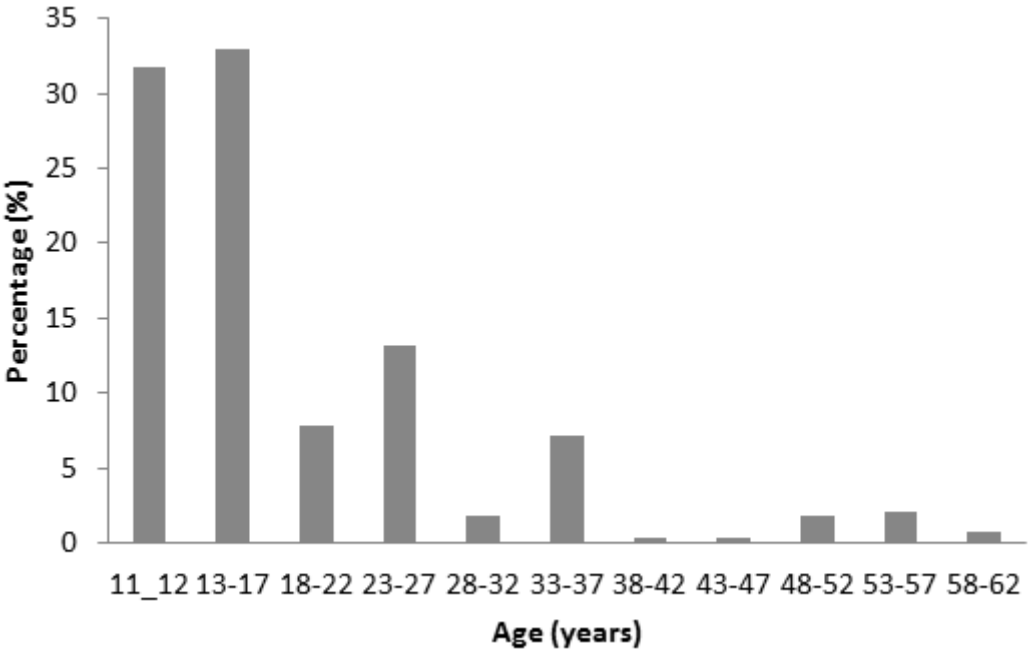


Figure 2: Mean age of pet owners attending community spay/neuter clinics in Gulu District

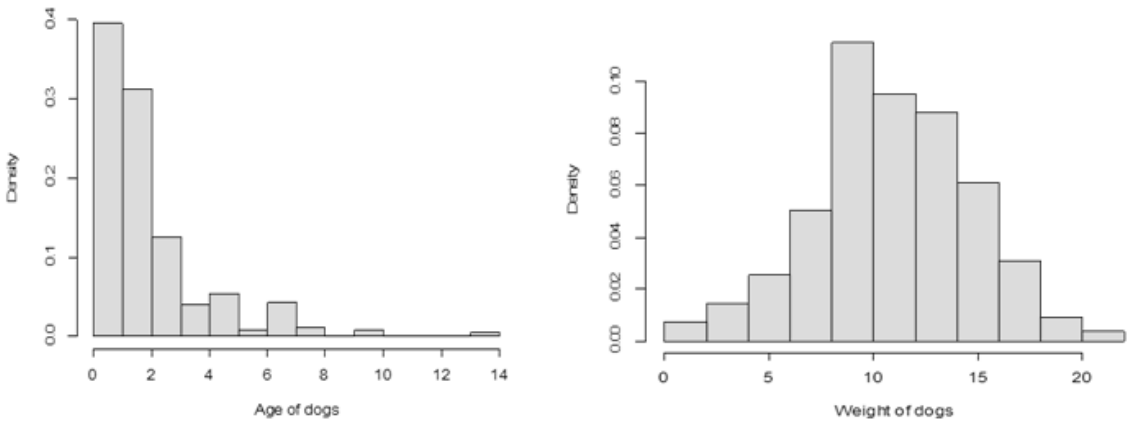


Figure 3: Age and weight of dogs presented for community neuter clinic in Gulu, Uganda

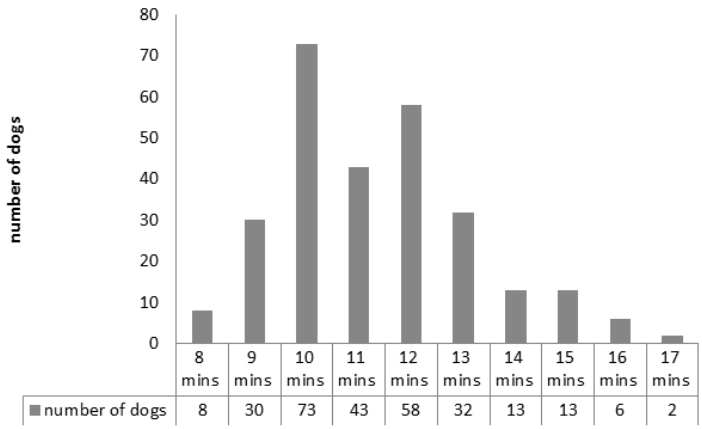


Figure 4: Duration of pinhole procedure in dogs

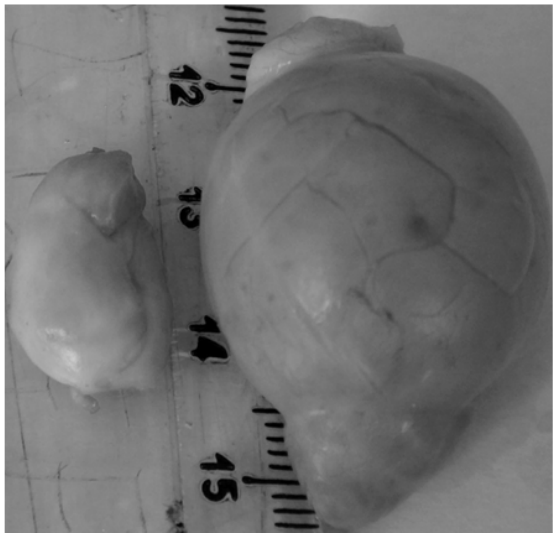


Figure 5: Atrophied (left) and un-atrophied (right) testes recovered from a dog three months after pinhole castration

above two years tended to have high frequency of complications as shown in figure 7.

Discussion

In the current study, attendants (owners) who presented dogs for castration were predominantly (95%) male (men and boys). This directly contrasts with a Finnish study, which showed that more women than men keep pets and that more men than women did not want pets at all10. It should be noted that in Finland; just like in other affluent societies, dogs are kept primarily for companionship and are largely regarded as members of the family11. Given that women are often tasked with caregiving responsibilities, they are more likely to be responsible for pet care12. This is unlike in poor communities

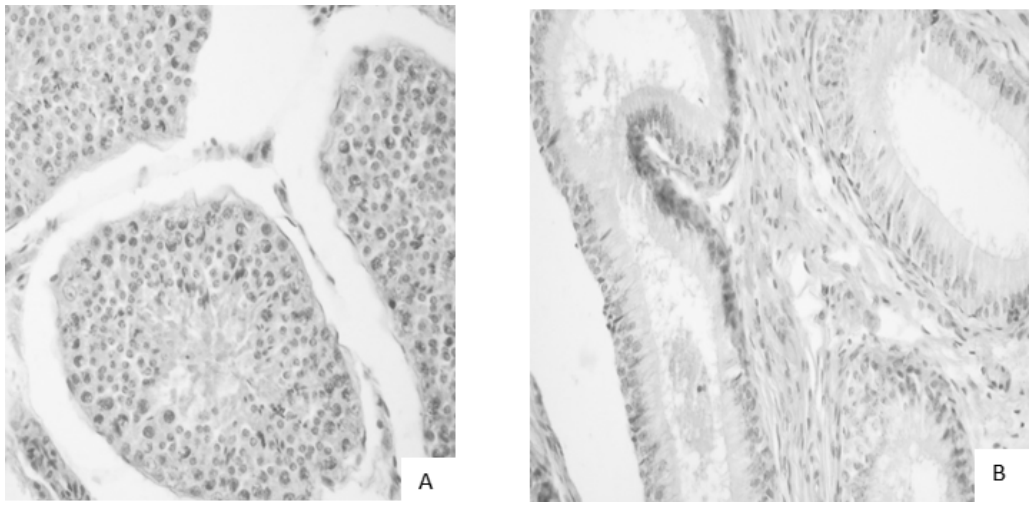


Figure 6: Micrographs showing normal tissue architecture in non-atrophied (A) and fibrosis in atrophied (B) testes.

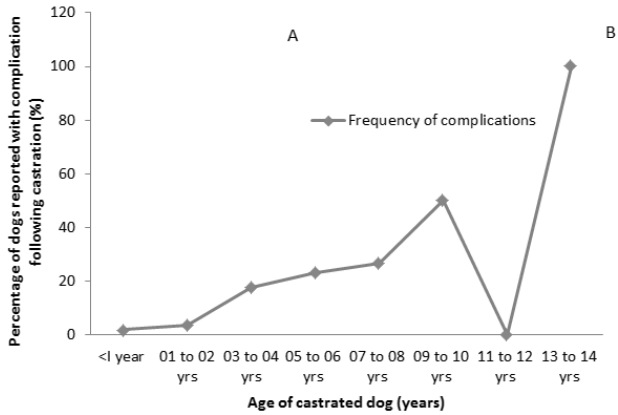


Figure 7: Effect of age on frequency of pinhole castration complications

where dogs are kept primarily for hunting and/or guard purposes13. Hunting is a male chore, hence the closer associating with dogs by men as witnessed in this study. It is hence advisable that successful community mass neuter campaigns in poor communities should target men for effective mobilization. Much as attendants were predominantly male, another staking demographic feature is that the majority (65%) of whom were youth under 18 years. This may be explained by the fact that our sensitization and animal welfare education in primary schools was a key component of mobilization for the mobile spay/neuter clinics. Through this, more school-going youths were directly. It is also probable that the youth predominance reflected the obtaining demographic structure of Uganda where children under 15 years comprise over 50% of the total national population14. Youths also tend to be more adventurous than old people hence will generally embrace new phenomena more easily15. Our spay/neuter clinics were the first ever dog population control campaign conducted in the whole of northern Uganda. Furthermore, it should be borne in mind that in rural African communities, livestock herding is generally a social responsibility of children16. This responsibility may be extended to the care of dogs as seen in this study. This implies that in rural Africa, companion animal intervention programs that engage children (8-15 years) are likely to be more successful.

Dogs presented for castration were mostly (73%) young i.e. under 2 years, less than 10% were 3 years or older. This could be an indication that younger dogs are more populous in communities in Gulu District. This may be attributed to high birth rates due to uncontrolled mating in rural communities where dogs are not confined and sterilization or birth control is a rarity3. It could also indicate that in this community, dogs live a shorter life span, most not reaching 3-4 years. The communities in Gulu where this study was executed are extremely poor to afford good care of their dogs in terms of feeding or requisite veterinary services. In contrast, in developed countries dogs generally leave up to 13 years because of the good welfare accorded to them17. It should however, be noted that castrating animals while still young is a common practice18. However, a systematic investigation is still warranted to give a better understanding of the dog demographic structure and welfare concerns in northern Uganda.

During the clinics, each pinhole procedure took about 11.4 minutes to complete. This closely compares with a recent experimental study that reported time duration of 14 minutes to complete a pinhole castration procedure in puppies4. The same study further reported that a standard surgical castration took thrice (35 minutes) longer than castration using the pinhole technique. This further strengthen the assertion that compared to standard surgical castration, pinhole castration is quicker and simpler to perform4,5. This implies that during mass sterilization of dogs, one is likely to castrate thrice more dogs using the pinhole technique than with the conventional knife castration method at a given time. Surgical castration is an invasive and tedious procedure requiring skilled/experienced personnel; it is in other words a time consuming exercise19. Interestingly, we observed that, duration of pinhole castration decreased with increasing weight of the dog. Pinhole castration is simple because the spermatic cord is easily accessible for manipulation within the scrotal skin and hence easy to ligate in situ1,5. Manipulation of spermatic cord within the scrotal skin it is much easier in heavy dogs than the small ones; weight in this case being primarily a factor of dog age. The cords in young dogs are still tiny, hence much more difficult to manipulate and ligate.

Cost is usually a major consideration in the choice of animal treatment and care options. Indeed, charitable development agencies are inclined to support community projects that are inexpensive but with demonstrable impact. These considerations are the basis of the quest for simple and cheap alternatives for animal sterilization1. At an estimated material cost of 2.12 US dollars per case, we demonstrate that pinhole procedure is a cheaper alternative to conventional surgical castration. It compares with a recent report that estimated the average material cost of 5.76 US dollars for a standard surgical castration in puppies4. They noted that material requirements and cost of surgical

castration was four times higher than that for pinhole castration. This is a testament that given a fixed budget, a community mass neuter project employing the pinhole sterilization technique may have four times the impact of a similar project opting for the standard surgical approach.

Some (8.3%) owners complained that dogs had painful swelling of the testes and were dull within 2-3 days after pinhole procedure. Similar reports of pain and dullness following pinhole castration has been previously reported5,6. In both studies, plasma cortisol assays showed that both surgery and pinhole methods elicited substantial cortisol responses; an indication of pain or stress evoked by either methods. They however noted that surgery elicited higher cortisol response than pinhole technique. This further augments the assertion that pinhole castration is a minimally invasive and less stressful technique that should be readily adopted as an alternative to the standard surgical technique of castration1,5.

Two cases (<1%) of unilateral testicular atrophy were recorded. All previous studies on pinhole castration reported bilateral atrophy in all (100%) of castrated animals1,5,6. However, these studies were controlled experiments involving very few experimental animals with procedures carefully followed to detail. The current study involved mass castration; where 15-25 dogs could be presented for castration by a clinician within 4-6 hours. The rush to complete all cases presented could have led to poor ligature application or the spermatic cords were completely missed during ligation. The spermatic cord may also be missed when using other techniques applied in food animal castration such as the burdizzo and the elastrator methods20. Overall, if well applied, the methods are generally considered effective. We conclude that pinhole castration is indeed a simple, cheap and effective alternative for mass dog castration that should be adopted for use in resource poor communities. This may help curb the rampant stray dog menace and associated rabies incidence in such communities.

Acknowledgments

This study was funded in part by the Swedish International Development Cooperation Agency (Sida) and Makerere University. Ms. Sarah Schmidt of the Central Valley Coalition for Animals, California provided technical advice and help with community mobilization. The funders however, had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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EVALUATION OF SOME FOWL POX, GUMBORO AND NEWCASTLE DISEASE VACCINES MARKETED IN NIGERIA

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Summary

The quality of live commercial fowl pox (FP), Gumboro disease (GD) and Newcastle disease (ND) vaccines manufactured by four laboratories and on sale in Nigeria were tested.

One of the nine vaccines yielded Aspergillus sp., two Salmonella sp. and three Escherichia coli when grown on culture media. All the four ND vaccines and one of the three FP vaccines tested contained the recommended virus titre. None of the two GD vaccines tested contained the recommended virus titre.

Two of the three FP vaccines were able to protect 100% of vaccinated chicks challenged with a virulent FP virus. All chicks vaccinated orally (PO) or intramuscularly (IM) with Two of the three vaccines had pox lesions when challenged while only 17% of chicks had pox lesions following IM vaccination with one of the three FP vaccines. Two weeks post vaccination (PV) only 17% and 33% of the chicks vaccinated with two of the three GD vaccines had precipitin antibodies (PAb) in their sera while 100% of the chicks vaccinated with one of the three GD vaccines had PAb. All the three GD vaccines caused reduction in body weight gain and atrophy of the bursa of Fabricius when administered at one or 10 times the normal dose. The haemagglutination inhibition (HI) antibody titre of ND vaccinated chicks was highest two weeks PV and in chicks vaccinated intraocularly with 10 times the normal dose. Only 50%, 56% and 78% of chicks vaccinated with three ND vaccines were protected when challenged with a local virulent ND virus.

Live viral vaccines are therefore possible sources of bacterial and fungal infections for poultry in Nigeria. Some commercial vaccines contain low virus titres, are poorly immunogenic or pathogenic even at recommended dose therefore are unable to protect all vaccinated chicks when exposed to virulent viruses. The aforementioned factors are some of the likely causes of outbreaks of FP, GD and ND in vaccinated flocks in Nigeria. There is the need to routinely reassess the quality of all commercial poultry vaccines and maintain their quality by proper handling, transportation and storage. Vaccines should also to be properly administered by poultry farmers.

Keywords: Safety, Poultry vaccines, Potency.

EVALUATION DE CERTAINS VACCINS VENDUS AU NIGERIA CONTRE LA VARIOLE AVIAIRE, LA MALADIE DE GUMBORO ET LA MALADIE DE NEWCASTLE

Résumé

Cette étude s'est penchée sur la qualité des vaccins vivants contre la variole aviaire (FP), la maladie de Gumboro (GD) et la maladie de Newcastle (ND) fabriqués par quatre laboratoires et commercialisés au Nigeria.

Un des neuf vaccins a fait apparaître Aspergillus sp., deux Salmonella sp. et trois Escherichia coli en milieux de culture. Tous les quatre vaccins contre la ND et l'un des trois vaccins contre la FP contenaient le titre du virus recommandé. Aucun des deux vaccins contre la GD étudiés ne contenait le titre viral recommandé.

Deux des trois vaccins contre la VA ont pu protéger 100% des poussins vaccinés infectés avec un virus virulent de la VA. Tous les poussins vaccinés par voie orale (PO) ou intramusculaire (IM) avec deux des

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trois vaccins présentaient des lésions de la variole après infection avec le virus, tandis que seuls 17% des poussins avaient des lésions de la variole après vaccination IM avec l'un des trois vaccins contre la VA. Deux semaines après la vaccination (PV : post-vaccination) seuls 17% et 33% des poussins vaccinés avec deux des trois vaccins contre la MG avaient des anticorps précipitines (PAb) dans leur sérum alors que 100% des poussins vaccinés avec l'un des trois vaccins contre la MG avaient des PAb.

Tous les trois vaccins contre la MG ont causé une réduction du gain pondéral et une atrophie de la bourse de Fabricius après administration d'une ou 10 fois la dose normale. L'inhibition de l'hémagglutination (IH) du titre d'anticorps des poussins vaccinés contre la MNC était la plus élevée à deux semaines PV et chez les poussins immunisés par inoculation intraoculaire avec 10 fois la dose normale. Seuls 50%, 56% et 78% des poussins vaccinés avec trois vaccins contre la MNC étaient protégés en cas d'infection avec un virus virulent local de la MNC.

Les vaccins à virus vivants sont donc des sources possibles d'infections bactériennes et fongiques pour les volailles au Nigeria. Certains vaccins commerciaux contiennent de faibles titres viraux, sont faiblement immunogènes ou pathogènes même à la dose recommandée, ils ne sont donc pas capables de protéger tous les poussins vaccinés lorsque ceux-ci sont exposés à des virus virulents. Les facteurs précités sont quelques-uns des causes probables de l'apparition de VA, de MG et de MNC chez les troupeaux vaccinés au Nigeria. Il est nécessaire de réévaluer régulièrement la qualité de tous les vaccins aviaires commerciaux et de maintenir leur qualité par la manipulation, le transport et le stockage appropriés. Les vaccins doivent également être correctement administrés par les éleveurs de volailles.

Mots-clés : Innocuité ; Vaccins aviaires ; Efficacité.

Introduction

Fowl pox (FP), Gumboro disease (GD) and Newcastle disease (ND) are endemic in Nigeria[1, 2, 3]. They are important because they cause reduction in production and high mortality in Nigerian poultry4. They are currently controlled primarily by routine vaccination5. However, outbreaks of these diseases have continued to occur in vaccinated birds[6, 7, 8, 9, 10, 11, 12, 13]. The administration of vaccines of poor quality has often been advanced as the main cause of the outbreaks in vaccinated birds[14, 15].

This study was conducted to determine, the sterility, viability (virus content), safety (pathogenicity, vaccination reaction), immunogenicity (antibody response) and potency (post vaccination challenge reaction) of some live FP, GD and ND vaccines sold to poultry farmers in Nigeria.

Materials and Methods

Chicks

Day-old chicks whose parents were vaccinated against FP and ND only during the first week of life and have remained unvaccinated for GD were used.

Housing

The chicks were housed for 3 weeks in a clean, disinfected and fumigated house and then housed in wire-floored cages.

Feeding

The chicks were fed on commercial chick mash for 3 weeks and then grower's mash ad libitum up to the end of the experiment.

Challenge Viruses

Fowl pox virus: The FP virus (FPV) was obtained from the scabs of the unfeathered skin of 8–week–old chickens that had suffered from FP. Morbidity in the flock was 100%. A 10% w/v homogenized clarified scab in phosphate buffered saline (PBS) pH 7.4 was used for challenge.

Gumboro disease virus: The GD virus (GDV) was obtained from the bursae of chicks naturally infected with GD. A 50% w/v of homogenized and clarified bursae in PBS pH 7.4 was used for challenge. The titre of the GDV was 6.8 Log10 bursal infective dose (BID) 50 per ml of undiluted viral suspension.

Newcastle disease virus: The ND virus (NDV) was isolated and characterized at the National Veterinary Research Institute (NVRI), Vom, Nigeria from the brain of a guinea fowl. It had a haemagglutination titre of 5 Log2. The intracerebral pathogenicity index, intravenous pathogenicity index, mean lethal dose, mean death time and mean embryo infective dose

were 1.69, 2.10, 8.0 Log2, 44.8 h and 8.36 Log10 respectively. Based on these characteristics it was identified as a velogenic strain of the NDV16. It killed 100% of 6-week-old ND susceptible chicks injected intramuscularly (IM) with 0.1 ml of virus suspension diluted from 107 Log10 in sterile PBS pH 7.4.

Vaccines

Live FP, GD and ND (La Sota strain) vaccines were brought off-the-shelf from Agro veterinary shops in Jos, Kaduna, Kano and Zaria, Nigeria. The vaccines were products of four different manufacturers and were designated in the paper as vaccines from source 1, 2, 3 and 4.

Haemagglutination test (HAT) and haemagglutination inhibition test (HIT)

The HAT and HIT were conducted as described by Hitchner et al. 17.

Agar gel precipitation test (AGPT)

The AGPT was conducted as described by Cullen 18 .

Sterility Tests

A dilution of each of the vaccine used was made in duplicate and sub cultured in blood agar (BA) and Sabouraud's dextrose agar (SDA) plates. The BA plates were incubated at 37oC while the SDA plates were incubated at room temperature. The plates were examined for growth after, 24, 48 and 72 hours (h) of incubation.

Viability Tests

The virus titre of 3 vials of each batch of vaccine was determined by inoculating 10-day-old embryonated chicken eggs (in the case of ND vaccines) or 12-day-old embryonated chicken eggs (in the case of FP vaccines) with 10-fold dilutions of vaccine from 10-3 to 10-8, each dilution was inoculated into five eggs. The mean embryo infective dose (EID 50) for ND vaccines was determined by the method of Karber 19. The mean pock forming unit for FP vaccine was determined by the method of Cunningham20. The mean tissue culture infective dose (TCID50) was determined using the method of Reed and Muench 21.

Safety Tests

The safety test for GD vaccines was conducted as recommended by Palya and is described briefly below 22. Twelve 2-week-old chicks were bled and their sera tested for antibodies (Ab) to GDV. Ten doses of the GD vaccine was administered intraocularly (IO) to 6 GDVAb negative chicks. Six unvaccinated chicks were kept separately from vaccinated chicks. Vaccinated chicks were observed for clinical signs and death. At 14 days post-vaccination (PV) all vaccinated and unvaccinated chicks were killed, each bird was weighed and its bursa of Fabricius (BF) removed and weighed. The bursal body weight ratio (BBR) of each chick was calculated by dividing the bursal weight in grams by body weight in grams and multiplying the ratio by 1,000. The bursal body weight index (BBI) was calculated by dividing the BBR of vaccinated chicks with that of unvaccinated chicks. Chicks with a BBI of less than 0.7 were considered as having atrophied BF.

The safety test for ND vaccines was conducted as described by Allan et al. 23. Briefly, one vial of a batch of vaccine was suspended in sterile PBS pH 7.4. Twelve 3-week–old chicks negative for NDVAb were inoculated with 10 doses each of the vaccine IO or per os (PO). Six 3–week–old ND susceptible chicks were kept in isolation as controls. Vaccinated chicks were observed for 2 weeks for clinical signs of disease and death. If less than 80% of the vaccinated chicks survived at the end of the test, the vaccine was considered as unsafe.

Potency Tests

The potency test for FP vaccines was conducted as described by Tripathy and Reed 24. Six 4-week-old chicks were vaccinated with FP vaccine intradermally (ID) using the stab method at the wing web. Another six 4-week-old unvaccinated chicks were kept in isolation as controls. Vaccinated chicks were examined 7 days PV for 'vaccine takes' at vaccination sites. At 14 days PV vaccinated and unvaccinated chicks were challenged with a virulent FPV by applying with a fine brush a virus suspension in an area of the thigh where feathers have been removed. All challenged chicks were examined at 7 days post challenge (PC) for swelling of the skin. Another set of six 4-week-old chicks

were vaccinated ID, PO or IM with each of the FP vaccines from 3 different manufacturers and challenged at 14 days PV as stated above. At least 80% of unvaccinated chicks should have pox lesions and at least 80% of vaccinated chicks should not.

To determine the potency of GD vaccines one field dose of GD vaccine was administered IO to 6 two-week-old chicks negative for GDVAb. At 14 days PV 6 vaccinated and 6 unvaccinated chicks were bled and challenged IO with 5.2 Log10 BID50 in 0.05 ml of a virulent GDV. Challenged chicks were observed for clinical signs and death. At 14 days PC all chicks were bled and killed, weighed and their BF removed and weighed. The BBR of the chicks was determined. The BBI was calculated using unvaccinated unchallenged chicks as negative controls. Vaccinated chicks were expected not to show clinical signs, die or have atrophied BF.

To test for the potency of ND vaccines, 3-week-old chicks were separated into three groups (1, 2 and 3) of 10 chicks per group. Chicks in group 1 were vaccinated with the recommended field dose of ND vaccine PO and group 2 with 1/10th of a filed dose of ND vaccine PO. The chicks were bled at 0, 2, 3 and 13 weeks PV and the NDVAb titre in their sera determined. At 3 weeks PV chicks vaccinated with 1 field dose of ND vaccine PO and unvaccinated chicks were challenged with 6 Log10 ELD50 in 0.12 ml per bird of a virulent NDV IM. All unvaccinated chicks were expected to die within 6 days PC and about 80% of vaccinated chicks be protected.

Results

Sterility Test

Bacterial growth were seen when all FP vaccines from all the 3 sources were grown on BA. No bacterial and fungal growth was detected in the GD vaccines from all the 3 sources. Two of the 3 ND vaccines were contaminated with bacteria. None of the 3 sources of vaccines was completely free of either bacterial or fungal contamination. One of the 9 vaccines tested yielded fungus while 5 yielded bacteria when grown on culture media. The bacteria isolated were Escherichia coli and Salmonella sp. and the fungus isolated was Aspergillus sp. (Table 1).

Viability Tests

All the vaccines have some viable virus in them. Variation in virus content was wide amongst FP vaccines (1.6 to 5.2 x 105 per ml pock forming unit-PFU) and narrow between GD vaccines and ND vaccines. The virus content for all the GD vaccines was low (4.2 to 4.7 Log10 TCID50 per ml) and high for all ND vaccines (6.8 to 10 Log10 EID 50 per ml). The haemagglutinating ability of two of the four ND vaccines tested was very low (<10 Log10) (Table 2).

Safety, Immunogenicity and Potency Tests:

All the chicks vaccinated ID with FP vaccines from source 1 and 3 reacted with swellings and scabs at the wing web 4 days PV. Only 67% of the chicks vaccinated ID with FP vaccine from source 2 had 'vaccine takes' (Table 3). None of chick vaccinated ID and challenged with a virulent FPV developed pox lesions. About 50% of the chicks vaccinated ID with FP vaccine from source 2 developed pox lesions while all chicks vaccinated PO or IM with the vaccine from the same source developed pox lesions. None, 17% and 100% of the chicks vaccinated ID, IM and PO respectively with FP vaccine from source 3 developed pox lesions following challenge. All unvaccinated chicks had pox lesions after challenge (Table 4).

None of the chicks vaccinated with GD vaccines from source 1, 2 and 4 with one or ten times the normal dose showed clinical sings or died of GD. Also, none of the chicks vaccinated showed clinical signs or died of GD following challenge. Three days PC unvaccinated chicks exhibited anorexia, somnolence, dropped wings, prostration, severe depression and yellowish–white diarrhoea. About 67% of them died and postmortem examination revealed dehydration, enlargement of the liver, kidneys and BF; congestion of skeletal muscles, liver, spleen, pancreas, lungs, kidneys, thymus and BF; turgidity and oedema of the BF and haemorrhages in the duodenum, caecal tonsils and leg muscles. Only 17% to 30% of chicks vaccinated with either 1 or 10 times the normal dose of GD vaccines from source 1

Table 1: Results of tests for bacterial and fungal contamination on live fowl pox, Gumboro disease and Newcastle disease vaccines from four different sources.

Vaccine			Culture media and growth	
1.	Fowl pox	Present	-[a]	+ [b]
2.	Fowl pox	Present	+ [c]	+ [d]
3.	Fowl pox	Lost	-	+ [d]
1.	Gumboro	Present	-	-
2.	Gumboro	Present	-	-
3.	Gumboro	Present	-	-
1.	Newcastle	Present	-	+[b]
2.	Newcastle	Present	-	-
3.	Newcastle	Present	-	+[b]

a, no growth; b, Escherichia coli grew; c, Aspergillus, sp. grew; d, Salmonella sp. grew

Table 2: Virus content of fowl pox and Gumboro disease vaccines and haemagglution titre and virus content of Newcastle disease vaccine obtained from different sources:

Source of vaccine	Fowl pox vaccine [a]PFU Log^{10} ($\times 10^5$ per ml)	Gumboro disease vaccine TCID50 (Log10 per ml)	Newcastle disease vaccine	
			HA titre	EID 50 (Log 10 per ml)
1	5.2	4.2	160	10.0
2	2.8	4.7	<10	6.8
3	1.6	ND[b]	<10	7.8
4	-[c]	-	160	8.0

a, pock forming unit; b, not determined; c, vaccine not obtained

and 2 had detectable precipitin Ab in their sera. All chicks vaccinated with either 1 or 10 times the normal dose of GD vaccine from source 3 had detectable precipitin Ab (Table 5). Chicks vaccinated with GD vaccines from the three sources at the normal or 10 times the normal field dose did not die of GD but had lower mean body weight, mean bursal weight and BBR than those of unvaccinated unchallenged chicks. The BBI of chicks vaccinated with the 3 GD vaccines either at 1 or 10 times the normal dose was lower (0.26 to 0.39) than the BBI of unvaccinated unchallenged chicks (1.0). The severely affected were chicks vaccinated with GD vaccine from source 4 and chicks vaccinated with two GD vaccines at 10 times the normal dose (Table 6).

None of the chicks vaccinated with all the 3 ND vaccines from the 3 sources at 1 or 10 times the normal dose developed clinical signs or died. At the time of vaccination the mean NDV Ab titre of chicks vaccinated with vaccines from source 1 and 3 was less than 1.0 Log2. At two weeks PV 100% of the chicks vaccinated with the 3 ND vaccines from the 3 different sources had detectable NDV Ab in their sera. The Ab titres were highest 2 weeks PV with vaccines from source 1, 2 and 3 being 5.6, 3.9 and 4.0 Log2 respectively. The percentage of chicks with NDV Ab was highest at 2 weeks PV for chicks vaccinated with vaccines from source 1 and 3 and highest at 3 weeks PV for chicks vaccinated with vaccine from source 2. By 13 weeks PV the mean NDV Ab titres of all vaccinated chicks had decreased to 2.0 Log2 (Table 8). The percentage of chicks positive for NDVAb of chicks vaccinated with ND vaccines from source 1 (94%) and 3 (95%) were comparable. The mean NDV Ab titre was highest (5.6 Log2) for chicks vaccinated with the ND vaccine from source 1. The NDV Ab titres were highest (5.8 Log2) in chicks vaccinated IO with 10 times the normal dose of the ND vaccines (Table 7). The percentage of chicks with detectable NDV Ab in their sera was lowest (69%) in chicks vaccinated PO with 1/10th the normal dose of the vaccines and the mean NDV Ab titre was lowest (2.6

Table 3: Results of vaccination of chicks with fowl pox vaccines from 3 different sources.

Source of vaccine	No. of chicks with 'vaccine takes'/no. vaccinated (%)
1	6/6 (100)
2	4/6 (67)
3	6/6 (100)
Unvaccinated	0/6 (0)

Table 4: Reaction to challenge with a virulent fowl pox virus 2 weeks post vaccination ID, PO or IM with fowl pox vaccines from 3 different sources.

Source of vaccine	Route of vaccination	No. with lesions/no. challenged (%)
1	ID	06/(0)
	PO	6/6 (100)
	IM	6/6 (100)
2	ID	3/6 (50)
	PO	6/6 (100)
	IM	6/6 (100)
3	ID	0/6 (0)
	PO	66/6 (100)
	IM	1/6 (17)
Unvaccinated	Unvaccinated	6/6 (100)

Table 5: Precipitin antibody response and mortality of chicks vaccinated with 1 or 10 times the normal dose of Gumboro disease vaccines from 3 different sources.

Source of vaccine	No. of field dose per bird	No. sero positive/ no. tested (%)	No. dead/no. vaccinated and challenged (%)
1	1	1/6 (17)	0/6 (0)
	10	2/6 (33)	
2	1	2/6 (33)	0/6 (0)
	10	1/6 (17)	
4	1	6/6 (100)	0/6 (0)
	10	6/6 (100)	
Unvaccinated	Unvaccinated	4/4 (100)	4/6 (67)

Table 6: Changes in the mean body and bursal weight of chicks vaccinated with Gumboro disease vaccines from 3 different sources at 1 or 10 times field dose.

Source of vaccine	No. of field dose	Mean body weight (g)	Mean bursal weight (g)	Mean bursal body weight ratio	Bursal body weight index
	1	178.8+ 26.4	0.26+0.09	1.4+0.31	0.33
	10	180.4+23.4	0.32+0.23	1.69+1.1	0.37
	1	152.9+24.3	0.26+0.09	1.68+0.42	0.39
	10	158.1+11.7	0.25+0.14	1.60+1.0	0.35
	1	153.3+13.4	0.21+0.09	1.30+0.49	0.30
	10	178.2+33.3	0.22+0.12	1.2+0.40	0.26
Unvaccinated challenged	Unvaccinated challenged	165.5+51.6 158.3+28.9	0.14+0.08 0.34+0.11	0.79+0.31 2.1+0.5	0.18 0.46
Unvaccinated unchallenged	Unvaccinated unchallenged	243.9+15.7 196.5+20.7	1.09+0.29 0.91+0.26	4.30+1.25 4.6+1.3	1.0 1.0

Table 7: Antibody titres of chicks 2 weeks post vaccination with ND vaccines from three different sources administered at various does using different routes

Source of ND vaccine	Route and dose of vaccine administered				
	PO[a] X1[b]	PO X1/10[c]	PO X10[d]	IO[e] X10	Mean
	Mean and standard deviation of ND HI Ab tire in Log_2 (% positive)				
1	2.8 +1.0 (100)	6.0 +3.3 (100)	4.8 +1.7 (80)	5.3 +0.5 (100)	5.6 +2.1 (94)
2	3.5 +1.3 (75)	1.0 +1.4 (33)	3.6 +1.5 (83)	5.5 +2.9 (100)	3.9 +2.4 (74)
3	1.8 +1.3 (100)	4.0 +3.9 (83)	3.5 +2.7 (100)	6.6 +2.9 (100)	4.6 +2.2 (95)
Mean	2.6 +1.3 (93)	4.5 +3.5 (69)	3.9 +1.9 (87)	5.8 2.4 (100)	4.2 +2.6 (87)

a, per os route; b, one normal field dose; c, one tenth of normal field dose; d, ten times of normal field dose; e, intraocular route

Table 8: Mean HI Ab titres of chicks before and after vaccination with ND vaccines from 3 different sources.

Source of ND vaccine	Weeks post-vaccination			
	0	2	3	13
	Mean and standard deviation ND HI Ab titre in Log_2 (%) positive)			
1	0+0 (42)	5.6+2.1 (94)	3.4+2.3 (63)	2.2+1.3 (71)
2	0.3+0.5 (67)	3.9+2.4 (74)	3.7+1.8 (100)	2.2+2.0 (100)
3	0.8+0.9 (83)	4.0+2.2 (95)	4.0+2.2 (91)	2.0+1.0 (83)

Log2) in chicks vaccinated PO with one field dose of the ND vaccines. Variation in the NDV Ab titres within vaccinated chicks was high between the chicks vaccinated PO with 1/10th of the normal dose of vaccines from source 1 and 3 and chicks vaccinated IO with 10 times the normal dose of the ND vaccines from source 2 and 3 (Table 8). Some vaccinated and all unvaccinated chicks became sick and died following challenge with a virulent NDV (Table 9). Clinical signs were observed 2 days PC with NDV while mortality commenced 5 days PC. The clinical signs observed in sick birds were depression, anorexia, sneezing, diarrhoea, torticollis, opisthotonus, backward movement and circling (Table 9). The lesions observed at necropsy included, severe congestion of the lungs, muscles, pancreas, liver, spleen, trachea; enlargement of the kidneys, spleen and liver and; haemorrhages in the gastrointestinal tract and caecal tonsils. Mortality ceased at 7 days PC while nervous signs persisted in chicks vaccinated with ND vaccines from source 1 and 2. The protection rates against ND were 0%, 50%, 56% and 78% for unvaccinated chicks, those vaccinated PO with one field dose of ND vaccine from source 1,3 and 2 respectively (Table 10).

Table 9: Morbidity, mortality and clinical signs of chicks challenged with a virulent NDV after vaccination with 3 ND vaccines from three different sources.

Day post challenge	Source of vaccine					
	1		2		3	
	% sick	% dead	% sick	% dead	% sick	% dead
4	20	0	11	0	22	0
5	40	20	9	18	0	13
6	25	25	0	0	0	0
7	33	17	14	0	14	43
24	40[a]	0	14[a]	0	0	0
	Source of vaccine					
Clinical signs	1		2		3	
Depression	+[b]		+		+	
Somnolence	+		+		+	
Anorexia	+		+		+	
Diarrhoea	+		+		+	
Sneezing	-[c]		-		+	
Nervous sings	+		+		-	

a, nervous sings; b, presence of clinical sign; c, absence of clinical sign

Table 10: Morbidity and mortality of chicks vaccinated with three ND vaccines after challenge with a virulent ND virus.

Source of ND vaccine	Morbidity (%)	Mortality (%)	Protection rate (%)
1	40.0	50.0	50.0
2	14.0	22.0	78.0
3	22.0	44.0	56.0
Unvaccinated	100.0	100.0	0

Discussion

Live FP and ND vaccines are possible sources of bacterial and fungal infections for poultry in Nigeria. To avoid the widespread dissemination of avian pathogens in the country, there is a need to reassess all commercial poultry vaccines imported before they are released for sale and subsequent use.

The minimum recommended virus titre for FP vaccine is 4.0 Log10 EID50 for GD vaccine 3.0 Log10 EID50 per ml or 6.0 to 8.5 Log10 CID50 and for ND vaccine 6.5 Log10 EID50 23, 25, 26, 27, 28. Based on these recommendations, all the ND vaccines and one of the FP vaccine used in this study met the required standards. As virus content in live vaccines influence the level of protection conferred on birds all the ND vaccines tested, none of the GD vaccines and one of the FP vaccines would be expected to protect vaccinated chicks that later become exposed to virulent viruses22, 23, 29. There is therefore a need to maintain adequate virus content of live vaccines by the addition of extra virus dose (80% or more) at the point of manufacture to compensate for losses that may occur during transportation, storage and administration. In addition live vaccines should be properly transported, stored and administered.

Chicks that received FP vaccine from source 1 and 3 showed evidence of complete successful reaction to vaccination while only 67% were successfully immunized by FP vaccine from source 2. Fowl pox outbreaks may therefore be expected in flocks vaccinated with FP vaccine from source 2. For FP vaccine from source 1 and 3 the ID route is the most

appropriate while the IM route could be used for vaccinating chicks with FP vaccine from source 3. The FP vaccine from source 2 was the poorest of the 3 FP vaccines in terms of resistance to challenge. At the recommended doses administered the oral route did not confer any resistant to FPV challenge irrespective of the source of the FP vaccine.

The 3 GD vaccines tested differed in their immunogenicity with one of them being more immunogenic even at the normal field dose. GD vaccines from some sources may therefore be unable to prevent GD outbreaks in vaccinated flocks as was previously reported by Adene et al. and Aba-Adulugba et al. 30, 31. Only highly immunogenic GD vaccines should be used for the routine vaccination of chicks in Nigeria. In terms of safety none of the GD vaccines tested qualified because all the caused reduction in weight gain and bursal atrophy although at varying degrees. Some commercial GD vaccines tested had earlier been reported to have caused the death of susceptible chicks and pathology in the BF of such chicks31, 32, 33, 34. The 3 GD vaccines tested are therefore unsafe for use in chicks devoid of MAAb. Although the amount of virus in live vaccines is important for adequate immune response and protection, the level of attenuation of the virus appeared to the most important for protection. Highly attenuated GD vaccines for example would not immunize chicks that have some MAAb while mildly attenuated vaccines would immunize such chicks but could cause bursal atrophy and death 29, 31, 35.

The level of NDVAb produced depends on the source of ND vaccine, route of administration and the dose of ND vaccine administered. The highest NDVAb level was obtained 2 weeks PV. Chicks vaccinated orally with 1 or 10 times the normal field dose of ND vaccine responded more uniformly than chicks vaccinated using other routes and doses. Vaccination with 10 times the normal field dose of the 3 ND vaccines tested was apparently safe and resulted in the production of a high NDVAb titre and high percentage of chicks with NDVAb in their sera. None of the 3 ND vaccines tested was able to protect all vaccinated chicks against NDV challenge but the ND vaccine from source 2 was the most protective. Revaccination with a mesogenic ND vaccine might have increased the protection rates of the lentogenic ND vaccines used in this study. This is important because all NDV isolated to date from sick, dead and apparently healthy Nigerian poultry are velogenic16, 36. In addition all vaccinated chicks were protected from challenge exposure when a mesogenic NDV strain was included in vaccination schedules by Shamaki et al. 37.

The following recommendations are made based on the findings of the present study. There is a need to reassess the quality of all commercial poultry vaccines from all sources before they are released for sale in the Nigerian market. Use only the ID route for all FP vaccines. Use mildly attenuated GD vaccines only in chicks with some MA and highly attenuated GD vaccines in chicks devoid of MA or 'intermediate' GD vaccines for all categories of chicks. Use only lentogenic ND vaccines that would engender 80% or more protection in vaccinated chicks. Boost with mesogenic ND vaccines all chicks vaccinated with lentogenic ND vaccines in order to maintain high Ab levels. ND vaccines produced from lentogenic NDV strains such as La Sota could be safely used at 10 times the normal field dose for better Ab response. Live FP, GD and ND vaccines should be properly transported, stored and administered.

Acknowledgements

The authors received material, moral, logistical and or technical support and assistance from Drs. K. A. Majiyagbe, L. H. Lombin, E. S. Haruna, M. Busman and A. O. Olabode, Mr. M. Okewu and G. O. N. Echeonwu of NVRI, Vom, Nigeria and Mr. U. Nwokeocha, D. Leo, H. Garba and L. Musa of Ahmadu Bello University, Zaria, Nigeria. The support is gratefully acknowledged.

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DESCRIPTION DE LA FILIERE AVICOLE TRADITIONNELLE EN AFRIQUE DE L'OUEST : CAS DU SENEGAL ET DU TOGO

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Resume

La filière avicole traditionnelle couvre plus de 70-80% de la production avicole dans les pays de l'Afrique de l'Ouest. Des enquêtes menées en 2010-2011 sur les marchés et les producteurs présents au alentour des marchés hebdomadaires ruraux ont permis de caractériser les acteurs de la filière, de recenser leurs activités, d'estimer le nombre moyens de volailles entrants et sortants dans ces marchés et d'apprécier le niveau de biosécurité dans ces marchés dans deux pays de l'Afrique de l'ouest notamment au Sénégal et au Togo.

L'enquête de la filière avicole dans les communautés rurales peut être décrite selon un circuit plus ou moins complexe, où les principaux acteurs de la filière sont : i) les producteurs qui sont des éleveurs vivant dans les villages et/ou les communautés rurales ; ii) les collecteurs primaires et secondaires, qui achètent les volailles et les revendent dans les marchés ruraux (hebdomadaires) ou de collecte (journaliers) ; et iii) les grossistes qui regroupent les volailles des collecteurs pour les acheminer vers des marchés plus importants notamment dans les marchés urbains et/ou à l'extérieur du pays. Les flux commerciaux de volailles vivantes entre les acteurs varient selon les pays. Le nettoyage dans les élevages a paru moyennement fréquent (3 à 4 par semaine) avec l'utilisation de désinfectants dans 15 à 25% des cas, la biosécurité dans les marchés hebdomadaires, acceptable (contrôle dès l'arrivée et nettoyage des cages hebdomadaires) et le taux de mortalité (30-40% chez les adultes et 60-70% chez les poussins) reste élevé. Dans notre étude, le retour des poulets invendus dans les fermes pourrait constituer l'une des pratiques à risque de propagation et de maintien des maladies infectieuses dans cette filière.

Mots-clés : Aviculture traditionnelle, Acteurs de la filière, circuit de commercialisation. Biosécurité.

Abstract

The traditional poultry industry covers more than 70-80% of the poultry production in West Africa countries. Surveys conducted in 2010-2011 on the market and producers present in the surrounding rural weekly markets have allowed to characterize the actors in the sector, to identify their activities estimate the average number of incoming and outgoing poultry in these markets, and to assess biosafety level in these markets in both West African countries including Senegal and Togo.

The survey of the poultry sector in rural communities can be described as a more or less complex circuit, where the major actors in the sector are: i) producers who are farmers living in villages or rural communities; (ii) the primary and secondary collectors who buy poultry and sell them in rural markets (weekly) or collection markets (daily); and iii) wholesalers who gather poultry from the collectors for delivery them to larger markets particularly in urban markets and/or outside the country. Trade flows of live poultry between actors vary according to countries.

Cleaning in the farms appeared moderately frequent (3-4 per week) with the use of disinfectants in 15-25% of cases, biosafety in weekly markets, acceptable (control at the arrival and weekly cage cleaning) and the mortality rate (30-40% among adults and 60-70% in chicks) remains high. In our study, the return of unsold chickens in the farms could be a practical risk of propagation and maintenance of infectious disease in this sector.

Key words : Traditional poultry, Chain actors, Marketing chain, Biosecurity.

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Introduction

L'élevage de volailles occupe une place importante en Afrique occidentale. Dans la plupart de ces pays, l'aviculture est divisée en deux sous-secteurs : i) le sous-secteur dit moderne qui se concentre aux alentours des grandes villes avec des poules de races améliorées qui représentent près de 20-30% des effectifs ; ii) le sous-secteur villageois ou traditionnel qui est relativement très présent dans les pays (70-80% des effectifs), permettant ainsi l'exportation de leur production notamment les races locales vivantes. Ainsi, deux types de circuits de commercialisation parallèles de volailles coexistent notamment les circuits des races améliorées et celui des races locales. Le circuit traditionnel constitue une source de revenus considérable pour les éleveurs et contribue à l'amélioration de la sécurité alimentaire des populations vulnérables rurales. Plus de 85% des ménages ruraux élèvent une ou plusieurs espèces aviaires (poulet, pintade, dindon, canard) et plus de 70% des propriétaires de poulets sont des femmes (Gueye, 1998). L'élevage de volailles traditionnelles constitue une « banque » dans laquelle le poulet est utilisé comme valeur de change dans les villages (Guèye, 2003). A cause de ses bonnes qualités organoleptiques, la viande de poulet traditionnel est de plus en plus prisée par les consommateurs urbains, ce qui rend cette spéculation de plus en plus rentable. Selon Fotsa et al. en 2007, environ 18 à 40% des poulets traditionnels produits localement sont destinés à la vente. L'aviculture villageoise est gérée par une multitude d'acteurs qui s'occupent de la collecte, du transport et de la commercialisation des poulets traditionnels à l'état vivant.

La fluidité du circuit de commercialisation, son faible coût logistique et la résistance à certaines maladies sont des atouts des ces volailles traditionnelles rustiques. Toutefois de nombreux facteurs freinent son développement notamment sa faible productivité et les maladies infectieuses comme la Newcastle et plus récemment l'Influenza aviaire hautement pathogène (IAHP).

Des neuf pays infectés par le virus Influenza de type A (H5N1) en Afrique, sept sont en Afrique subsaharienne. L'IAHP est apparue, pour la première fois dans la région ouest-africaine, au Nigéria (Février 2006). Des foyers ont été rapportés plus tard au Cameroun (Février 2006), au Burkina Faso, en Côte d'ivoire (Mars 2006), au Ghana (Avril 2007), au Togo (juin 2007) et au Bénin (Novembre 2007). Les derniers foyers ont été recensés au Nigéria en Septembre 2008. De nombreuses études ont été conduites pour comprendre le mode de dispersion de la maladie dans les différents pays, le fonctionnement des filières avicoles, les différentes chaines de commercialisation intra et inter-pays (Bebay, 2006). Concernant la circulation du virus, il s'est fait principalement par les échanges commerciaux des volailles et des produits aviaires à l'intérieur des pays et entre les pays (Fasina et al., 2009 ; Ekong et al., 2012).

L'origine du virus n'a pas été formellement prouvée, mais il est néanmoins acquis que la volaille domestique, notamment celle du secteur commercial exploitant les races améliorées était en première ligne en ce qui concerne l'apparition des foyers. Toutefois, le rôle ou la menace concomitante que représente la volaille villageoise du fait de ses effectifs importants, de sa proximité avec les fermes avicoles modernes et surtout du circuit commercial sans véritable contrôle sanitaire dont elle fait l'objet doit être pris en compte.

Cette étude a pour objectif de décrire la filière avicole traditionnelle, d'identifier les principaux acteurs, de préciser leurs rôles et activités, et de comprendre l'importance des circuits commerciaux ruraux pour déterminer les flux de poulets traditionnels entre les différents acteurs dans deux pays ayant connu ou non une épizootie de l'IAHP en Afrique de l'Ouest.

Materiels Et Methodes

Zones d'étude

L'étude a eu lieu dans deux communautés rurales de l'Afrique de l'ouest (Togo et Sénégal) choisis selon 2 critères : i) être une zone de production de volailles traditionnelles et ii) avoir déjà été infecté par l'IAHP (plusieurs fois de préférence) ou pas. C'est sur cette base qu'Adétikopé a été

choisi dans un pays rapporté infecté par l'IAHP (Togo) et Wack gouna dans un pays non infecté (Sénégal). La division administrative est pratiquement la même dans ces deux pays de l'Afrique de l'ouest. Ils sont subdivisés en villages (unité primaire), communautés rurales (ou cantons), communes, départements et régions. Le nombre d'habitants dans les communautés rurales est variable selon les pays.

Au Togo (capitale Lomé, population totale : 6 961 049 habitants et 3 644 villages, soit 1 910 habitants/village ; Statistiques Togo, 2011), l'élevage de poules locales représente prés de 9 millions de têtes soit 67% du cheptel avicole et cette spéculation est non uniformément répartie sur le territoire national. Trois régions (Savanes, Kara et Maritime) sur 5 rassemblent 80% du cheptel traditionnel national. L'IAHP est apparue dans la localité de Sigbehoué (préfecture des lacs, Région Maritime) en juin 2007 dans un élevage de poules pondeuses. D'autres foyers ont été confirmés à Abgata (Juillet 2007), Agodeka et Tonoukati. En août 2008, un autre foyer est réapparu à Agbata, quelques semaines après des foyers de Kano et Katsina au Nigeria. Adétikopé (environ 12 km d'Agbata) est une communauté rurale de la commune de Tsévié faisant parti de la préfecture de Zio (région maritime) qui se trouve à près de 30 km de Lomé. Il existe un marché rural (hebdomadaire) à Adétikopé et un marché de collecte (journalier) à Tsévié.

Au Sénégal (capitale Dakar, population totale : 13 567 338 habitants et 13 544 villages, soit 1 001 habitants/village ; Ansd, 2013), l'élevage traditionnel représente près de 56% du cheptel avicole en 2009 (Direction de l'élevage 2010 selon Teno, 2010), soit environ 22,500 millions de têtes de volailles. Aucun foyer n'a été déclaré et des mesures de prévention ont été prises notamment l'arrêt de toutes importations de volailles. Ces mesures ont relancé la production locale de poulets de races améliorées et traditionnels. La communauté rurale de Wack Ngouna, situé à 40 km de Kaolack, dans le département de Nioro du Rip (région de Kaolack), a été choisie pour notre étude. Cette communauté rurale comporte un marché rural et de collecte hebdomadaire.

Collecte des données

Afin de recueillir des informations sur la filière avicole traditionnelle, deux questionnaires standardisés ont été élaborés respectivement pour les éleveurs et pour les acteurs des marchés (collecteurs et grossistes). A notre connaissance, il n'existe pas de statistiques sur le nombre d'éleveurs et d'acteurs de volailles traditionnelles dans les deux pays. Ces questionnaires ont été pré-testés et corrigés selon les observations faites par des acteurs.

Ces enquêtes ont été conduites entre Novembre 2010 et Février 2011 dans les communautés rurales d'Adétikopé au Togo et Wack Ngouna au Sénégal. Ces communautés rurales sont réputées être parmi les plus actives dans le commerce de la volaille traditionnelle.

La collecte des informations a été faite auprès de 30 éleveurs de volailles locales dans les villages environnant les marchés et de 20 acteurs dans les marchés de chaque localité des deux pays. Le choix des éleveurs et des acteurs du marché ont été fait de manière aléatoire le jour de notre visite, soit près de 100 personnes sondées au total.

Après l'enregistrement des caractéristiques sociodémographiques, les questions adressées aux éleveurs étaient reparties en 4 thèmes principaux : les sources d'approvisionnement des volailles, l'identification des acheteurs et le nombre de vente mensuel, l'hygiène des poulaillers. Quant au questionnaire administré aux acteurs du marché, il a comporté des questions relatives à la description des acteurs, le nombre de volailles entrant dans les marchés et des informations sur les ventes de volailles, la biosécurité.

Analyses statistiques

Les réponses ont été codées pour faciliter leur saisie. La saisie des données a été faite avec Epidata version 3.1. La description des variables a été faite à l'aide des statistiques descriptives et présentée sous forme de tableau de fréquence. Les associations de variables ont été déterminées par le test de chi-deux avec le logiciel R version 2.15.2.

Resultats et Discussion

Organisation de la filière avicole traditionnelle

Comme Issa et al., 2012, les principaux acteurs de l'approvisionnement des volailles dans le secteur traditionnel (figure 1) sont les suivants :

Producteurs

Ce sont des éleveurs de poulets traditionnels de race locale principalement et avec quelques sujets de race améliorée. Le mode de production est de type extensif avec la divagation des volailles. Ces producteurs constituent environ 70-90% de la population des villages et des communautés rurales et utilisent peu d'intrants. Certains producteurs nourrissent quelques fois les volailles avec des restes d'aliments ou des termites et construisent un poulailler de fortune pour protéger les volailles de la pluie et des prédateurs. Les producteurs qui ont des métis issus de croisements entre poulets locaux et améliorés, construisent en général un poulailler pour abriter les animaux. Ces métis sont très prisés en raison de meilleurs rendements. Ce sont, pour la plupart, les femmes qui entretiennent ces poulaillers. La commercialisation des volailles est l'une des rares occasions pour les personnes vivant en milieu rural de gagner rapidement des revenus pour subvenir à leurs besoins immédiats (Aklilu et al., 2007 ; Gueye, 2010 ; Issa et al., 2012). Parmi les 60 personnes interviewées dans les deux pays, 10 (16,6%) personnes dites « gros éleveurs » possèdent des effectifs de plus de 50 poulets (maximum : 395) et le reste « petits éleveurs » a des effectifs variant de 2 à 50. Les gros éleveurs sont principalement des hommes et se trouvent pour la plupart dans les communautés rurales.

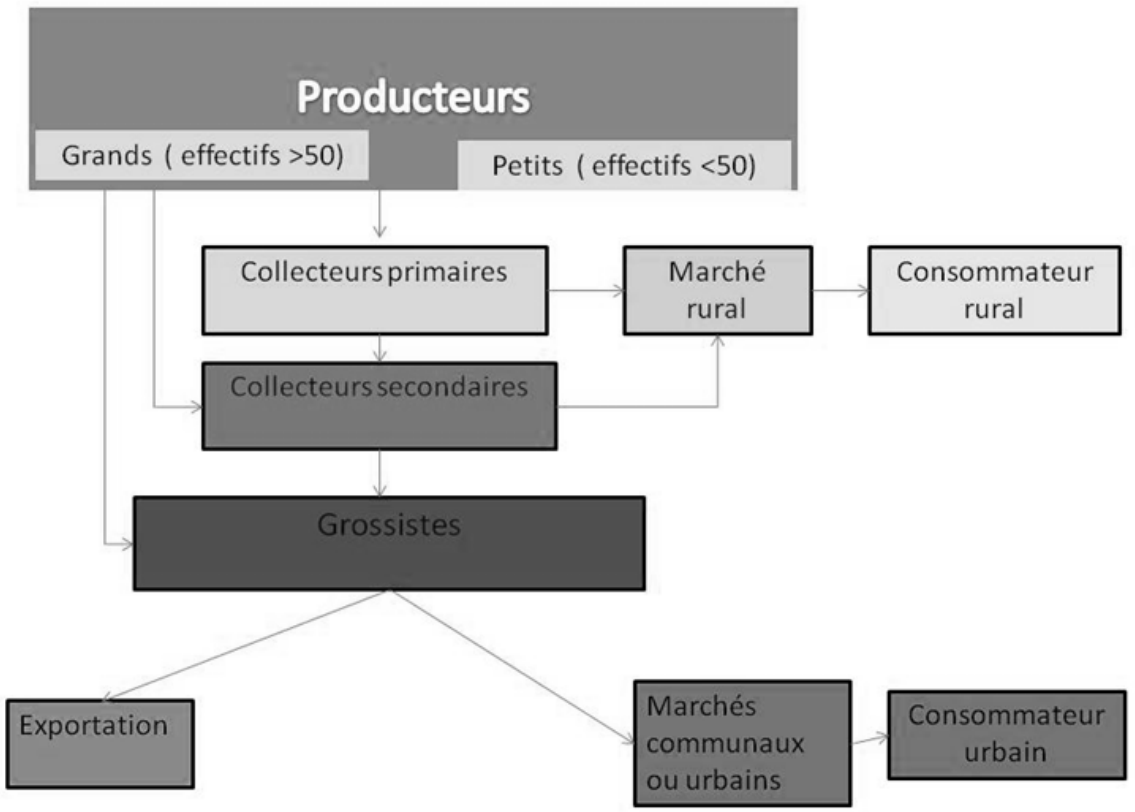


Figure 1 : Circuit de commercialisation des volailles traditionnelles en Afrique de l'Ouest, 2012 (les flèches indiquent uniquement les liens entre les compartiments et non les flux).

Collecteurs :

Deux types de collecteurs ont été rencontrés : les collecteurs primaires et secondaires.

Les collecteurs primaires ou villageois (« Banabanas ou Salou-Saloum » au Sénégal) achètent les volailles chez les petits éleveurs traditionnels et les revendent aux consommateurs ruraux, aux collecteurs secondaires et aux grossistes. Plusieurs moyens de déplacement, fonction de la distance à parcourir, sont utilisés par ces acteurs pour se rendre dans les villages. Les déplacements se font à pied, en chariot, en bicyclette, en motocyclette et en véhicules (Gondwe et al., 2005 ; Emuron et al., 2010 ; Mopaté et Djimé, 2012). Ce sont essentiellement des hommes qui assurent cette activité et on les rencontre dans les marchés hebdomadaires ruraux.

Les collecteurs secondaires se trouvent dans les marchés ruraux et de collecte (dont 15% de femmes au Togo et 0% au Sénégal) et sont ravitaillés par les éleveurs venant vendre les poulets lors des marchés hebdomadaires et par les collecteurs primaires. La vente des volailles est effectuée par près de 40 à 58% des femmes en Ethiopie (Aklilu et al., 2007), de plus de 50 % au Nigeria (Alabi et al., 2006) et de 88% au Botswana (Emuron et al., 2010). Ces collecteurs secondaires revendent leurs volailles aux grossistes. Dans certains marchés, il existe des revendeurs (ou détaillants) qui écoulent les invendus des collecteurs. Ils n'ont aucun stock mais ils jouent le rôle d'intermédiaires entre les collecteurs et les acheteurs avec un petit pourcentage sur le prix de vente les jours de marchés.

Grossistes :

Ces acteurs achètent de grandes quantités de volailles soit à de gros producteurs ou à des collecteurs secondaires. Ils acheminent ces volailles à l'extérieur ou vers des plus gros marchés notamment celui de la capitale. Ce sont uniquement des hommes dans les deux pays. En général, les grossistes emploient certains collecteurs primaires et/ou secondaires et exercent principalement leurs activités dans les marchés de collecte journaliers. Ce sont généralement des personnes de la même famille ou des employés.

Consommateurs :

Les premiers consommateurs sont les éleveurs eux-mêmes qui consomment environ 2 à 50 % de leur production selon qu'ils soient gros ou petit éleveurs. Les autres poulets destinés à la vente sont principalement les mâles, vendus soit au collecteur primaire ou dans les marchés, soit troqués contre des chèvres ou des chiens (consommés surtout au Togo). Il existe aussi des rôtisseurs ou des tenants de maquis (consommateurs professionnels locaux) qui viennent s'approvisionner dans les marchés ruraux (hebdomadaires) ou communaux (journaliers).

Le circuit de commercialisation de volailles traditionnelles tel qu'appliqué dans les deux pays sondés est présenté à la figure 1. Dans ce circuit, les petits producteurs (plus nombreux), produisent une part importante de la volaille locale au niveau des communautés rurales. Les collecteurs primaires et secondaires achètent la quasi-totalité de la production de ces petits producteurs et les vendent, soit en petite quantité dans les marchés hebdomadaires de ces communautés rurales (directement aux consommateurs ruraux ou indirectement par des revendeurs) soit en plus grande quantité à des collecteurs secondaires installés dans ces marchés communaux. Ces collecteurs secondaires revendent tous leurs produits à des grossistes qui sont basés dans les communes. Les gros producteurs peu nombreux, vendent leur production soit à des collecteurs secondaires ou à des grossistes qui approvisionnent à leur tour les marchés régionaux et urbains (surtout de la capitale) plus importants. Ce circuit est appliqué dans la plupart des communautés rurales productrices de volailles locales et approvisionne in fine les marchés des grandes villes et de la capitale, pour satisfaire le consommateur urbain friand de cette viande qu'il est disposé à payer plus cher que le poulet de chair issu des élevages modernes péri-urbains.

Caractéristiques sociodémographiques des interviewés

Deux types de personnes sont interviewées, les unes dans les élevages (producteurs) et les autres dans les marchés (collecteurs et grossistes).

Dans les élevages, sur les 60 personnes interviewées dans les deux pays, 54,4% sont de sexe masculin et 45,6% de sexe féminin. Ces résultats sont identiques à ceux de Aboe et al., 2006 au Ghana mais différents de ceux de Missohou et al., 2002 en Haute Casamance au Sénégal où les femmes étaient plus nombreuses (52%). La plupart des personnes rencontrées étaient mariées (88,5%) et seulement peu d'entre elles ont été scolarisées (8,4 % chez les hommes et 2,8% chez les femmes). Les poulets sont les espèces aviaires les plus présentes (98,5%) dans les deux pays. Les petits éleveurs représentaient 85% des personnes sondées et les gros éleveurs 5%. Concernant les autres espèces, les proportions diffèrent entre les deux pays. Ainsi, les canards (11%), les pintades (9%) et les pigeons (4%) sont les plus élevés au Togo. Quant au Sénégal, l'élevage des autres espèces aviaires est marginal et inférieure à 1%. Plus de 60% des personnes enquêtées, affirment que l'élevage et l'agriculture constituent leur activité principale.

Dans les marchés, l'âge moyen des personnes enquêtées (collecteurs et grossistes) a été de 48 ans (± 5,4). L'âge minimum rencontré était de 20 ans et la personne la plus âgée (de sexe masculin) avait 75 ans. Teno en 2010, avait fait une étude dans les marchés de la ville de Dakar, où l'âge moyen des 34 commerçants sondés était de 47 ans (min : 22 et max : 72). Peu de jeunes exercent cette activité. Cette observation corrobore avec celle de Teno à Dakar en 2010. Les femmes étaient plus fortement représentées au Togo (65%) qu'au Sénégal (0%) dans les marchés. Aucune femme exerçant le métier de collecteurs n'avait aussi été recensée dans l'enquête de Teno en 2010 à Dakar, confirmant ainsi nos résultats. Dans notre échantillon, 78% des personnes sondées étaient mariées, 85% n'avaient pas été scolarisées et 5,2% de ces personnes ont un niveau scolaire secondaire. Pour la plupart des personnes enquêtées (98,7%) dans les marchés, la vente de volailles constitue la principale activité. De ces personnes, 90% l'exercent à leur propre compte, 6,4% en association avec un ou plusieurs individus et 3,6% travaillent pour une tierce personne. Nos résultats indiquent que les commerçants du marché (collecteurs et grossistes) pratiquaient cette activité depuis longtemps ou constituaient une activité familiale. Teno (2010) a indiqué aussi que certains commerçants pratiquaient cette

Tableau I : Activités des producteurs par pays en Afrique de l'Ouest, 2013.

Paramètres étudiés	**Aditekope (Togo)**	**Wack Ngouna (Sénégal)**
Source approvisionnement (%)		
Auto-approvisionnement	40	34
Vendeur du marché	26	24
Revendeur itinérant	14	15
Autre éleveur traditionnel	10	21
Don et héritage	10	6
Age moyen (mois) des poulets lors achat	3-6	3-18
Acheteurs (%)		
Collecteur primaire	20	32
Collecteur secondaire	39	28
Consommateurs	9	10
Autoconsommation	32	30
Nombre de ventes/mois : moy (min – max)	8 (0-50)	12 (0-68)
Taux de mortalité moyen (%)		
Poussins	70	60
Adultes	30	40
Nettoyage poulaillers/semaine : moy (min-max)	3 (0-7)	4 (0-7)
Utilisation de désinfectants (%)	16,7	25,4

Tableau II : Activités des collecteurs par pays en Afrique de l'ouest, 2013.

Paramètres étudiés	**Aditekope (Togo)**	**Wack Ngouna (Sénégal)**
Nombre moyen par marché de		
Petits producteurs	26	26
Grands producteurs	4	4
Nombre moyen par marché de :		
Collecteurs primaires	30-40	35-55
Collecteurs secondaires	60-70	45-65
Nombre de marchés à détail	**3**	**2**
Nombre de grossistes / marché	**2**	**3**
Tenue des marchés / semaine	**1**	**1**
Biosécurité :		
Contrôle sanitaire à l'arrivée (oui/non)	Oui	Oui
Nombre de nettoyage cage-sol/semaine	1	1
Origine des volailles (%)		
Villages environnants	80	85
Autres marchés	10	10
Elevages commerciaux	10	5
Nombre moyen de volailles achetées : moy (min-max)	**17 (4-60)**	**20 (2-40)**
Nombre moyen de volailles vendues : moy (min-max)	**15 (3-20)**	**8 (1-15)**
Races de volailles vendues	**Locale**	**Locale/métis**

activité depuis au moins 23 ans.

Activités des producteurs

Les activités des producteurs sont listées dans le tableau I. De manière générale, les producteurs nettoyaient leur poulailler entre 3 et 4 fois par semaine et une proportion de 16 à 25% utilisaient des désinfectants. Ainsi, la fréquence de nettoyage des poulaillers et du sol est plus importante ($\chi 2$= 21,4 ; p=0,015) chez les gros producteurs par rapport aux petits producteurs. Aucune différence statistiquement significative n'a été observée entre la fréquence de nettoyage et l'âge, le sexe et le niveau scolaire. La source d'approvisionnement chez les gros éleveurs est de l'auto-approvisionnement (100%) contrairement aux autres producteurs. Les principales sources d'approvisionnement des petits producteurs restent l'auto-approvisionnement (80%) et l'achat de sujets au marché (20%), généralement pour améliorer les performances génétiques des poulets locaux. Les critères de poids et de taille sont les seuls mis en jeu lors de la vente d'un poulet aux restaurateurs tandis que la couleur du plumage et le sexe sont plus recherchés par les clients sacrificateurs (Gueye, 1998 ; Teno, 2010). De plus, les gros producteurs ont tendance à prendre plus soin de leurs activités que les petits producteurs, en ce qui concerne l'hygiène et les soins aux poulets. Les petits producteurs indiquaient que l'autoconsommation, les collecteurs primaires et secondaires se partageaient 90% de la production et les ventes oscillaient entre 0 et 50 par mois selon le type de producteurs en dehors des périodes de fête.

L'âge des poulets à l'achat pour les producteurs varie selon les pays entre 3 à 18 mois. Les mortalités sont plus importantes chez les petits producteurs (60-90%) que chez les gros producteurs (5 à 30%). Au Ghana, les pertes étaient de l'ordre de 75% (Aboe et al., 2006). En Casamance (Sénégal), les pertes étaient plus élevées chez les poussins (43%), modérés chez les poulets en croissance (16%) et faible chez les adultes (3%) selon Missohou et al., 2002.

Activités des collecteurs et grossistes

Les collecteurs enquêtés dans les marchés indiquent qu'il y a entre 4 et 6 gros producteurs à Aditekope et Wack Ngouna (Tableau II). Le nombre de collecteurs primaires (30-55) et secondaires (45-70) est sensiblement le même dans les deux communautés rurales ainsi que le nombre de grossistes (2 à 3). Ce sont principalement des collecteurs secondaires qui ont été interviewés dans les marchés. Les mesures de biosécurité semblent bonnes avec minimalement un contrôle sanitaire des volailles à l'arrivée et un nettoyage fréquent des cages et poulaillers. Plus de 80% volailles proviennent principalement des petits éleveurs venant des villages environnants. Ainsi, prés de 15 volailles en moyenne sont achetées (min : 2 et max : 60) et une dizaine vendue en moyenne aux consommateurs et quelque fois plus d'une trentaine aux grossistes les jours de marché. Selon Issa et al., 2012, les collecteurs secondaires seraient approvisionnés à 50% par des intermédiaires (collecteurs primaires) en Ouganda et ces collecteurs primaires génèrent 65% plus de bénéfices que les producteurs. Ces intermédiaires paieraient un tiers du prix des poulets vendus sur les marchés urbains. Les Invendus sont ramenés pour plus de 98% au domicile du collecteur et/ ou des revendeurs car ce sont des marchés hebdomadaires. Le retour des volailles invendues du marché vers la maison pourrait constituer une pratique à risque majeur de propagation des maladies à contact direct notamment les maladies infectieuses virales telles que l'Influenza aviaire hautement pathogène et la maladie de Newcastle.

Conclusion

Cette étude montre l'importance de la filière avicole traditionnelle dans l'approvisionnement en protéines animales et l'impact socio-économique qu'elle a dans les ménages ruraux des pays de l'Afrique de l'Ouest. Malgré la diversité des acteurs présents dans cette filière, des similitudes existent dans l'organisation et le circuit de distribution de cette spéculation en Afrique de l'ouest au niveau des communautés rurales et des communes. Le nombre de volailles acheté dans un marché hebdomadaire semble faible, mais ce nombre croit rapidement lorsqu'on arrive au niveau des grossistes qui ravitaillent non seulement les capitales à forte densité de population et même les pays environnants par les exportations. Toutefois, une profonde réflexion doit être faite pour les poulets invendus qui sont ramenés dans les fermes sans aucune mesure de biosécurité (quarantaine) et pourrait être une pratique pouvant aggraver le tableau épidémiologique lors d'épizooties.

Remerciements:

Nous tenons à remercier le Centre de recherche pour la conservation des zones humides méditerranéennes (Tour le Valat), les agents des Directions des Services Vétérinaires du Sénégal et du Togo en particulier Mr Jamil Brassier et enfin le Dr Alain Kamaga Waladjo pour sa relecture de l'article.

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EFFECT OF LOW PROTEIN-METHIONINE-AND-LYSINE-SUPPLEMENTED DIETS ON PERFORMANCE, IMMUNE RESPONSE AND CARCASS CHARACTERISTICS IN BROILERS

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Abstract

Two experiments were conducted to investigate the effect of supplementing low CP diets with methionine and lysine on broiler performance, carcass measure and their immune response against Infectious Bursa Disease (IBD) virus. In Experiment 1, ten diets were formulated. Diet 1 (control diet) contained 23.0% CP and 0.6% methionine. The remaining 9 test diets were formulated to contain 20, 17 and 14% CP each with 0.6, 1.0 and 1.4% methionine in a 3x3 factorial arrangement. Three hundred and twenty 1-d-old broiler chicks were weighed and randomly allocated to the 10 diets with four replicates and eight birds each. In Experiment 2, 10 diets were also formulated. Diet 1 (control diet) contained 23% CP and 1.2% lysine. The rest 9 diets were similar to those in Experiment 1 but lysine was used to supplement the CP at levels 1.2, 1.6 and 2.0% inclusion. One hundred and sixty 1-d-old broiler chicks were weighed and randomly allocated into the 10 diets with four replicates of four birds each. In Experiment 1, Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) in control and 20% CP diet with 1.0 methionine were similar. Dressed weight, weights of thigh, breast, and drum stick in the control and 1.0% methionine diets were similar There was no effect of methionine, CP or their interaction on the antibody titre against IBD. In Experiment 2, increasing lysine and decreasing CP significantly ($P<0.01$) reduced BWG and FI, but 1.6% lysine had similar effect on FCR as the control diet. There was no effect of lysine, CP or their interaction on the antibody titre against IBD virus. Results suggest that supplementation of broiler diets of CP less than 23% with 1.0% methionine and 1.6% lysine resulted in similar performance as control diet and the bird's immune response against IBD virus was not compromised.

Keywords: Immune response, Ideal methionine to lysine ratio, Performance indices, Antibodies, Carcass characteristics.

EFFET DE LA SUPPLÉMENTATION DES RÉGIMES PAUVRES EN PROTÉINES AVEC LA MÉTHIONINE ET LA LYSINE SUR LA PERFORMANCE, LA RÉPONSE IMMUNITAIRE ET LES CARACTÉRISTIQUES DE CARCASSE DES POULETS DE CHAIR

Résumé

Deux expériences ont été réalisées dans le but d'étudier l'effet de la supplémentation des régimes faibles en PB avec la méthionine et la lysine sur la performance et les caractéristiques de carcasse des poulets de chair ainsi que leur réponse immunitaire contre le virus de la bursite infectieuse (BI). Dans l'Expérience 1, dix régimes ont été formulés. Le Régime 1 (régime témoin) contenait 23,0% de PB et 0,6% de méthionine. Les 9 régimes d'essais restants ont été formulés de manière à contenir 20 ; 17 ; et 14% de PB chacun, respectivement avec 0,6 ; 1,0 ; et 1,4% de méthionine selon un plan factoriel 3x3. Trois cent vingt poussins de chair âgés d'un jour (1 J) ont été pesés et répartis de manière aléatoire dans les 10 régimes avec quatre répétitions et huit oiseaux chacun. Dans l'Expérience 2, 10 régimes ont été également formulés. Le Régime 1 (régime témoin) contenait 23% de PB et 1,2% de lysine. Les 9 régimes restants étaient similaires à ceux de l'Expérience 1, mais la lysine a été utilisée pour compléter les PB aux niveaux d'inclusion de 1,2 ;

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1,6 et 2,0%. Cent soixante poussins de chair d'un jour ont été pesés et répartis de manière aléatoire dans les 10 régimes avec quatre répétitions de quatre oiseaux chacun. Dans l'Expérience 1, le gain pondéral (GP), la prise alimentaire (PA) et l'indice de consommation (IC) dans le régime témoin et le régime à 20% de PB avec supplément de méthionine à 1,0 étaient similaires. Le poids de carcasse, les poids de cuisse, de poitrine et de pilon dans le régime témoin et les régimes avec supplément de méthionine à 1,0% étaient similaires. La méthionine, les PB ou leur interaction n'ont pas eu d'effet sur le titre de l'anticorps dirigé contre la BI. Dans l'Expérience 2, l'augmentation de la lysine et la réduction des PB ont diminué de manière significative (P <0,01) le GP et la PA, mais la lysine à 1,6% a eu un effet similaire à celui du régime témoin sur l'IC. La lysine, les PB ou leur interaction n'ont pas eu d'effet sur le titre de l'anticorps dirigé contre le virus de la BI. Les résultats laissent entendre que la supplémentation des régimes alimentaires de poulets de chair ayant des PB à moins de 23% avec de la méthionine à 1,0% et de la lysine à 1,6% a produit une performance similaire à celle du régime témoin et que la réponse immunitaire des oiseaux contre le virus de la BI n'a pas été compromise.

Mots-clés : Réponse immunitaire ; Ratio méthionine/lysine idéal ; Indices de performance ; Anticorps ; Caractéristiques de carcasse

Introduction

Nutrition is a critical determinant of immune responses, and malnutrition is the most common cause of immunodeficiency worldwide. Nutrients can influence the maturity of the immune system (Cook, 1991; Latshaw, 1991). The quality of dietary protein is an essential factor, which influences the synthesis of immunoglobulins and their function. Protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocytic function, complement system, secretory immunoglobulin A antibody concentration, and cytokine production (Chandra, 1997). Increasing dietary crude protein content will increase daily feed consumption and weight gain of broilers (Jahanian, 2009). Diets low in crude protein content without adequate amino acid supplementation will negatively affect the immunologically performance of the birds. Their ability to fight infection will be reduced and consequently will affect performance (weight gain, feed intake and feed conversion ratio) and carcass quality of the birds. Antibodies being proteins, deficiency of any essential amino acid particularly in the growing phase in chicken results in poor immune competence (Latshaw, 1991). Protein deficiency inhibited antibody production and the development of antibody producing cells in response to T-dependent antigens (Carlomagno et al., 1980). According to the report of Jahanian (2009), diets low in crude protein decreased ($P < 0.001$) relative weights of thymus and bursa of Fabricius. Skin reaction to phytohaemaglutinin P was also impaired when the diets were low in crude protein. Nutritional strategy aimed at reducing the crude protein requirement of broilers is the partial replacement of intact protein (e.g., soybean meal) with crystalline free amino acids, by which excess dietary amino acids are minimized in relation to their requirements, bringing the dietary protein closer to ideal protein and in turn decreasing the dietary CP content.

The development of amino acid supplementation allows meeting the essential amino acid needs at low protein levels (Dirain and Waldroup, 2002). Schutte (1987), Stillborn and Waldroup, (1988) reported that if low-CP diets were supplemented with adequate amounts of essential and nonessential amino acids to meet the nutritional requirements of birds, performance can be produced similar to that observed with the conventional standard (high CP) diets. Research has suggested that levels of lysine and methionine in excess of NRC (1994) recommendation may result in enhanced performance, especially in regards to breast meat yield (Schutte and Pack, 1995). Adequate dietary levels of methionine and lysine are needed to support immune response, optimum growth and carcass yield of fast-growing commercial broilers. Information on the exact requirement of methionine and lysine needed for optimal immune response of broilers is still scanty. This study describes two experiments conducted to determine the effect of supplementation of methionine and lysine to graded CP levels on performance,

carcass measures and immune response to IBD virus in broilers.

Materials and methods

Diets and animals

Ten diets were formulated: diet 1 (control diet) contained 23% CP and 0.6% methionine (Experiment 1) and 23% CP and 1.2% lysine (Experiment 2). The nine other experimental diets consisted of 20, 17 and 14% CP each with 0.6, 1.0 and 1.4% methionine (Experiment 1) and same level of CP but 1.2, 1.4 and 1.6% lysine (Experiment 2). The composition diets for Experiment 1 is shown in table 1 and composition of diets for Experiment 2 is shown in table 2. In Experiment 1 320 one-day-old broiler chicks (Arbor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing-branded, weighed (Blocked by weight) and randomly allotted to 40 pens of 4 replicates with 8 birds per replicate. A group of 4 replicates was fed any of the 10 experimental diets in a factorial arrangement (4 levels of CP and 3 levels of methionine). The birds were housed in a well illuminated and ventilated poultry house. Feed and water were provided ad libitum. The chicks were vaccinated against Infectious Bursal Disease (IBD) virus on d 10 posthatch via drinking water. The birds were fed the diets for 42 days. In Experiment 2, 160 one-day-old broiler chicks (Abor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing-branded, weighed and randomly allocated to the 10 diets on weight basis with 4 replicate pens and 4 birds per replicate. The chicks were also vaccinated against IBD virus at d 10 post-hatch. The study lasted 35 days.

Sample collection, antibody determination, carcass measures and chemical analysis

The proximate composition of the diets was determined according to the methods of AOAC (2000). In both studies, blood samples were collected from two chicks per pen through the jugular vein at weekly interval pre and post vaccination for period of the study. Serum was separated by centrifugation (8000 revolution per minute for 5 minutes) and antibodies specific for IBD was detected in the sera using an ELISA kit (ProFLOKR, Synbiotics Corporation, CA, USA) according to manufacturer's instructions. One hundred microliters of each sample were used for the assay. Absorbance was measured at 405nm using an ELISA reader (SUNRISE Absorbance Reader, TECAN) by standard procedures, (Snyder et al., 1988). For carcass measure, two birds per replicate were randomly selected, weighed, sacrificed and defeathered. Carcass parts were weighed and their weights expressed as percent of body weight to determine the effect of the experimental diets on the cut parts.

Statistical analysis

Data in both studies were analysed by the two-way analysis of variance procedure appropriate for completely randomized design with a factorial arrangement using the General Linear Models (GLM) procedure of SAS (SAS, 1999). Means were further separated using Duncan Multiple range test.

Results

Experiment 1 (Methionine supplementation)

The analysed nutrient composition for diets in Experiment 1 is shown in table 3 and the analysed composition for diets in Experiment 2 is shown in Table 4. Diets contained CP levels of between 14.2-22.7% and crude fibre levels of 2.0-6.0%. The results of performance characteristics are shown in Tables 5. Body weight gain and feed intake were significantly reduced by decreasing CP levels and increasing methionine level beyond 1.0%. There was no significant effect on performance of the birds on either 0.6 or1.0% methionine diet (Table 6). Results of the effect of methionine, crude protein and their interaction on carcass parts are presented in Table 7. There was no significant effect of 0.6 and 1.0% methionine levels and 23 and 20% CP on carcass parts. Methionine above 1.0% significantly ($P< 0.05$) decreased weight of carcass parts. Decrease in CP level beyond 20%, significantly ($P<0.05$) reduced dressed weight, drum stick and thigh. Table 8 shows the result of antibody titre against IBD. There was no significant effect of CP, methionine or their interaction on IBD antibody titre. Nevertheless, birds on the 20% CP and 1.0% methionine had higher antibody

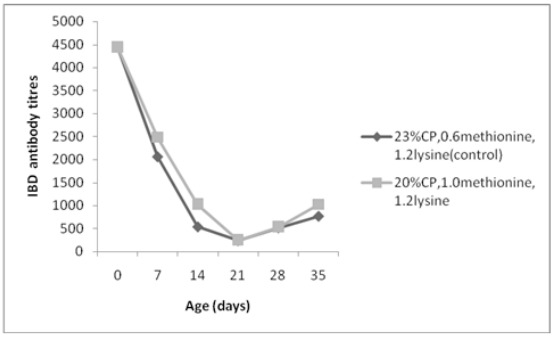


Figure 1: Infectious bursal disease virus antibody titre in broilers on 23% crude protein, 0.6% methionine with 1.2% lysine and 20% crude protein, 1.0% methionine with 1.2% lysine

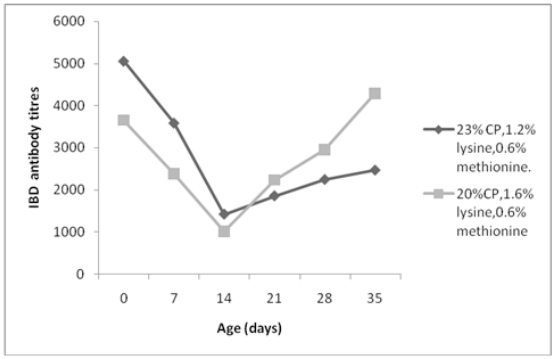


Figure 2: Infectious bursal disease virus antibody titre in broilers on 23%CP, 1.2%lysine with 0.6%methionine and 20%CP, 1.6% lysine with 0.6%methionine

titre compared to birds on the control and other diets as further shown in figure 1.

Experiment 2 (Lysine supplementation)

Crude protein in the diets was between 14.41 and 22.54% while the crude fibre value ranged from 3.0 to 4.0% (Table 4). The results of performance of the birds are shown in Table 9 and the effect of CP, lysine and their interactions on body weight gain, feed intake and feed conversion ratio are as presented in Table 10. Increasing lysine levels significantly ($P<0.05$) lowered body weight gain, feed intake and feed conversion ratio. Crude protein significantly ($p<0.05$) decreased the parameters as well. There was no significant difference in the feed intake of birds on 23 and 20% crude protein diets. Interaction between CP and lysine significantly affected feed intake but not weight gain and feed conversion ratio. The results of weights of carcass parts are shown in Table 11. Increasing lysine levels significantly ($P<0.05$) lowered the weight of thigh, drumstick and breast. There was no significant difference in the weight of thigh in birds on 23 and 20% CP diets. Interaction of lysine and crude protein had no significant effect on any of the carcass parts.

The results of the effect of CP, lysine and their interactions on antibody titre against IBD virus are as shown in Table 12. Lysine levels had no significant effect on the antibody titre. The highest antibody titre was produced in birds on the 17% CP diet. Interaction between CP and lysine had no effect on the antibody titre in the birds. Figure 2 shows the effect of antibody titre against Infectious bursal disease virus in broilers on 23% CP, 1.2% lysine with 0.6% methionine control diet and broilers on 20% CP, 1.6% lysine with 0.6% methionine diet, showing that birds on 20% CP, 1.6% lysine with 0.6% methionine diet had better immune response than birds on the control diet and other diets in the study.

Discussion

The NRC (1994) requirements for amino acids and crude protein are designed to support maximum growth and production in healthy birds kept under ideal conditions. The recommended levels for methionine in poultry depend on species, stage of production, environmental condition and amount of energy in feed. The low concentration of methionine in high-protein corn-soybean diets has led to wide use of synthetic methionine supplementation in poultry feed. An improvement in broiler performance when methionine was added to a corn-soybean diet was reported by Virtanen and Rosi (1995) and Hesabi et al. (2006). In the present study, birds on 20% CP with 1.0% methionine had better weight gain, and feed conversion ratio than birds on 23% CP with 0.6% methionine control diet and the other experimental diets. This is in support of earlier reports of (Lipstein et al., 1975; Schutte, 1987; Parr and Summers, 1991; Deschepper and De Groote, 1995; Yamazaki et al., 1996, 1998 and Aletor et al., 2000) that reduced crude protein-

Table 1: Gross composition (g/100gDM) of experimental diets. (Study 1)

Crude protein	23	20	20	20	17	17	17	14	14	14
Methionine	0.6	0.6	1.0	1.4	0.6	1.0	1.4	0.6	1.0	1.4
Lysine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Ingredients										
Maize	36.00	45.50	45.50	45.50	48.00	48.00	48.00	56.00	56.00	56.00
Soybean meal	41.00	32.00	32.00	32.00	21.00	21.00	21.00	12.00	12.00	12.00
Wheat Offal	13.00	12.00	12.00	12.00	20.00	20.00	20.00	21.00	21.00	21.00
Palm oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	2.14	2.40	2.22	2.00	2.50	2.45	2.05	2.50	2.19	2.00
Limestone	2.09	2.22	2.00	1.82	2.35	2.00	2.00	2.09	2.00	1.79
*Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Met	0.27	0.30	0.70	1.10	0.34	0.74	1.14	0.38	0.78	1.18
Lysine	-	0.08	0.08	0.08	0.31	0.31	0.31	0.53	0.53	0.53
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

CP-crude protein *Premix supplied the following information kg of diet:Vitamin A (12,500,000 I.U),Vit D3 (2,500,000 I.U),Vit E (40,000mg) Vitamin K3 (2,000mg),Vit B, (3,000mg),Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg).

Table 2: Gross Composition (g/100gDM) of experimental diets. (Study 2)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Crude protein	23	20	20	20	17	17	17	14	14	14
Lysine (%)	1.2	1.2	1.6	2.0	1.2	1.6	2.0	1.2	1.6	2.0
Methionine (%)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Ingredients										
Maize	32.00	44.00	44.00	44.00	50.00	50.00	50.00	57.00	57.00	57.00
Soybean meal	40.00	32.00	32.00	32.00	22.00	22.00	22.00	12.00	12.00	12.00
Wheat offal	18.00	13.00	13.00	13.00	17.00	17.00	17.00	20.00	20.00	20.00
Palm oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	2.10	2.45	2.23	2.00	2.29	2.00	1.94	2.00	2.00	1.75
Limestone	2.14	2.60	2.40	2.20	2.50	2.36	2.00	2.50	2.08	1.90
*Vitamin-minerals premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.26	0.30	0.30	0.30	0.34	0.34	0.34	0.38	0.38	0.38
Lysine	-	0.15	0.57	1.00	0.37	0.80	1.22	0.62	1.04	1.47
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*Premix supplied the following information kg of diet:Vitamin A (12,500,000 I.U),Vit D3 (2,500,000 I.U),Vit E (40,000mg) Vitamin K3 (2,000mg),Vit B, (3,000mg),Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg).

Table 3: Proximate composition (g/100gDM) of experimental diets. (Study 1)

	1	2	3	4	5	6	7	8	9	10
Crude protein	23	20	20	20	17	17	17	14	14	14
Methionine	0.6	0.6	1.0	1.4	0.6	1.0	1.4	0.6	1.0	1.4
Dry matter	92.60	92.00	92.60	91.20	92.23	92.40	91.58	91.20	91.10	91.42
Crude protein	22.68	20.93	20.67	20.35	17.75	17.38	17.23	14.16	14.88	14.80
Ash	10.00	10.00	9.50	9.00	8.00	7.00	7.50	8.00	9.00	8.50
Ether extract	7.00	7.00	8.00	7.00	8.50	8.00	7.50	8.00	8.50	7.00
Crude fibre	3.00	2.00	4.00	4.00	4.00	6.00	4.00	4.00	4.00	5.00
NFE	47.23	60.07	57.83	59.65	61.75	62.62	63.77	65.84	63.62	64.70

NFE=Nitrogen Free Extract

Table 4: Proximate composition (g/100gDM) of experimental diets (Study 2).

	1	2	3	4	5	6	7	8	9	10
Crude protein	23	20	20	20	17	17	17	14	14	14
Lysine	1.2	1.2	1.6	2.0	1.2	1.6	2.0	1.2	1.6	2.0
Dry matter	92.00	90.50	91.50	91.50	90.50	91.50	91.50	92.50	91.50	92.50
Crude protein	22.54	20.59	20.22	20.02	17.22	17.21	17.11	14.57	14.41	14.57
Ash	7.00	7.00	9.00	5.00	7.00	8.00	6.00	9.00	6.00	7.00
Ether extract	10.00	11.00	10.00	11.00	11.50	12.00	12.00	14.00	13.00	14.00
Crude fibre	4.00	3.00	4.00	3.00	4.00	3.00	4.00	3.00	4.00	3.00
NFE	56.46	58.41	56.78	60.98	60.28	59.79	60.89	59.43	62.59	61.43

NFE-Nitrogen Free Extract

amino acid supplemented diets improved growth and feed consumption of broilers. Fasuyi and Aletor (2005) also reported that better performance can be obtained with adequate supplementation of essential amino acids especially methionine which has been identified to be in marginal quantities in most poultry diets. Garlich (1985) found that feed conversion was better when diets were supplemented with methionine. As reduction in crude protein exceeded 3 percentage units and methionine inclusion was above 1.0%, there was a reduction in the growth performance of the birds. Excess methionine has been reported to decrease average daily feed intake and negatively affect growth performance (Katz and Baker, 1975; Harter and Baker, 1978 and Han and Baker, 1993). According to the work of Haper et al. (1984) and Nawaz et al. (2006) many amino acids, when fed in excess to growing chickens cause symptoms of toxicity such as decreased feed intake, weight gain and increased mortality.

In study two, birds on 20% CP with 1.2 or 1.6% lysine had similar body weight gain and feed conversion as those on the control (23% CP with 1.2% lysine) diet. This is in agreement with findings of Labadan et al. (2001) that lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency. At normal CP level, high lysine content has been reported to increase growth rate in broilers (Holsheimer and Veerkamp, 1992). This is also in agreement with the findings of Zarate et al. (2003) that formulation with commercially available purified essential amino acids to meet broiler requirements not only improves their overall balance but also allows for reduction in CP while improving the overall performance of broiler birds. Corzo et al. (2005) found that lysine supplementation significantly improved the live body weight and feed conversion efficiency of broilers, while Si et al. (2001) evaluated the relationship of dietary lysine in diets for broilers and found that increase in the level of essential amino acids resulted in significant improvements in feed conversion. As reduction in crude

protein exceeded 3 percentage units and lysine inclusion was above 1.6%, the performance of the birds was poorer has also been reported by Haper et al. (1984) and Nawaz et al. (2006). Broilers do not seem to do as well when intact protein is reduced to a greater extent and replaced with crystalline essential amino acids (Pinchasov et al., 1990).

In the methionine study, results of carcass measures showed no significant differences between the carcass measures of birds on 23% CP and 0.6% methionine (control diet) and birds on 20% CP supplemented with any of the methionine levels. This is consistent with the report of Hickling et al. (1990) who suggested that amino acid levels in excess of NRC recommendations improved breast meat yield of broilers. The observed significant ($P<0.05$) reduction in weight of carcass parts in response to CP levels lower than 20% and at 1.4% methionine is in agreement with the findings of Nawaz et al. (2006) of significant decrease in carcass weight of broilers fed low CP diets. Dai (2002) had reported that animals require not only free amino acids, but also small peptides to support growth, and the peptides may be the essential nutrients, and the low-protein, amino acid supplemented diet may have been deficient of these small peptides.

Similarly results of carcass measures in the lysine study showed no significant differences in the carcass parts of birds on 23 and 20% CP at 1.2% lysine inclusion. This is consistent with the report of (Kamran et al., 2004) that dietary protein level for broilers could be reduced from 23 to 20%, with beneficial effects on growth performance and carcass characteristics and increased economic returns. Hesabi et al. (2008) also evaluated the effects of different levels of lysine and methionine on carcass characteristics in broilers and suggested that additional methionine and lysine may improve performance and carcass traits in broiler chicks. Sibbald and Wolynetz (1986) reported that increasing dietary lysine levels has been reported to increase carcass protein retention. In the present study, lowering the dietary CP level below 20% and increasing lysine level above 1.6% significantly reduced carcass weight ($p<0.05$). This agrees with the findings of Nawaz et al. (2006) who observed

Table 5: Performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

	1	2	3	4	5	6	7	8	9	10		
Crude protein(g/100g)	23	20	20	20	17	17	17	14	14	14		
Methionine (g/100%)	0.6	0.6	1.0	1.4	0.6	1.0	1.4	0.6	1.0	1.4		
Parameters											**SEM**	**P-Value**
Initial weight	40.2	40.4	40.3	40.1	40.2	40.5	40.2	40.2	40.2	40.2		
Final weight (g/b/ 35 days)	753.13^{ab}	740.63^{ab}	775.06^{a}	678.13^{bc}	741.96^{ab}	692.19^{abc}	625.45^{c}	722.50^{ab}	715.63^{ab}	475.25^{d}	26.81	<0.0001
Weight gain(g/b/d)	20.37^{ab}	20.00^{ab}	20.99^{a}	18.23^{bc}	20.05^{ab}	18.62^{abc}	16.72^{c}	19.49^{ab}	19.30^{ab}	12.32^{d}	26.82	<0.0001
Feed intake (g/b/d)	47.05^{a}	44.46^{a}	44.29^{a}	43.13^{a}	45.18^{a}	44.82^{a}	37.08^{b}	45.98^{a}	46.34^{a}	34.56^{b}	46.46	<0.0001
Feed conv. ratio	2.31^{bcd}	2.22^{de}	2.10^{e}	2.36^{bc}	2.26^{cd}	2.41^{b}	2.22^{de}	2.36^{bc}	2.41^{b}	2.82^{a}	0.04	<0.0001

^{a,b,c,d}Means within the row with different superscripts are significantly different ($p<0.05$)
SEM-Standard error of mean. P-Probability value`

Table 6: Effect of methionine, crude protein, and their interaction on performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

Treatment	Final weight (g/b)	Weight gain (g/b)	Feed intake (g/b)	FCR
Methionine (g/100g)				
0.6	739.55[a]	19.98[a]	45.67[a]	2.29 [b]
1.0	727.63[a]	19.64[a]	45.15[a]	2.31[b]
1.4	591.61[b]	15.76[b]	38.26[b]	2.47[a]
SEM	14.82	14.82	25.68	0.02
Crude protein(g/100g)				
23	753.13[a]	20.37[a]	47.05[a]	2.31[b]
20	731.27[ab]	19.74[ab]	43.96[b]	2.23 [b]
17	686.53[bc]	18.47[bc]	42.36[b]	2.30 [b]
14	636.46[c]	17.03[c]	42.30[b]	2.53[a]
SEM	18.96	18.96	32.85	0.03
Factorial Effects		**P (ANOVA)**		
Methionine	<0.0001	<0.0001	<0.0001	<0.0001
Crude Protein	0.0018	0.0018	0.2371	<0.0001
Met x CP	0.0046	0.0045	0.0026	<0.0001

Means within the same column with different superscripts are significantly different (p<0.05)

Table 7: Effect of methionine, crude protein, and their interaction on carcass measure (g/100gliveweight)

Treatment	Dressed weight	Drum stick	Thigh	Breast	Back	Wing
Methionine	**(g/100g)**					
0.6	514.59[a]	73.88[a]	84.38[a]	145.38[a]	130.81[a]	78.08
1.0	491.71[a]	70.88[a]	78.83[a]	143.54[a]	125.25[a]	72.25
1.4	369.79[b]	52.88[b]	61.13[b]	103.75[b]	95.46[b]	54.33[b]
SEM	21.29	3.05	3.19	6.77	5.84	3.56
Crude Protein	**(g/100g)**					
23	536.25[a]	77.13[a]	89.75[a]	149.38	131.25	76.13
20	476.83[ab]	69.88[ab]	78.21[ab]	137.29	118.33	69.04
17	442.83[b]	64.00[b]	73.21[b]	127.25	118.29	67.88
14	449.21[b]	62.68[b]	71.13[b]	126.79	114.75	66.46
SEM	27.24	3.90	4.08	8.66	7.47	4.56
Factorial	**Effects**		**P (ANOVA)**			
Methionine	0.0002	0.0002	0.0002	0.0003	0.0006	0.0003
Crude Protein	0.6323	0.3563	0.3327	0.6737	0.9721	0.8668
Met x CP	0.0688	0.0855	0.0578	0.0644	0.0825	0.0793

[a,b.]Means within the same column with different superscripts are significantly different (p<0.05)

Table 8: Effect of CP, Methionine and their interaction on Antibody titre against IBD

Treatment	Antibody titre
Methionine (g/100g)	
0.6	895.8
1.0	981.4
1.4	950.9
SEM	80.08
Crude Protein (g/100g)	
23	768.2
20	1025.8
17	830.4
14	1014.4
SEM	102.45
Factorial Effect	P (ANOVA)
Methionine	0.9324
Crude Protein	0.2369
Met x CP	0.5488

significant decrease in carcass responses of broiler fed low CP diets. Metabolically, excess lysine cannot be used efficiently because the receptors for lysine become saturated.

In chickens, it has been shown that shortage or excess of dietary protein (Payne et al., 1990) or amino acids (Bhargava et al., 1971b) alters immune responses. Amino acids are needed to trigger such a response, which consists in clonal proliferation of lymphocytes and establishment of germinative centers in the bursa of Fabricius to refine immunoglobulin affinity. Amino acids are also involved in the recruitment of new bone marrow monocytes and heterophils and synthesis of effector molecules (immunoglobulins, nitric oxide, lysozyme) and communication molecules such as cytokines and eicosanoids (Rubin et al., 2007). In study one, the results of the immune response showed that there were no significant differences between the antibody titre of the birds on 23% CP, 0.6% methionine control diet and birds on the other experimental diets. However, numerically birds on 20% CP and 1.0% methionine diet had higher antibody titre value (1174.6) against IBD virus, compared with those on the control diet (768.2). Tsiagbe et al. (1987) suggested that the requirement for

Table 9: Performance of broilers on low crude protein methionine supplemented diets (n=4 replicates of 8 birds each)

	1	2	3	4	5	6	7	8	9	10		
Crude protein(g/100g)	23	20	20	20	17	17	17	14	14	14		
Methionine (g/100%)	0.6	0.6	1.0	1.4	0.6	1.0	1.4	0.6	1.0	1.4		
Parameters											**SEM**	**P-Value**
Initial weight	40.2	40.4	40.3	40.1	40.2	40.5	40.2	40.2	40.2	40.2		
Final weight (g/b/ 35 days)	753.13^{ab}	740.63^{ab}	775.06^{a}	678.13^{bc}	741.96^{ab}	692.19^{abc}	625.45^{c}	722.50^{ab}	715.63^{ab}	475.25^{d}	26.81	<0.0001
Weight gain(g/b/d)	20.37^{ab}	20.00^{ab}	20.99^{a}	18.23^{bc}	20.05^{ab}	18.62^{abc}	16.72^{c}	19.49^{ab}	19.30^{ab}	12.32^{d}	26.82	<0.0001
Feed intake (g/b/d)	47.05^{a}	44.46^{a}	44.29^{a}	43.13^{a}	45.18^{a}	44.82^{a}	37.08^{b}	45.98^{a}	46.34^{a}	34.56^{b}	46.46	<0.0001
Feed conv. ratio	2.31^{bcd}	2.22^{de}	2.10^{e}	2.36^{bc}	2.26^{cd}	2.41^{b}	2.22^{de}	2.36^{bc}	2.41^{b}	2.82^{a}	0.04	<0.0001

^{a,b,c,d}Means within the row with different superscripts are significantly different ($p<0.05$)
SEM-Standard error of mean. P-Probability value`

Table 10: Effect of CP, lysine and their interaction on performance of broilers (n=4 replicates of 4 birds each)

Treatment	Final Weight (g/b)	Weight Gain (g/b)	Feed Intake (g/b)	Feed Conversion Ratio
Lysine (g/100g)				
1.2	8331.56[a]	21.36[a]	58.68[a]	2.98[b]
1.6	727.08[b]	18.33[b]	54.29[b]	3.30[b]
2.0	562.50[c]	13.68[c]	48.45[c]	3.82[a]
SEM	24.61	24.17	9.86	0.14
Crude Protein (g/100g)				
23	1035[a]	27.12[a]	62.75[a]	2.31[c]
20	920[b]	23.87[b]	63.39[a]	2.74[bc]
17	710.42[c]	17.85[c]	55.30[b]	3.21[b]
14	422.92[d]	9.73[d]	41.37[c]	4.37[a]
SEM	31.48	30.91	12.61	0.18
Factorial Effect			**P-ANOVA**	
Lysine	0.0001	0.0001	0.0001	0.0001
Crude Protein	0.0001	0.0001	0.0001	0.0001
Lys x CP	0.0316	0.0323	0.0001	0.4317

[a,b,c,d]means within the row with different superscripts are significantly different ($p<0.005$) g/b- gram / bird

Table 11: Effect of CP, lysine and their interaction on broiler carcass parts (g/100gliveweight)

Treatment	Dressed weight	Drum stick	Thigh	Breast	Back	Liver	Lung
Lysine (g/100g)							
1.2	437.81[a]	67.50[a]	72.03[a]	129.53[a]	96.78[a]	25.03[a]	3.97[a]
1.6	370.83[b]	58.17[b]	64.63[b]	106.00[b]	89.33[a]	25.21[a]	3.92[a]
2.0	281.67[c]	41.38[c]	49.63[c]	73.33[c]	67.08[b]	20.04[b]	2.96[b]
SEM	13.23	2.43	2.44	3.83	4.00	1.41	0.24
Crude Protein (g/100g)							
23	552.50[a]	85.13[a]	89.38[a]	175.25[a]	121.75[a]	28.25[a]	5.50[a]
20	481.67[b]	76.08[b]	80.38[a]	139.79[b]	108.46[a]	26.42[a]	4.38[a]
17	363.75[c]	55.75[c]	64.08[b]	101.54[c]	86.63[b]	26.17[a]	3.33[c]
14	206.67[d]	29.33[d]	36.04[c]	52.29[d]	50.42[c]	16.63[b]	2.63[c]
SEM	17.29	3.10	3.12	4.90	5.11	1.80	0.31
Factorial Effect							
Lysine	0.0001	0.0001	0.0001	0.0001	0.0011	0.0480	0.0411
Crude Protein	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Lys x CP	0.0380	0.0599	0.0611	0.0041	0.0566	0.7719	0.0118

Table 12: Effect of crude protein, lysine and their interaction on Antibody titre

Treatment	Antibody titre
Lysine (g/100g)	
1.2	3856.7
1.6	3163.1
2.0	3150.5
SEM	269.71
Crude Protein (g/100g)	
23	2467.2[b]
20	3512.6[ab]
17	3903.0[a]
14	2980.7[ab]
SEM	345.02
Factorial Effect	P (ANOVA)
Lysine	0.2146
Crude Protein	0.0615
Lys x CP	0.5312

[a,b]Means within the row with different superscripts are significantly different ($p<0.05$)
SEM-Standard error of mean.

methionine for maximum antibody titers was greater than that for growth. This means that the IgG levels did increase in birds fed increasing percent of methionine. This may indicate that extra methionine is important for the synthesis of the IgG antibodies or perhaps required for thymus-derived T–cell helper function. Also Swain and Johri (2000) and Shini et al. (2005) reported that methionine requirements are higher when the purpose is to maintain optimal immunity levels, as compared to growth and that lower supply of sulphur amino acids like methionine and cysteine results in a severe lymphocyte depletion in the intestinal tissues (Peyer's patches) and in the lamina propria. Deficiency or excesses of dietary protein or amino acids has previously been said to alter immune response in chickens (Konashi et al., 2000, Chen et al., 2003), but among the various experimental diets fed in study two, birds on 20% CP and 1.6% lysine diet produced the highest antibody titre against infectious bursal disease vaccine. This is in support of the work of Konashi et al. (2000), which reported that lysine, one of the key amino acids for protein synthesis and muscle deposition is involved in the synthesis of cytokines, proliferation of lymphocytes and thus in the optimum functioning of immune system in response to infection. According to Chen et al. (2003), inadequate supply of lysine would reduce antibody response and cell-mediated immunity in chicken.

Conclusions

Results of the current study showed that the growth performance and carcass measures of broilers on 20% CP diet with 1.0% methionine or 1.6% lysine was similar to those on a control diet containing 23% CP with 0.6% methionine or 1.2% lysine. Antibody titre values in birds on the 20% CP with 1.0% methionine or 1.6% lysine were higher than those on 23% CP and 0.6% methionine or lysine. Better immune response in broilers can be obtained with adequate supplementation of methionine and lysine, which have been identified to be in marginal quantities in most poultry diets. Typical poultry feeds, in which much of the protein may contain less than the desired quantity of methionine and lysine necessitates their supplementation of such diets for improved immune response against Infectious Bursal Disease virus.

Impact

The results of the research will educate poultry farmers that they may not need to include up to 23%CP in broiler diets in accordance to NRC (1994) recommendation, but that a lower inclusion rate of 20% CP supplemented with 1.0% methionine and 1.6% lysine will produce growth performance and carcass characteristics as a 23% CP, 0.6% methionine and 1.2% lysine diet. Furthermore, such lower CP diet supplemented with methionine and has no adverse effect on immune response against IBD. The results point to the fact that methionine and lysine in excess of NRC recommendations are required for optimum antibody formation.

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IN VITRO ACTIVITY OF AZADIRACHTA INDICA EXTRACTS ON SOME PATHOGENIC FUNGI

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Abstract

The objective of this study was to evaluate the activity of Azadirachta indica extracts on some pathogenic fungi. Hexane, methanol and aqueous extracts of A.indica seeds and leaves were screened for their antifungal activity against T. mentagrophytes, T. soudanense M. audouinii, M.canis, , A. flavus Curvularia lunata and C. albicans using agar diffusion method. The obtained results indicated that both methanol and hexane extracts revealed antimicrobial activity against tested fungi at different concentrations used. The alcohol extracts were found most effective than the aqueous extract.

Among the pathogenic fungi tested, Dermatophyte species were found to be most sensitive to the extracts than A. flavus. A.indica extracts did not inhibit the growth of A. flavus but affect its sporulation while C. albicans was found insensitive to the extracts. Thus the microbial inhibition potential of A.indica leaf and seed extracts which was observed in this study opened the way for perspective use of A.indica to treat dermatophyte infection. Hence, the use of A.indica is highly recommended for treatment of infectious diseases caused by the test pathogens.

Keywords: Azadiracta indica, in vitro activity, pathogenic fungi, natural products

ACTIVITE IN VITRO DES EXTRAITS D'AZADIRACHTA INDICA SUR CERTAINS CHAMPIGNONS PATHOGENES

Résumé

L'objectif de cette étude était d'évaluer l'activité des extraits d'Azadirachta indica sur certains champignons pathogènes. Des extraits d'hexane, de méthanol et aqueux et des graines et feuilles d'A. indica ont été examinés pour déterminer leur activité antifongique contre T. mentagrophytes, T. soudanense M. Audouin , M.canis, A. flavus Curvularia lunata et C. albicans en utilisant la méthode de diffusion sur gélose. Les résultats obtenus indiquent que les extraits de méthanol et d'hexane ont révélé une activité antimicrobienne contre les champignons testés à différentes concentrations utilisées. Les extraits d'alcool se sont révélés plus efficaces que l'extrait aqueux.

Parmi les champignons pathogènes testés, les espèces de dermatophytes se sont révélé les plus sensibles aux extraits par rapport à A. flavus. Les extraits d'A.indica n'ont pas inhibé la croissance de A. flavus mais ont affecté sa sporulation alors que C. albicans s'est révélé insensible aux extraits. Ainsi, le potentiel d'inhibition microbienne des extraits des feuilles et graines d'A.indica, qui a été observé dans cette étude, ouvre la voie à la perspective de l'usage d'A.indica pour le traitement de l'infection aux dermatophytes. Par conséquent, l'utilisation d'A.indica est fortement recommandée pour le traitement de maladies infectieuses causées par les agents pathogènes testés.

Mots-clés : Azadiracta indica ; Activité in vitro ; Champignons pathogènes ; Produits naturels

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Introduction

Azadirachta indica A. Juss is a tree in the Mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India, Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions. Locally in the Sudan it is known as neem tree. The Neem tree has enjoyed a revival, being rediscovered by the people of India where it is now the centre of a thriving industry, producing goods ranging from cosmetics and medicines to natural insecticides (Cole, 2002).

Tropical climate especially in the coastal regions of India creates the kind of humid hot house atmosphere that favors the growth of fungi. Traditionally, in Ayurveda, Neem seed oil, aqueous extracts of Neem leaf, Neem leaf powder, the smoke from burning dried Neem leaves, and Neem leaf pastes have been used for prevention and treatment of fungal conditions in India (Mishra et al., 1995).

Athlete's foot, ringworm, and Candida, which causes vaginal yeast infections and thrush, are some of the more common fungi that attack humans ((Kannan et al., 2006).

In Sudan, Muna et al. (2003), studied the parasitic effect of seed kernel, oil and water extract of A. indica on Argas persicus. Decrease egg hatching and mortality of larvae were shown.

There are two medicinal compounds in the Neem leaf, gedunin and nimbidol, have been clinically proven to control these fungi. Jock itch, another fungal infection that attack humans, has been treated traditionally in India for thousands of years with Neem seed oil and leaf aqueous extracts. Creating medicinal smoke by burning dried Neem leaves is an ancient practice in Ayurveda for purifying the atmosphere around a seriously ill patient. A clinical study examining the efficacy of this ancient practice found that smoke from burning dried Neem leaves exerted an extreme suppression of fungal growth and germination. Clinically, Neem is proven to be an effective analgesic; anti-inflammatory, anti arthritic; antipyretic; hypoglycaemic; antigastric ulcer; spermicidal; antifungal; antibacterial; diuretic; antimalarial; antitumour; and immunomodulatory agent (Biswas et al., 2002). These qualities along with the above mentioned properties of Neem is what makes Neem so effective against serious skin conditions like eczema, psoriasis, acne, dermatitis, shingles, jock itch, athletes foot, ring worm, scabies, lice and Candida infection. Moreover, lotions and creams containing Neem oil and Neem leaf extracts are found effective externally while Neem leaf extract, capsules, and teas are useful internally because they effectively detoxify the blood from impurities which cause skin problems (Selvester,1999).

The Science of Neem:

Active ingredients of neem include: Nimbin (has an anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal effect); Nimbidin (anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-fungal); Ninidol (anti-tubercular, anti-protozoan, anti-pyretic); Gedunin (vasodilator, anti-malarial, anti-fungal) ; Sodium nimbinate (diuretic, spermicide, anti-arthritic); Quercetin (anti-protozoal) ; Salannin(insect repellent) and Azadirachtin(insect repellent, anti-hormonal).

The highest concentrations of the active ingredients are found in the seed and oil, however the active ingredients are also found in lesser amounts in the bark and the leaves (Selvester, 1999).

Materials and Methods

Isolates of A. flavus, C. albicans, T. mentagrophytes, T. soudanense M. audouinii, M.canis, , M. canis and Cu.rvularia lunata were obtained from culture collection of Mycology Department at Central Veterinary Research Laboratories Centre (CVRLC), Khartoum, Sudan.

Neem seeds and leaves were collected from Elsrourab district located at North Omdurman, Khartoum state, Sudan. They were left to dry at room temperature (30oc) for 6 days and then ground into powder using mortar and pestle. The powdered plant parts were kept for further work.

Preparation of extracts:

Extraction of neem was carried out

according to method described by (Harborne, 1984). Neem leaves and seeds were successively extracted with n. hexane and methanol using soxhlet extractor apparatus for about 6h for hexane and 72h for methanol. Extraction was continued till the color of the solvent in the last siphoning returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus and the yield percentages were calculated.

Water extract:

Water extract was prepared by adding 10ml of boiled distilled water to a sample of 10gm of coarsely powdered plant material in a beaker with occasional shaking for 4h at room temperature. The extract was then filtered and the precipitate was washed with small volume of distilled water and the filtrate was immediately used (Almagboul, 1992).

Susceptibility test of neem extract:

Preparation of fungal suspensions:

Tested fungi were sub cultured on Sabouraud's Dextrose agar (SDA) and incubated at 25oc for 4 days. Fungal growth was harvested and washed with 100 ml of sterile normal saline. Suspensions were stored in the refrigerator until used.

Agar diffusion test:

In vitro test:

Aspergillus, Candida and dermatophytes were maintined in pure cultures on SDA slant for evaluation of antifungal activity of A. indica leaves and seeds extracts. Concentrations of 50, 100, 200 mg /20 ml medium were prepared from neem's seeds and leaves extracts. The test samples and untreated control were inoculated with equal inoculum of the tested fungi. After 2 weeks when good growth in untreated control was obtained, the test was read (Elfadil et al., 2002).

Results

The yield percentage of neem methanolic and hexane extracts was calculated as follows:

Yield percentage =
Weight of extract / weight of plant sample * 100

From 60 g of neem leaves and 100 g neem seeds obtained after extraction the yield is shown below (Table 1).

Agar diffusion test:

In vitro test:

It was shown that the growth of the pathogenic fungi was inhibited with alcoholic extract of seeds and leaves of A. indica (Table 2). It was evident that the growth inhibition of fungi was more pronounced with methanol and hexane extracts of both leaves and seeds than aqueous extract.

Hexane extract of seeds:

The growth of M. audouinii, M. canis, T. mentagrophytes and T. soudanense was completely inhibited with 50 and 100 mg/ml of the hexane extract (Table 2). On the other hand, 100 mg/ml of neem seed was found to be fungistatic for A. flavus where white colony was obtained (fig. 3). A slide mounted in Lacto Phenol Cotton Blue (LPCB) revealed sterile hyphae without spores. Furthermore, both extracts affect the growth of C. lunata.

Methanol extract of seeds:

M. canis, M. audouinii, T. mentagrophytes and T. soudanense were found sensitive to 20% methanol extract. Also, 200 mg methanol extract was found to affect the growth and sporulation of A. flavus. A white colony with sterile hyphae was shown on LPCB slide mount.

Hexane extract of leaves:

100 mg/ml of leaves hexane extract was found to affect the growth of M. canis, T. mentagrophytes while 50 mg/ml retard the growth of A. flavus. Sterile hyphae were seen on slide mounted in LPCB.

Methanol extract of leaves:

M.canis, T. mentagrophytes, T. soudanense and M. audounii were found susceptible to leaves methanol extract where complete inhibition of growth was observed (Table 3; fig 1&2).

Table 1: yield percentage of extracted leaves and seeds of A. indica.

Plant	Leaves		Seeds	
Solvent extract	**Weight (g)**	**Yield %**	**Weight (g)**	**Yield %**
Hexane	1.876	3.127%	22.976	
	22.976%			
Methanol	8.6	14.333%	7.3	7.3%

Table 2: Activity of hexane and methanol extracts of A. indica seeds on dermatophyte species.

Extract	Concentration mg/ml	Microorganisms M.canis	Growth M. audounii	inhibition T. mentagrophytes	T. soudanense
Hexane	200	+	+	+	+
	100	"	"	"	"
Methanol	200	"	"	"	"
	100	"	"	"	"
Control	0.00	_	_	_	_

Table 3: Activity of hexane and methanol extracts of leaves on dermatophyte species.

Extract	Concentration mg/ml	Microorganisms M.canis	Growth M. audounii	inhibition T. mentagrophytes	T. soudanense
Hexane	200	+	+	+	+
	100	"	"	"	"
Methanol	200	"	"	"	"
	100	"	"	"	"
Control	0.00	_	_	_	_

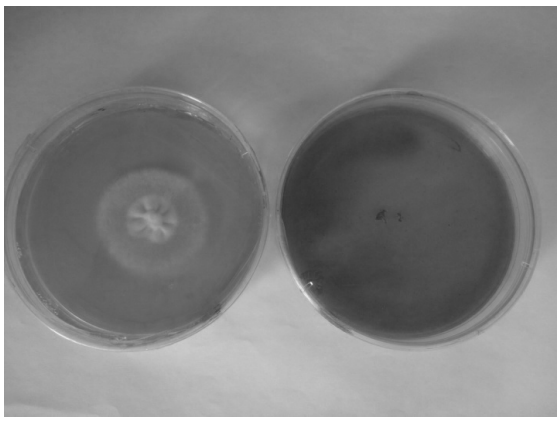


Figure 1: Control plate (left) showing growth of M.canis on SDA after 10 days of incubation at room temperature. Growth inhibition of M.canis in hexane extract of A. indica leaves at concentration of 100mg/ml after 10 days of incubation at room temperature (×40) (right). .

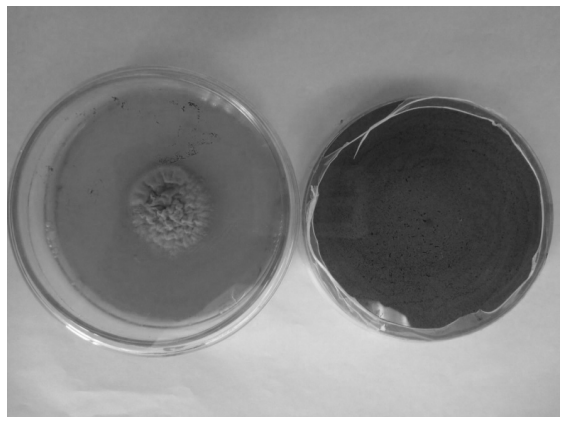


Figure 2: Control plate (left) showing growth of T. soudanense on SDA after 10 days of incubation at room temperature (×40). Growth inhibition of T. soudanense in hexane extract of A. indica leaves at concentration of 100mg/ml after 10 days of incubation at room temperature (×40) (right).

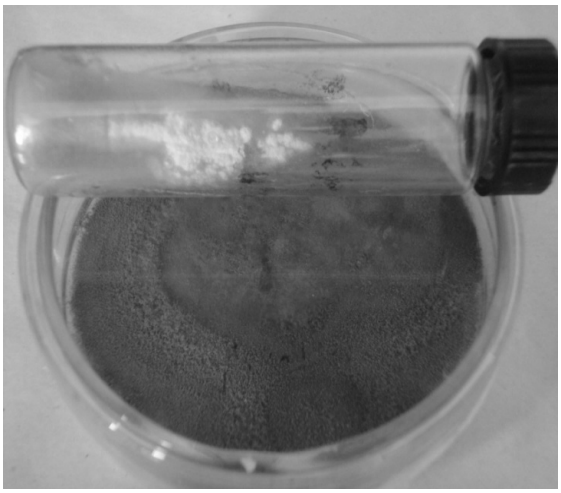


Figure 3: White colony of A. flavus on SDA at concentration of 100mg/ml Hexane extract of A. indica at concentration of 100mg/ml (vial) and green colony of the control (plate) after 7 days of incubation at room temperature (×40).

Candida albicans was found to be resistant to all extracts at different concentrations used. Thus, A. indica extracts were found to have no effect on C. albicans when compared with the positive control (Nystatin).

Discussion

The activity of hexane and methanol extracts of A.indica against the growth of Aspergillus flavus, Candida albicans and some selected dermatophytes was tested in vitro. The use of n.hexane is also suggested by Liauw et al. (2008).

The present results revealed inhibition of radial growth of Aspergillus and dermatophytes by alcoholic leaves and seeds extracts of A. indica, suggesting the presence of antifungal substances in the plant. This finding is in an agreement with Mondali et al. (2009). This result shows that A.indica extract is well endowed with biologically active compounds that some dermatophytes were sensitive to it.

Inhibition of sporulation of A.flavus which observed in this study is similar to that reported by Joudo (2009) while resistance of C.albicans to A.indica extracts is not. This might be due to strain resistant or other factors such as age of leaves, solvent used for extraction and process of extraction.

Aqueous extracts of the plant was found to have no effect on fungi tested. This might be due to in solubility of active ingredients in water which have an antifungal action. The alcoholic extract was more potent than the water extract of A.indica. This finding is on line with Mondali et al. (2009).

Conclusion

The effect of hexane, methanol and water extracts of A. indica against some pathogenic fungi was evaluated by agar diffusion method. The obtained results revealed that the alcoholic extract posses the stronger antifungal activity compared to water extract. Dermatophytes showed high sensitivity to alcoholic leaves and seeds extracts followed by Aspergillus flavus. Thus, this result confirms the efficacy of A. indica as a natural antimicrobial and suggests the possibility of employing it as a drug for treatment of infectious fungal infections.

Acknowledgements

The authors are grateful to Sudan Academy of Science (SAS) for permission to carry this study. Great thanks are due to the Staff of Mycology Department, Veterinary Research Institute for technical help. Comments and revision of the manuscript carried by Dr. Hayat Mahgoub, Biochemistry Department, are highly appreciated.

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PREVALENCE OF FLEAS IN SHEEP AND GOATS IN OGUN STATE, NIGERIA

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Abstract

This study evaluated the association of factors (breed, sex, and age); location (village, peri-urban and sales point) and prevalence of fleas in sheep and goats in five Local Government Areas in Ogun State, Nigeria. A total of 300 sheep and 300 goats were selected at random from the three locations. The estimated age, sex, and breeds of each animal were determined. Thereafter, they were examined for presence of fleas and/or skin lesions. Flea samples were collected into universal bottles containing 1% formaldehyde. Location and severity of the flea on each animal were also recorded. The fleas were identified up to species level. Data on the animals, management factors and the prevalence of fleas were processed into contingency tables to establish the association between the prevalence of fleas and factors (age, sex, breed, and location) using Chi-square(X2) test statistics. Fleas (Ctenocephalus felis) were observed to infest sheep in the study area. There were no significant associations (P > 0.05) of all the animal factors with prevalence of fleas. It was therefore recommended that effective ectoparasites control measures should be instituted to prevent adverse effects of these parasites on the health and production of these animals.

Key Words: Prevalence, Fleas, Sheep and Goats, Ogun State.

PREVALENCE DES PUCES CHEZ LES OVINS ET CAPRINS DE L'ETAT D'OGUN AU NIGERIA

Resume

Cette étude a évalué l'association de facteurs (race, sexe et âge) ; la localité (village, zone péri-urbaine et point de vente) et la prévalence des puces chez les ovins et caprins dans cinq collectivités locales de l'Etat d'Ogun au Nigeria. Au total, 300 moutons et 300 chèvres ont été sélectionnés de manière aléatoire dans les trois sites. L'âge estimatif, le sexe et la race de chaque animal ont été déterminés. Par la suite, ils ont été examinés pour rechercher la présence de puces et / ou de lésions cutanées. Des échantillons de puces ont été recueillis dans des bouteilles universelles contenant 1% de formaldéhyde. En outre, l'endroit et la sévérité des puces sur chaque animal ont été enregistrés. Les puces ont été identifiées jusqu'au niveau de l'espèce. Les données sur les animaux, les facteurs de gestion et la prévalence des puces ont été traitées en tableaux de contingence pour établir l'association entre la prévalence des puces et les facteurs (âge, sexe, race et emplacement) à l'aide des statistiques du test de Chi carré (X2). On a constaté que les puces (Ctenocephalus felis) infestaient les moutons dans la zone d'étude. On n'a pas relevé d'associations significatives (p> 0,05) entre les facteurs animaliers et la prévalence des puces. Ainsi, il a été recommandé que des mesures de contrôle efficaces des ectoparasites soient adoptées pour prévenir les effets néfastes de ces parasites sur la santé et la production de ces animaux.

Mots-clés : Prévalence ; Puces ; Ovins et Caprins ; État d'Ogun

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Introduction

Sheep and goats form an important part of livestock industry in the Sub-Sahara Africa. They serve as valuable supplement to cattle in terms of animal protein supply for the teaming population including the provision of manure for field crops. It has also been established that over 90% of sheep and goats in the Sub-Saharan Africa are found in East and West Africa (Jatau et al., 2011). These animals are important source of investment especially in the rural areas of the country where livestock is seen as saving in the form of bank. Parasitism continues to be the major constrains to livestock production in Sub-Saharan Africa (Ajayi et al., 1987; Bell-Sakyi et al., 2004; Okaiyeto et al., 2008).

Though small ruminants are important components of the Nigerian farming system, their contribution to food production, rural income and export income are far below the expected potential. This is because small ruminant production is constrained by the compound effects of diseases, poor feeding and poor management (Getachew, 1995).

Skin problems caused by ectoparasites result in serious economic loss to smallholder farmers, the tanning industry and the country as a whole. They can result in mortality, decreased production and reproduction, downgrading and rejection of skins. Hides and skins accounts for 12–16% of the total value of exports in Nigeria (Asfaw, 1997).

According to Bayou (1998), skin problems due to external parasite cause 35% of sheep skin and 56% of goat skin rejections. Feeding may cause direct damage to skin and other sub-cutaneous tissues, inflammation and significant blood loss. This activity is usually associated with pruritis, erythema, excoriation, papules, scaly and crusting and self-trauma. Wounds may be subject to secondary infestation or bacterial infection (Hart, 2000). The salivary and faecal antigens produced by fleas as they feed can stimulate immune responses in some individuals leading to hypersensitivity (Van den Broek et al., 2003).

Since fleas can act as vectors of important zoonotic diseases and also diseases to animals, it is therefore important, to undertake a study on the prevalence of fleas of sheep and goat in Ogun State Nigeria.

Materials and Methods

Study area

The survey was conducted in Ogun State in four Local Government Areas, namely Abeokuta South, Abeokuta North, Odeda and Obafemi/Owode Local Government Areas. Sheep and goats managed under village, peri-urban and sales point locations in all these areas were used for the study.

Sample collection

A total of 300 sheep and 300 goats were randomly selected from the three locations (peri-urban, village and sales point). The estimated age, sex, breed and body condition of each selected animal was determined. The animals were clinically examined for presence of fleas and/or skin lesions. Care was taken to minimize discomfort and pain to the animals during the process of sample collection. The location and severity of the fleas on each animal were recorded. Fleas were collected and physically counted at every search by combing the coat of the animal (Jeong-Hyun et al., 2008). Identification of fleas was performed on the basis of the keys provided by Macy and Berntzen (1977) and Wall and Shearer (1997). Animals infested with fleas were noted and the fleas on each of the animals were collected into universal bottles containing 1% formaldehyde.

Laboratory examination of the samples

Laboratory examination of the samples was done at the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Fleas collected from the infested animals were separated and identified using MAFF (1987) identification keys.

Statistical analysis of data

Data on animal and management factors and the prevalence of fleas were processed into contingency tables to establish the association between prevalence and the various factors of sheep and goat production system using Chi-square(X^2) test statistics of Genstat package (Genstsat Release 7.2 DE, Copyright 2007, Lawes Agriculture Trust, Rothamstal Station).

Means were separated using Duncan Multiple range Test in same Statistical Package while significant associations were further expressed in Descriptive Statistics to show the trend.

Data Sheet for Infestation:

0 - None: if no flea is found.
1 - Low: if few fleas are found only at the interdigital spaces.
2 - Moderate: if fleas are found at the interdigital spaces and the head region.
3 - Severe: if fleas are present in the interdigital spaces, perineal, body trunk and the head region.

Results

In addition, fleas (Ctenocephalus felis) were observed to infest sheep and goats in the study area. Chi-square analyses of some animal factors associated with flea species of goats and sheep in the study area are shown in Table 1. There was no significant association ($P<0.05$) of all the animal factors with prevalence of fleas in goats.

Chi-square analysis of some animal factors associated with prevalence of flea species of sheep in the study area is shown in Table 1. There were significant associations ($P<0.05$) between breeds of sheep and the prevalence of fleas as shown in Table 1. There were however, no significant associations of age and sex of sheep with prevalence of flea species and also between location of sheep and prevalence of fleas as revealed in Table 1.

Figures 1 shows prevalence of fleas in sheep while figure 2 shows prevalence of fleas in goat which is 2% in both species (sheep and goat). Figure 3 shows significant association of breeds of sheep with prevalence of fleas ($P=0.001$). Only some proportions of the O'uda breed (14.7%) had low level of infestation while none of the WAD and Yankassa breeds had flea infestation.

Table 1: Chi-Square Analysis of some Animal factors associated with Prevalence of fleas in Goats and sheep.

	Animal species	
	Goat	**Sheep**
Age	0.3 (P = 0.581; 1)	2.47 (P = 0.116; 1)
Sex	0.03 (P = 0.865; 1)	0.1 (P = 0.619; 2)
Breed	2.5 (P = 0.286; 2)	*39.8 (P < 0.001; 2)
Location	0.4 (P = 0.819; 2)	5.3 (P = 0.071; 2)

*- Significant associations

Note: Values in parentheses are corresponding Probability levels (P) and degrees of freedom, respectively.

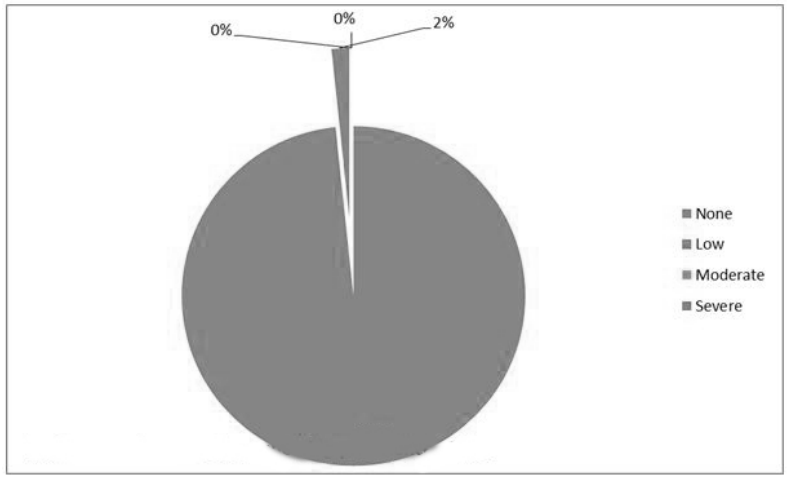


Figure 1: Percentage of Sheep showing prevalence of flee

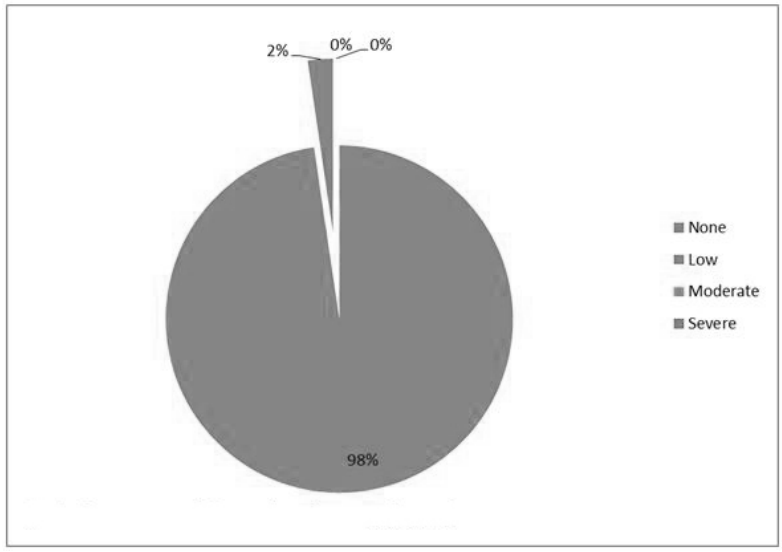


Figure 2: Percentage of Goats showing prevalence of flee

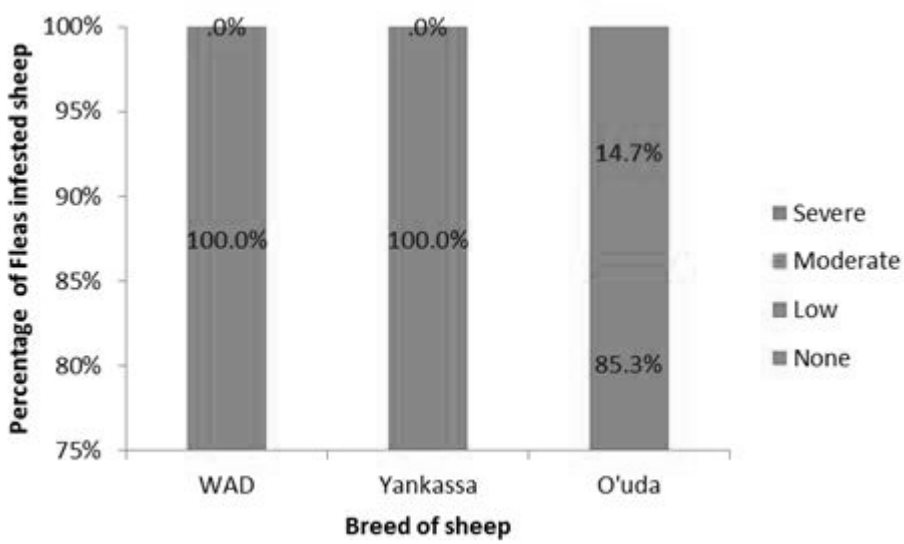


Fig. 3: Significant Association of Breed of Sheep with Prevalence of Fleas

Discussion

These results were similar to those of Dipeolu (1975) and Abdullahi et al. (2000) obtained for Northern Nigeria. Plant (2006) similarly identified this flea species as being of economic importance in Australia. Also, sheep were found to have same susceptibility to flea infestation as goats, dissimilar to the reports of Fagbemi (1982) who reported a higher susceptibility in sheep. This may be due to the fact that the sheep were not flocking with cattle. This result agrees with Webb and David (2002), Schwalbach et al. (2003). There was also no association between all the animal factors and flea infestation in goats. Yeruham (1989) similarly pointed out that fleas are generally not considered to be important ectoparasites of livestock and that, this may not be the case in a number of countries in the Mediterranean region. These were similar to the results obtained by Dipeolu (1975) and Abdullahi et al., (2000) for Bauchi area.

This result corroborated with the study of Ofukwu and Akwuobu (2010), in which management system significantly affect the prevalence and distribution of ectoparasites. Prevalence of flea was reported to be high in extensive management system and lowest in intensive management system (Ofukwu and

Akwuobu, 2010). The findings of this study may simply imply that the goats and sheep in the village in Ogun state are probably not raised under extensive management system but semi-intensively. External parasites cause extensive losses among small ruminants, especially in humid areas. Flea infestation was more severe in sheep than in goats. The heavier infestation of fleas in sheep may be because they are more hairy than goats, making it easier to find these fleas in them than in goats. Finally, the infestation of sheep and goat fleas were not high compare to that reported in Makurdi (Ofukwu and Akwuobu, 2010).

Conclusion

The animal factors of 'breed' were found to be significantly associated with flea infestation in sheep. WAD and Yankassa breeds had zero prevalence while the prevalence in O'uda had 14.7% prevalence rate. All the factors had no significant association with prevalence of fleas in the goats under this study.

Impact

Flea infestation in this study area showed that goats are more susceptible than sheep, however, it is suggested that regular ectoparsite control be instituted on small ruminant Farms as this will prevent the problems associated with infestation of fleas and other ectoparasite which is manifested in poor skin quality, diseases, poor body-score, low carcass yield, loss of productivity and sometimes death. Control measures in animal by the use of ectoparasitic agents such as pour-on preparations and ivermectins; also control of the environment are encouraged for maximum performance and productivity.

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RESULTATS D'UNE ENQUETE SERO-EPIDEMIOLOGIQUE SUR LA BRUCELLOSE DANS LES ELEVAGES BOVINS LAITIERS EN ZONE PERI-URBAINE DU DISTRICT DE BAMAKO

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Resume

Suite à l'explosion démographique, les besoins des populations urbaines du Mali en lait et produits laitiers en particulier se sont considérablement accrus.

Le développement au cours de ces dernières décennies de l'élevage bovin laitier dans les zones périurbaines du District de Bamako et d'autres grandes villes du Mali a permis de faire face en partie aux dits besoins. Cependant, cet élevage est confronté à des contraintes d'ordre sanitaire parmi lesquelles figurent certaines pathologies de l'élevage laitier.

Cette étude, réalisée en 2011 avait comme objectif d'évaluer la prévalence sérologique de la brucellose dans les élevages bovins laitiers situés en zone périurbaine du District de Bamako et de formuler des recommandations pour un meilleur contrôle de la maladie.

Elle a permis de tester 684 sérums issus de 10 élevages bovins dans lesquels les promoteurs souhaitaient un dépistage de la brucellose en vue de l'assainissement de leurs troupeaux. Le taux de prévalence sérologique a été évalué à 11,1% (76 sérums positifs).

Cette prévalence varie selon : l'élevage (site), l'âge (les sujets de plus de 10 ans sont plus infectés), le sexe (taux plus élevé chez les femelles) et l'état clinique des animaux (taux plus élevé chez les femelles qui ont avorté).

L'étude a en outre permis de formuler des recommandations pratiques en vue de mieux contrôler la brucellose dans les élevages bovins laitiers du Mali.

Mots clé : Brucellose, Bovins, Sérologie, Bamako

Summary

Following the demographic explosion, the needs of urban populations in Mali in milk and dairy products in particular have increased substantially.

Development during the last decades of dairy farming in periurban areas of the District of Bamako and other major cities in Mali has enabled us to address those needs in part. However, this livestock is faced with constraints, including some health conditions of dairy farming.

This study, conducted in 2011 was aimed to evaluate the seroprevalence of brucellosis in dairy cattle farms located in periurban area of the District of Bamako and make recommendations for better control of the disease.

The study has permitted to test 684 sera from 10 cattle herds whose promoters wanted a brucellosis for their clean. The individual prevalence rate was estimated to 11.1% (76 positive sera).

It was established that the rate of brucellosis prevalence varied depending of farming (site), age (higher among subjects aged over than 10 years), sex (rates significantly higher in females) and clinical status of animals (maximum number of cases in animals that had aborted).

The study allowed also to formulate practical recommendations for a better control of brucellosis in dairy cattle farms in Mali.

Keywords: Brucellosis, Cattle, Serology, Bamako

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Introduction

Au Mali, pays à vocation agro-pastorale, l'élevage constitue une source précieuse de revenus et de protéines, surtout dans le contexte de l'explosion démographique où, les besoins des populations urbaines en lait et produits laitiers se sont considérablement accrus.

Au cours de ces dernières décennies , l'élevage bovin laitier a connu un essor considérable dans les zones périurbaines du District de Bamako et d'autres grandes villes du Mali. Ceci a permis de satisfaire en partie les besoins des populations en lait de consommation.

Cependant, cet élevage est confronté à des contraintes d'ordre sanitaire parmi lesquelles figurent certaines pathologies de l'élevage laitier dont la brucellose.

Une étude effectuée par TOUNKARA et al. (1997) a permis d'obtenir un taux de prévalence de 23,3 % chez les bovins et d'isoler les premières souches de Brucella abortus.

Une autre étude faite par Bonfoh et al. (2003) a permis d'obtenir une prévalence individuelle variant de 8,7 % à 19,5 % et une prévalence troupeau variant de 31,4 % à 65 % en brucellose bovine.

Par ailleurs, les résultats de travaux de diagnostics effectués par le Laboratoire Central Vétérinaire pendant la période 2008-2010, montrent que sur 933 échantillons de sérums bovins soumis pour le sérodiagnostic de la brucellose 106 (10,8%) se sont revelés positifs.

Les données des différentes études réalisées sur la brucellose bovine au Mali sont fragmentaires et méritent d'être réactualisées.

L'objectif de la présente étude était d'évaluer la prévalence sérologique de la maladie dans les élevages bovins laitiers situés en zone périurbaine du District de Bamako et de formuler des recommandations pour un meilleur contrôle de la maladie.

Materiel et Methodes

Période et type d'étude

Il s'agit d'une étude exploratoire de type transversale qui a été réalisée pendant la période 2009-2011.

Choix des sites et matériel de l'étude

Les principaux critères retenus pour le choix d'un élevage étaient l'accessibilité de la zone, être situé dans un rayon de 50 km autour de Bamako, l'adhésion du promoteur à l'esprit d'assainir son troupeau de la brucellose, avoir au moins un effectif de 10 têtes.

La situation géographique des sites est illustrée sur la figure 1.

Les troupeaux retenus étaient composés essentiellement de bovins appartenant à des races locales (N'Dama, zébu), à des races importées (Montbéliard, Holstein) ou à des sujets issus de croisements entre les animaux de races locales et exotiques. Les sujets de l'échantillon appartenaient à cinq tranches d'âges : de 6 à11mois, de 1 à 3 ans, de 3 à 6 ans, de 6 à 10 ans et plus de 10 ans. Les animaux étaient parqués dans des enclos (murs en banco, en ciment ou en bois). Ils étaient composés en majorité d'animaux n'ayant présenté au préalable aucun signe clinique suspect de la brucellose, même si deux troupeaux avaient hébergé des sujets qui avaient avorté.

Choix des élevages et collecte des échantillons

Les troupeaux concernés par l'étude (10 au total) étaient répartis sur les principaux axes routiers qui mènent au District de Bamako : Bamako-Koulikoro, Bamako-Ségou, Bamako-Sikasso. Cependant, en raison des difficultés objectives d'un échantillonnage aléatoire (réticence de certains promoteurs d'élevage à la réalisation du prélèvement sanguin), l'échantillonnage par choix raisonné, a été retenu.

Le prélèvement de sang (5-10ml) a été effectué à la veine jugulaire dans des tubes vacutainer stériles. Les sérums ont été ensuite repartis en aliquotes et conservés à -20°C avant de subir les examens sérologiques.

Technique de diagnostic de laboratoire

Pour le diagnostic sérologique de la brucellose bovine, plusieurs techniques sont proposées : le test de fixation du complément (FC), la séroagglutination de Wright, l'Epreuve à l'Antigène Tamponnée (Test au Rose Bengale),

la technique Elisa ainsi que l'Epreuve de polarisation en fluorescence (FPA).

Dans le cadre de la présente étude, nous avons utilisé l'Epreuve à l'Antigène Tamponné, améliorée par l'emploi d'un antigène tamponné acide qui a augmente sa spécificité.

Le kit a été fourni par Symbiotics Corporation (Lot 193233).

L'antigène utilisé est une suspension de Brucella abortus (souche 99 de Weybridge) inactivée par la chaleur et le phénol (0,5%), diluée en tampon acide puis colorée par le Rose Bengale.

Le test au Rose Bengale détecte à la fois les IgG (preuve d'une infection ancienne) bovines, même si l'activité hémagglutinante des IgM (preuve d'une infection récente) est fortement réduite. Il s'agit d'un test simple, rapide à exécuter et offrant une grande sensibilité. Par conséquent, elle est principalement utilisée comme test de dépistage. C'est ainsi que la même technique a été utilisée par d'autres auteurs comme Godfroid et al.(2003) et Schelling et al. (2004) dans le cadre d'enquêtes épidémiologiques.

Analyse statistique des données

Les données ont été saisies sur le logiciel Microsoft Excel 2007 et analysées à l'aide du logiciel SPSS 16.0 pour Windows.

Le test de CHI-2 a été utilisé pour comparer la différence qui existe au niveau des taux de prévalence de la brucellose en fonction des sites, de l'âge, de la race des bovins et de l'état clinique des animaux.

Resultats

Au cours de cette étude, 684 sérums de bovins issus de 10 troupeaux ont été analysés à l'aide du test d'agglutination rapide sur lame (Test au Rose Bengale). Soixante Seize (76) sérums (11,1%). ont répondu positivement au test.

Ce taux varie en fonction de l'élevage, de l'âge et de l'état clinique des bovins testés.

Sur les 10 troupeaux testés 9 (90 %) hébergeaient au moins un animal positif.

Les résultats obtenus sont illustrés dans les tableaux I, II, III, IV et V.

Prévalence de la brucellose selon le site

Les résultats sur la variation de la prévalence en fonction du site, figurent dans le tableau I.

De l'examen du tableau I, il ressort que le taux de prévalence sérologique le plus élevé a été obtenu à Niamana (27 %) suivi de Banankoro avec 17 %. Par contre, aucun cas de brucellose n'a été mis en évidence dans les élevages de Katibougou Mandé.

L'analyse statistique (test du Chi-2) montre que les variations de prévalence sérologiques selon le site (élevage) sont significatives (Chi-2= 54,13 > 3,84).

Prévalence de la brucellose selon la race des bovins

Les résultats de la variation de la prévalence sérologique selon la race, figurent dans le tableau II.

Les résultats dudit tableau montrent qu'il n'y a pas de différence significative entre le taux de prévalence chez les sujets issus de croisement et ceux issus de races locales (11,7 et 10,6%).

Il ressort des analyses statistiques, qu'il n'y a pas une corrélation entre la race et le taux d'infection (Chi-2= 1,02 < 3,84).

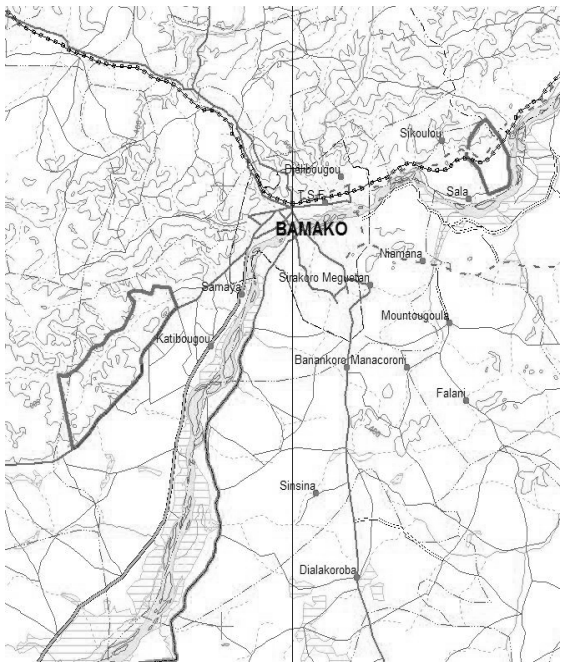


Figure 1 : Situation géographique des différents sites de l'étude (*Source : Institution Géographique du Mali, Bamako*).

Tableau I : Prévalence serologique globale et variations selon le site d'étude

Sites	Nombre total de sérums	Nombre de sérums Positifs	
		Nb	%
Banankoro	39	7	17,9
Dialakoroba	132	4	3,0
Falani	90	8	8,8
Kasséla	200	33	16,5
Manancoroni	19	1	5,2
Katibougou Mandé	38	0	0
Mountougoula	32	2	6,2
Niamana	37	10	27,0
Sala	62	10	16,1
Sikoulou	35	1	2,8
Total	**684**	**76**	**11,1**

Tableau II : Variation de la prévalence sérologique selon la race

Sites	Nombre total de sérums	Nombre de sérums Positifs	
		Nb	%
Sujets issus de race locale	385	41	10,6
Sujets issus de croisements	299	35	11,7
Total	**684**	**76**	**11,1**

Tableau III : Variation de la prévalence sérologique selon le sexe

Sites	Nombre total de sérums	Nombre de sérums Positifs	
		Nb	%
Mâle	146	4	2,7
Femelle	538	72	13,3
Total	**684**	**76**	**11,1**

Tableau IV : Variation de la prévalence sérologique selon l''âge

Sites	Nombre total de sérums	Nombre de sérums Positifs	
		Nb	%
0-1 an	24	1	4,1
1-3 ans	231	10	4,3
4-6 ans	269	36	13,3
7-9 ans	121	22	18,1
Plus de 10 ans	39	7	17,9
Total	**684**	**76**	**11,1**

Prévalence de la brucellose selon le sexe des animaux

Les résultats de la variation de la prévalence sérologique en fonction du sexe, figurent dans le tableau III.

Les différences de prévalence sérologique entre mâles et femelles sont stastistiquement significatives : 13,3% chez les femelles et 2,7% chez les males (Chi-2= 14,50 > 3,84).

Tableau V : Variation de la prévalence sérologique selon l'état clinique des animaux

Sites	Nombre total de sérums	Nombre de sérums Positifs	
		Nb	%
Avortement	35	14	40,0
Néant	649	62	9,5
Total	**684**	**76**	**11,1**

Prévalence de la brucellose selon l'âge des animaux

L'analyse de la variation de la prévalence sérologique en fonction de l'âge, figure dans le tableau IV. Le taux de prévalence le plus élevé a été observé chez les animaux de la tranche d'âge 7-8 ans (18,1 %)(Chi-2 = 24,66 > 3,84). Par contre, les taux de prévalence les moins importants ont été mis en évidence chez les jeunes appartenant aux groupes d'âge 0-1 an et 1-3 ans.

Prévalence de la brucellose selon l'état clinique des bovins testés

L'analyse des résultats sérologiques en fonction de l'état clinique des animaux montre que la prévalence la plus élevé est observée chez les animaux qui avaient avorté (40%) (tableau V).

Cependant, des sérums positifs (9,5%) ont été détectés chez des animaux ne présentant aucun signe clinique suspect de brucellose. Les résultats des tests statistiques confirment la présence d'une différence significative (Chi-2 = 33,89 > 3,84).

Discussion

La présente étude a permis d'obtenir une prévalence en anticorps brucelliques de 11,1% Cette prévalence varie selon les différents sites de l'étude.

Diverses études menées en Afrique ont permis d'évaluer l'importance de la brucellose bovine. Ainsi, d'après Sanogo et al (2008), Delafosse et al. (2002) ont respectivement obtenu des prévalences sérologiques de 2,6% au Tchad et de 15,8% en Ouganda.

Le taux d'infection de 11,1% que révèle notre étude est supérieur à ceux obtenus par Schelling et al. (2004) au Tchad chez les bovins (7%), Sanogo et al., (2008) en Cote d'Ivoire (5,1%) et largement inférieur à celui obtenu par Akakpo en 1987 en Afrique tropicale chez les bovins (22,5%) par la même technique d'agglutination rapide sur lame .Ce phénomène pourrait s'expliquer par divers facteurs dont le mode d'élevage. Par exemple, l'intensification de l'élevage semble favoriser l'extension de la maladie (Domenech et al., 1980)..

Steinmann et al (2006), ont montré que, des systèmes de production animale ont été développés en milieu urbain et périurbain pour satisfaire la forte demande des populations en viande et produits laitiers. Ce développement est accompagné d'amélioration génétique par croisement avec des races bovines exotiques (Montbéliard, Holstein, Rouge des Steppes). Ils conclurent que la brucellose animale a été à plusieurs reprises rencontrée dans le bétail malien et dans la population humaine. Ils ont estimé que la fécondité des troupeaux infectés par Brucella est réduite de 20% et la production de lait de vaches infectées peut être réduite de 15%. Les anticorps spécifiques à Brucella ont été récemment détectés dans le lait de vache malien à un taux de 30% dans les échantillons de lait en vrac à partir d'exploitations bovines autour de Bamako (Dao et al., 2009))

Selon Godfroid et al (2003), des études menées en Afrique du Sud au cours des années 1960, ont permis d'obtenir un taux de prévalence sérologique des anticorps brucelliques agglutinants de l'ordre de 10 à 16 %, taux qui est supérieur à celui obtenu dans notre étude.

Au Zimbabwe, au cours des années 1990, la séroprévalence de la brucellose a varié entre 14 et 29% chez le buffle et l'infection brucellique s'est maintenue dans ces populations, sans qu'il y ait eu contact avec des sujets infectés. Dans ce pays, le buffle est considéré comme un réservoir de germes potentiel pour les bovins, vu l'absence de control strict. (Madsen et ., Anderson., 1995).

Les résultats de la présente étude ont montré des taux sensiblement égaux

chez les bovins appartenant aux races locales et les sujets issus de croisement. Compte tenu de la faible taille de l'échantillon des races importées, nous ne pouvons tirer une conclusion sur la réceptivité de cette espèce sur la brucellose. L'analyse statistique sur la race des animaux infectés à la brucellose n'a pas permis d'établir une corrélation entre les deux paramètres. Cette conclusion est similaire à celle faite par Plommet et al. (1973), qui ont mené une étude sur la brucellose bovine expérimentale. Selon ces auteurs, il n'existe pas de races bovines plus résistantes que d'autres à l'infection brucellique. De même, aucune étude en conditions contrôlées n'a montré que les males sont plus résistants que les femelles, bien que cela ait été suggéré.

Le sexe a été lié à la brucellose. Ceci est probablement lié à la prédominance des vaches et des génisses dans les différents troupeaux visités. Delafosse et al (2002), ont obtenu des résultats similaires suite à la réalisation d'une étude dans la région du lac Tchad. Ces derniers ont trouvés que près de la moitié des femelles infectées par la brucellose avaient avorté. Au Burkina Faso, au Rwanda et au Togo, Akakpo (1987), a mis en évidence des taux de prévalence sérologique plus élevés chez les femelles par rapport aux males. Cependant, l'auteur, a conclu qu'il n'est pas possible de dégager la participation intrinsèque et exclusive du facteur sexe car il ne peut être dissocié des autres facteurs extrinsèques.

Notre enquête a permis d'établir que les sites de Niamana et de Kasséla se distinguent par des prévalences élevés. Dans ces sites, les troupeaux sont composés essentiellement de sujets issus de croisements entre les animaux de race locale et exotiques. Doumbia (2009) (données non publiées), montra à travers une enquête qui a porté sur quatre localités du Mali , que Bamako avait un taux de positivité plus élevé (14,2%) par rapport aux autres localités. Selon l'auteur la maladie est surtout fréquente dans les zones périurbaines de Bamako et Koulikoro où l'élevage semi intensif est pratiqué avec une grande proportion de métis.

La maladie a été associée à l'âge et le taux de prévalence le plus élevé a été obtenu chez les animaux de plus de 10 ans. Nous constatons que plus l'animal avance en âge plus le taux d'infection augmente. Les animaux dont l'âge est compris entre 10 et plus ont beaucoup plus de chance de contracter la brucellose. Des résultats similaires ont été obtenus par Akakpo (1987) qui conclue que plus l'animal vieillit, plus il a des chances d'être infecté, de le demeurer et d'être infectant pour les autres animaux. Tout en n'excluant pas l'influence du mode d'élevage, le maintien des animaux dans des parcs et enclos souillés, dont la contamination est régulièrement entretenue par des décharges bactériennes des animaux porteurs, augmente les chances d'infection des animaux sains.

L'analyse des résultats en rapport avec l'état clinique des bovins montre qu'il existe un lien étroit entre l'avortement et le résultat sérologique. Les autres symptômes essentiels de la brucellose (hygromas, arthrites) n'ont pas été rencontrés au cours de notre étude. Par ailleurs, au Tchad, Domenech (1987) a observé dans certains foyers de brucellose un taux d'avortement annuel de 20%.

Akakpo (1987) citant les travaux de Konté au Sénégal, estime le taux d'avortement brucellique chez les bovins à 1,7% (736 cas sur 43274 gestations). En Côte d'Ivoire, Camus estima que dans 40% des troupeaux, les avortements concernent environ 2% des femelles en gestation.

La technique de l'Epreuve à l'Antigène Tamponné (Test au Rose Bengale) a été retenue comme méthode de diagnostic en raison de sa spécificité et sa capacité à mettre en évidence à la fois les'IgM (preuve d'une infection récente) et les IgG (preuve d'une infection ancienne).

Simple et rapide, ce test est donc surtout utilisé en dépistage (Godfroid., 2003). .

Les résultats de notre étude indiquent qu'il existe un lien statistiquement significatif entre l'âge, le sexe, le site, le signe clinique et la séroprévalence de la brucellose. Le principal facteur qui favorise la persistance de la brucellose bovine est l'absence de conditions indispensables à l'application stricte des mesures d'ordre sanitaire.

Conclusion

Cette étude a eu pour objectif d'apporter de nouvelles connaissances sur

l'épidémiologie de la brucellose bovine au Mali. Elle a permis de confirmer que la brucellose demeure une contrainte majeure dans les élevages bovins laitiers de la zone péri-urbaine du District de Bamako..

L'étude a en outre montré que les taux d'infections étaient étroitement liés au site, au sexe, à l'âge et à l'état clinique des animaux testés.

Pour mieux contrôler la brucellose animale et humaine, un programme national de lutte contre la brucellose doit être mis en place en vue de créer les conditions nécessaires pour l'application stricte des textes législatifs et réglementaires en matière de prévention et de lutte contre la brucellose bovine (dépistage sérologique des animaux suspects, réduction de la dissémination de la maladie par le respect strict des mesures d'hygiène individuelle et collective, application des bonnes pratiques d'élevage par l'instauration de la mise bas en boxe isolée, incinération des placentas et des avortons, désinfection de tous les objets et matériels souillés et surveillance des chiens, pasteurisation systématique du lait provenant des animaux infectés avant la consommation, prévention de la contamination des exploitations indemnes de la maladie par l'interdiction d'introduire un animal infecté ou provenant d'une exploitation infectée par la brucellose)

Remerciements

Les auteurs adressent leurs sincères remerciements aux services techniques d'encadrement et aux éleveurs du District de Bamako pour leur entière disponibilité qui a permis de conduire la présente étude.

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CARACTERISATION ET EPIDEMIOLOGIE DES SOUCHES DE MYCOBACTERIES ISOLEES DES CARCASSES DE BOVINS A L'ABATTOIR FRIGORIFIQUE DE BAMAKO

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Resume

A l'abattoir frigorifique de Bamako au Mali, la tuberculose bovine demeure une des causes de saisies les plus fréquentes, en 2010 elle a représenté 2,50 % des saisies totales (Rapport 2010 Abattoir frigorifique de Bamako).

Déterminer la fréquence de Mycobacterium bovis à l'abattoir frigorifique de Bamako.

Méthodologie : Cette étude pilote de type prospectif, réalisée de Mars à Octobre 2011 a porté sur 55 des 100 carcasses bovines saisies à l'abattoir frigorifique de Bamako pendant la période de l'étude. Les analyses de laboratoire ont été effectuées au Laboratoire Central Vétérinaire (LCV) et au Laboratoire National de Référence (LNR) de la tuberculose du Mali.

Durant la période d'étude, 100 carcasses de bovins ont été saisies suite à la présence de lésions évocatrices de tuberculose sur 3713 bovins abattus. L'examen bactériologique de 55 échantillons a permis de confirmer le diagnostic de présomption de tuberculose bovine. L'identification a mis en évidence 14 cas d'infection par M. bovis, 2 cas de co-infection par une souche de M.bovis et une souche de mycobactérie atypique et 17 infections par une mycobactérie atypique. L'étude a en outre permis d'établir sur les 16 échantillons positifs en culture de Mycobacterium bovis, la distribution de la maladie en fonction de la race, du sexe, de l'âge des bovins et de faire des recommandations en vue de mieux contrôler la tuberculose bovine au Mali.

La tuberculose bovine constitue une contrainte majeure à l'Abattoir Frigorifique de Bamako. Il est indispensable de mettre en place un programme national de lutte contre cette infection chez les animaux d'abattage.

Mots-clés : Mycobacterium bovis- Bovins-Abattoir-caractérisation-Mali.

Abstract

In the refrigerated slaughterhouse in Bamako, Mali, bovine tuberculosis remains the most common cause of seizures (bovine tuberculosis in 2010 represents 2.50% of total seizures).

This work is a pilot study looking kind conducted from March to October 2011 and to determine the frequency of M. bovis strains isolated from cattle carcasses in refrigerated slaughterhouse Bamako goal.

The microbiological examination of 55 samples from cattle carcasses with tuberculosis lesions confirmed the diagnosis of bovine tuberculosis.

Isolation and characterization of isolates has highlighted 16 Mycobacterium bovis.

The study also helped to establish the 16 samples positive culture of Mycobacterium bovis distribution of M. bovis based on race, sex, age of cattle and make recommendations to better control bovine tuberculosis in Mali.

Keywords: Mycobacterium bovis-Cattle-Slaughter-characterization-Mali

Introduction

La tuberculose bovine est une maladie infectieuse chronique due à une bactérie du genre Mycobacterium (de l'Ordre des Actinomicetales, et de la Famille des Mycobacteriaceae) appelée Mycobacterium bovis. La bactérie est étroitement apparentée aux mycobactéries responsables de la tuberculose humaine et de la tuberculose aviaire. Cette maladie affecte pratiquement tous les mammifères, et se manifeste par l'apparition de la toux, de l'amaigrissement et une détérioration de l'état général, entraînant la mort. C'est une zoonose majeure parmi les maladies animales transmissibles reconnues par l'Organisation Mondiale de la Santé Animale (O.I.E) et par l'Organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO, 1994).

La tuberculose animale a des conséquences sérieuses sur le commerce des animaux et des produits d'origine animale. A la différence des humains, il n'existe pas de traitement économiquement efficace chez les animaux. Cependant, des programmes basés sur le dépistage et l'abattage des animaux infectés ainsi que la pasteurisation du lait ont considérablement réduit l'incidence de la tuberculose chez le bétail et chez l'homme dans les pays développés. Par contre, la tuberculose reste largement répandue dans les pays en développement. Sa nature insidieuse (longue période d'incubation, évolution chronique) associée aux conditions socio-économiques particulières dans ces pays font qu'elle demeure négligée des programmes de contrôle. On estime que près de 50 millions de bovins seraient infectés dans le monde (Chantal, 2001).

Cette étude pilote a été initiée pour apporter des informations nécessaires, actualisées et utiles sur la tuberculose bovine, afin d'élaborer de nouvelles stratégies de diagnostic pour lutter efficacement contre cette maladie.

Materiel et Methodes

Echantillonnage

Il s'agit d'une étude prospective réalisée pendant la période allant de Mars à Octobre 2011, à l'abattoir frigorifique de Bamako où des prélèvements ont été effectués.

Les travaux d'isolement et d'identification des germes ont été réalisés à l'Institut National de Recherche en Santé Publique de Bamako (INRSP).

L'échantillonnage s'est fait de façon exhaustive. Un échantillon représentatif a été prélevé parmi les carcasses portant des lésions suspectes de tuberculose à l'abattoir. Ces lésions suspectes sont caractérisées par la formation de granulomes nodulaires ou tubercules dans n'importe quel tissu du corps, mais plus fréquemment dans les nœuds lymphatiques de la tête et du thorax, des poumons, des intestins, du foie, de la rate, de la plèvre et du péritoine etc.

Dans chaque cas de collecte des prélèvements, une fiche de renseignement était dûment remplie et comportait les informations relatives à la date de prélèvements, au numéro d'identification, à la provenance des animaux (le marché d'approvisionnement), à la race, au sexe, à l'âge et à la nature des prélèvements.

Préparation des échantillons

Collecte à l'abattoir frigorifique de Bamako

Avec une pince, la partie de l'organe à prélever a été fixée et à l'aide d'un bistouri ou de ciseaux stériles, environ 200g ont été découpés et placés dans des sachets individuels stériles identifiés par organe et par animal. Les échantillons aussitôt après la collecte ont été conservés sous glace jusqu'à leur acheminement au laboratoire central vétérinaire.

Au Laboratoire Central Vétérinaire de Bamako

Pour la préparation des échantillons, 90 g d'organe par prélèvement ont été placés dans une boîte de Pétri contenant 10 ml d'eau distillée stérile. Le morceau d'organe a ensuite été découpé à l'aide de ciseaux stériles puis homogénéisés. Le contenu de la boîte de Pétri a ensuite été transvasé dans un flacon stérile de 50 ml. Le numéro d'identification de l'animal et le nom de l'organe prélevé ont été portés sur le flacon avant d'être envoyé au Laboratoire National de Référence à l'INRSP pour la culture et les tests d'identification.

Au Laboratoire National de Référence des mycobactéries à l'INRSP

Les fragments de tissus ont été broyés à l'aide de billes de verre. Le broyat obtenu a ensuite été décontaminé par la méthode de Petroff avec la soude à 4 %, neutralisé par une solution tampon phosphate à 0,067 mol/l, puis concentré par centrifugation. A partir du culot de centrifugation, la bactérioscopie a été faite par la technique de coloration du Zielh Neelsen à chaud. L'inoculation du culot a été faite sur le milieu liquide MGIT Bactec 960 et sur les milieux solides de Loweinstein Jensen avec et sans glycérol.

Les souches isolées ont été identifiées d'abord à partir des aspects morphologiques des colonies sur milieu solide et des bacilles acido-alcolo-résistants (BAARS) à la culture, à partir du temps de croissance des cultures. Les identifications antigénique et biochimique respectivement par le test immunochromatographique rapide de détection de l'Ag MPT64 spécifique des mycobactéries du groupe tuberculosis (SD Bioline MPT64) et le test colorimétrique de réduction du nitrate (test de Virtanen) ont été ensuite réalisées.

Les données ont été saisies, traitées et analysées sur Epi-Infos 3.5.1. Les taux de signification (P) ont été calculés.

Afin d'établir les particularités épidémiologiques de M .bovis, nous avons procédé à l'étude de la distribution des cas positifs selon la race bovine, le sexe et l'âge des animaux.

Resultats

Les cultures réalisées sur 55 prélèvements, ont donné 33 positives (60%). La bactérioscopie après concentration, était positive à un taux de 21,81%. Parmi les souches isolées, 16 ont été identifiées comme appartenant au complexe tuberculosis par le test rapide immunochromatographique sur milieu liquide. Les colonies de 14 des souches identifiées sont apparues sur milieu solide entre 4 et 13 semaines. Elles avaient l'aspect des colonies de M. bovis, petites, brillantes. Le test de réduction du nitrate était négatif pour ces souches ce qui nous a permis de les classer comme l'espèce bovis. Deux cultures contenaient un mélange d'une souche atypique et d'une souche de M. bovis. Ces deux souches de M. bovis ont été identifiées par l'aspect de leur BAARS en cordes après coloration au Zielh Neelsen (ZN), par le test rapide et par le temps de croissance sur milieu liquide. Les colonies pigmentées, apparues sur milieu solide étaient celles de mycobactéries non tuberculeuses, à croissance rapide. Les mycobactéries non tuberculeuses isolées sont toutes apparues entre une et trois semaines, elles étaient pigmentées chez 12 des 19 souches atypiques isolées. L'identification des 33 cultures positives, a donc mis en évidence 14 infections par M. bovis, 2 co-infections par M.bovis et une mycobactérie atypique (MNT) et 17 infections par une mycobactérie atypique. Le ganglion rétro pharyngé était l'organe le plus atteint par la maladie (Tableau 1).

L'étude de la distribution de M. bovis en fonction de la race a donné une fréquence élevée à 81,25% d'infection à M. bovis chez les animaux appartenant à la race « Zébu Peulh » (13/ 16). Par contre, il a été enregistré beaucoup moins d'infection à M. bovis chez la race N'dama avec une fréquence de 6,25 % (1/16).

Même si l'analyse statistique n'a pas permis d'établir un lien étroit entre la prévalence de M. bovis et le sexe des animaux ($p=0,14 > 0,05$ seuil de signification). Il ressort que sur 16 animaux positifs à M. bovis, 10 étaient des mâles soit 62,50% et six des femelles soit 37,50 %. (Tableau 3).

La répartition des souches de M. bovis isolées en fonction de l'âge des animaux, a donné trois positifs dans le groupe d'âges de 1-3 ans, un dans le groupes d'âges de 3-5 ans, 11 dans le groupe d'âges de 5-8 ans et un dans le groupe d'âges de 8 ans et plus (Tableau 4). Cependant, il est difficile de tirer une conclusion sur la prévalence chez les animaux de ces groupes d'âge en raison de la faible taille de l'échantillon.

Discussion

Au cours de cette étude, 100 échantillons présentant des lésions suspectes de tuberculose ont été collectés à l'inspection

Tableau I : Fréquence d'isolement des mycobactéries dans les différents prélèvements testés

Nature organes	M. bovis	MNT	Fréquence d'isolement
Ganglion préscapillaire	2	4	2
Ganglion retropharyngé	6	4	6
Foie	0	7	0
Ganglion pulmonaire	1	1	1
Ganglion mammaire	1	0	1
Ganglion pré crural	2	1	2
Poumon	4	1	4
Pancréas	0	1	0
Total	**16**	**19**	**16**

Légendes :
M. bovis : Mycobacterium bovis ;
MNT : Mycobactéries Non Typiques

Tableau 2 : Distribution de M. bovis en fonction de la race des bovins

Race	Négatif	M. bovis	MNT	Total
N'dama	1	1	2	4
Zébu Maure	7	2	1	10
Zébu Peulh	14	13	14	41
Total	**22**	**16**	**17**	**55**

Tableau 3: Distribution de M. bovis en fonction du sexe

Sexe	Négatif		M. bovis		MNT		Total
	Nombre	%	Nombre	%	Nombre	%	
Femelle	4	18,18	6	37,50	5	29,41	**15**
Mâle	18	81,82	10	62,50	12	70,58	**40**
Total	**22**	**100,00**	**16**	**100,00**	**17**	**100,00**	**55**

Tableau 4: Distribution en fonction de l'âge des bovins des échantillons positifs à M. bovis

Groupes d'âge	Négatif		M. bovis		MNT		Total
	Nombre	%	Nombre	%	Nombre	%	
1-3 ans	1	4,55	3	18,75	1	5,88	**5**
3-5 ans	1	4,55	1	6,25	0	0,00	**2**
5-8 ans	19	86,36	11	68,75	13	64,7	**43**
8 ans et plus	1	4,55	1	6,25	3	17,64	**5**
Total	**22**	**100,00**	**16**	**100,00**	**17**	**100,00**	**55**

de 3713 animaux abattus à l'Abattoir Frigorifique de Bamako avec une prévalence de lésions suspectes de 2,69%.

Cette prévalence relativement faible était due d'une part à la période de l'étude et d'autre part au fait que pratiquement tous les animaux abattus étaient des animaux d'embouche. Malgré cette faible prévalence, la tuberculose bovine reste un problème de santé publique. Par exemple pour une carcasse de 200kg si une personne consomme 300g de viande, 666 personnes consommeront cette carcasse ce qui est significatif pour une maladie comme la tuberculose bovine.

La présente étude a permis d'établir que M. bovis circule parmi les bovins abattus à l'abattoir frigorifique de Bamako avec une prévalence de 1,48 %.

La microscopie après concentration a donné un taux de 21,81%. Ce faible taux s'explique par le fait que cette technique est peu sensible, il faut un minimum de 104 bacilles/ml de suspension pour obtenir un frottis positif en BAAR (Digimbaye et al., 2006).

Par contre la culture sur les différents milieux, a donné globalement un taux de positivité de 60%. Parmi les trois milieux de culture utilisés, le milieu de LJ sans glycérol s'est avéré le plus sensible. Le milieu de LJ contenant le glycérol est plutôt favorable à la croissance de l'espèce tuberculosis alors qu'aucune souche de M. tuberculosis n'a été isolée dans l'étude. Le retrait du glycérol connu comme favorable à la croissance de M. bovis nous a permis de récupérer sept échantillons échappés à la culture sur milieu de LJ avec glycérol et sur milieu liquide de MGIT. La sensibilité du milieu de LJ sans glycérol s'est avérée plus élevée dans notre étude par rapport au milieu liquide. La plupart des échantillons étant pauci bacillaires, cette différence de sensibilité pourrait s'expliquer par le temps du protocole d'incubation plus long pour le milieu solide par rapport au milieu liquide.

Ce résultat est presque identique à celui obtenu à l'abattoir d'Abidjan Port- Bouët (Côte d'Ivoire) avec un taux de 22,11% à la bactérioscopie et 54, 80 % pour la culture et l'identification par la PCR multiplex (Cissé et al., 2008).

Cette étude a donné une prévalence de 1,48 % pour M. bovis qui est inferieure à celle obtenue chez les bovins à l'abattoir de Ndjamena au Tchad qui était de 7% (Diguimbaye et al ., 2006). Une étude menée en 2009 à l'abattoir de Sarh au sud du Tchad a aussi donnée une prévalence réelle estimée de 8% par la méthode bayésienne (Müller et al., 2009). Une étude faite à l'abattoir d'Addis Abeba en Ethiopie a trouvé une prévalence de 2% (Asseged et al., 2002) et une autre à l'abattoir municipal Hossana a donné une prévalence de 5% (Teklul et al., 2004). La faible prévalence obtenue dans notre étude pourrait s'expliquer aussi par le fait que la majorité des animaux abattus pendant cette période étaient des animaux d'embouche qui bénéficient d'un bon suivi et aussi par la faible taille de l'échantillon.

Dans cette étude la tranche d'âge qui a enregistré le plus grand nombre de cas de M. bovis a été la tranche d'âge comprise entre cinq et huit ans soit 68,75%. Une étude faite à Ouagadougou a montré une augmentation de la prévalence en fonction de l'âge soit 17% à deux ans et 33,7% à 6 ans (Traoré et al., 2004). Ce résultat montre la fréquence plus élevée de la maladie chez les animaux âgés que chez les plus jeunes. La race la plus sensible a été le Zébu Peulh chez laquelle le taux de prévalence a été de 81,25%. Une étude menée au Tchad a trouvé que la race Mbororo est plus sensible à la tuberculose (Ngandolo et al., 2009).

Conclusion

La tuberculose bovine constitue une contrainte majeure à l'Abattoir Frigorifique de Bamako. Mycobacterium bovis est la principale espèce de mycobactérie isolée en culture et son taux de prévalence varie en fonction de la race, du sexe et de l'âge des animaux.

En vue d'un meilleur contrôle de la tuberculose bovine à l'abattoir frigorifique de Bamako, il est indispensable de mettre en place un programme national de lutte contre cette maladie dans les élevages de provenance des animaux.

Remerciements

Nous adressons nos sincères remerciements à la Direction des Services Vétérinaires du District de Bamako, à l'ensemble des bouchers qui abattent leurs animaux à l'Abattoir Frigorifique de Bamako, à l'administration et à l'équipe de l'inspection de l'Abattoir Frigorifique de Bamako pour la franche collaboration. Les travaux de recherche ont été effectués au Laboratoire Central Vétérinaire et à l'Institut National de Recherche en Santé Publique de Bamako auxquels nous exprimons notre profonde gratitude.

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MICROBIOLOGICAL AND SEROLOGICAL STUDIES OF SOME POULTRY PATHOGENS IN WILD BIRDS IN SUDAN

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Abstract

Microbiological and Serological surveillance of 24 different species of wild water birds living around water sewage plants and fresh wetland water area in Khartoum state (Sudan) were carried out in the period from September 2011 to March 2012 during ringing operation. The presence of selected avian diseases including Newcastle disease (ND), avian influenza (AI), Infectious Bursal Disease (IBD) and Avian encephalomyelitis (AE) in addition to some selected bacteria were studied.

A total of 369 different samples (42 sera, 76 tracheal swabs, 77 cloaecal swabs and 174 organs) were examined. Newcastle disease virus was isolated from 3 bird species in water sewage plants while Newcastle disease virus antibodies were detected in the sera of 4 bird species in the same area. E coli, Staph.aureus, enterococcus spp and bacillus cereus were isolated from different species of birds from wetland area . In conclusion the results obtained suggest that wild birds might play an important role in the epidemiology of the circulating ND outbreaks in poultry flocks in the country . No evidence that wild birds in the study areas are infected with AI, IBD or AE.

We concluded that wild birds harbour some pathogenic organisms which are capable to produce different disease conditions in both human and animals.

Keywords: poultry pathogens , Water bird , wetland, sewage station ; Sudan.

ETUDES MICROBIOLOGIQUES ET SÉROLOGIQUES DE CERTAINS AGENTS PATHOGÈNES DES VOLAILLES CHEZ LES OISEAUX SAUVAGES AU SOUDAN

Résumé

La surveillance microbiologique et sérologique de 24 espèces différentes d'oiseaux aquatiques sauvages vivant autour des stations d'épuration d'eau et de la région d'eau fraîche des zones humides de l'Etat de Khartoum (Soudan) ont été effectuées dans la période de septembre 2011 à mars 2012 au cours de l'opération de baguage. La présence de certaines maladies aviaires, dont la maladie de Newcastle (MN), l'influenza aviaire (IA), la bursite infectieuse (BI) et l'encéphalomyélite aviaire (EA), en plus de quelques bactéries sélectionnées, a été étudiée.

Au total, 369 échantillons différents (42 sérums, 76 écouvillons trachéaux, 77 écouvillons cloacaux et 174 organes) ont été examinés. Le virus de la maladie de Newcastle a été isolé chez 3 espèces d'oiseaux dans les stations d'épuration d'eau, tandis que des anticorps du virus de la maladie de Newcastle ont été détectés dans les sérums de 4 espèces d'oiseaux dans la même zone. Les bactéries E. coli, Staph.aureus, Enterococcus spp et Bacillus cereus ont été isolés chez différentes espèces d'oiseaux dans la zone humide. En conclusion, les résultats obtenus laissent entendre que les oiseaux sauvages peuvent jouer un rôle important dans l'épidémiologie des foyers de MNC circulant dans les troupeaux de volailles dans le pays. Il n'y a pas de preuve que les oiseaux sauvages dans les zones d'étude sont infectés par l'IA, la BI ou l'EA.

Nous avons conclu que les oiseaux sauvages sont porteurs de certains organismes pathogènes capables d'engendrer différentes maladies à la fois chez l'homme et les animaux.

Mots-clés : Agents pathogènes des volailles ; Oiseau aquatique ; Zone humide ; Station d'épuration ; Soudan

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Introduction

Sudan host different species of resident and migratory wild birds . Wild birds are the natural reservoir of several viruses and bacteria that can infect a diversity of wild and domestic animals and human (Reed et al.,2003; Heeney 2006). The possible dissemination of numerous pathogenic microorganisms by migratory birds is well documented (Bahl et al.,1975; Hubalek .,2004; Munster etal 2005). Beside wet land areas, sewage treatment plants have been recognized as significant water bird habitat (Duffield .,1986; Hamilton, A.J. & Taylor,2005). Different viral and bacterial pathogens were circulating between both domestic and wild bird, Avian influenza virus has been isolated from 12 orders and 88 species of free –living birds mostly from Anseriformes and charadriiformes(Gilchrist, 2005). All Heamagglutinin (HA) and Neuraminidase (NA) subtypes can be found as low pathogenic avian influenza virus strains in aquatic wild birds as described by Fouchier et al (2005) but only strains carrying the H5 or H7 gene can mutate into highly pathogenic avian influenza (HPAI) strains that cause high mortality rates in domestic poultry (Alexander .,2000) .Currently migratory birds were considered to be responsible for long distance dispersal of the virus (Boyce et al., 2009; Kilpatrick et al 2006) .

ND is the most economically important disease in poultry especially in village chicken in developing countries. The virus can infect many species of domestic and wild birds (Philemon and Wambura.,2010). Kaleta and Baldauf (1988) stated that susceptibility of free living birds to the disease was varied among the species and the duration of virus shedding varies with the species of birds , being short in galliformes and quite long in psittaciformes, columbiformes and Passeriformes (Gilchrist,2005). There are no reports of isolation of IBD virus from free living species but some serological surveys performed by Ogawa et al (1998) in Japan indicate a probable role of wild bird in the epidemiology of IBD.

Wild Birds faeces contained many genera of zoonotic bacteria including Escherichia coli, Pseudomonas spp., Staphylococcus spp., Streptococcus spp., and Yersinia spp. (Brittingham .,1998). Dissemination of E. coli by migratory birds is of concern regarding drug resistance and public health ,wild birds are considered as an avian reservoir of bacteria that are enteric human pathogens; such as Campylobacter ,Salmonella and toxin - producing strains of Escherichia coli that may be transmitted to humans in the context of ringing and migration as mentioned by Hussein et al (2007) . Multiresistant E. coli strains were isolated from different avian species . therefore wild birds might constitute a potential hazard to human and animal health by transmitting multiresistant strains to waterways and other environmental sources via their faecal deposits (Sebastian et al .,2010).

The objectives of this study were to investigate avian pathogens in wild birds which constitute risk to domestic poultry flocks in the study area and to identify zoonotic bacteria from wild birds.

Materials and Methods

Study Area

Three areas were studied; two water sewage plant in Khartoum state (Waddafeeaa and Soba) and one fresh water wetland area (Umshigera Island). Waddafeaa Station was surrounded by different commercial poultry farms, while Soba Station was located near a highly populated area . Umshigera Island is a Fresh water wetland area it was accommodates large sedentary and seasonal bird populations of different types.

Samples and sampling techniques

Samples were collected during the late rainy season over a period of 7 months. Mist nets were used for capturing birds during the night which erected before sunset near roosting sites .While the team chasing birds towards the invisible nets after sunset, birds were captured , sampled and released. Tracheal and cloacal swabs were placed

in a viral transport media. Blood samples were collected aseptically from each bird and kept at room temperature overnight then the sera were separated . Organs were collected aseptically during post-mortem examination of birds .

A total of 116 wild birds of different 24 species were sampled. Samples include 41 sera samples ,77 Tracheal swabs (TS), 76 Cloacal swabs (CS), and 127 organs collected from 29 birds of different species (Table 1). Birds were of different age, sex and breeds (Table 2). Samples were transported in icebox to the laboratory and kept frozen till use . All birds investigated in this study were apparently healthy.

Serological tests

Heamagglutination Inhibition Test (HI): All sera samples were tested for the presence of avian influenza H5 , H7 and H9 subtypes and Newcastle disease virus antibodies by HI test after pre-treatment of serum as described by Ilaria Capua and Dennis J Alexander (2009). The HI titer for each bird was determined and expressed in log2 and HI titer ≤ 4log2 was considered positive.

Agar Gel Immunodiffusion Test (AGID): Samples were tested for the presence of AE antibodies by AGID test as described by Ikeda (1977) and according to OIE Manual (2008) for monitoring IBD antibodies.

Microbiological study:

Viral isolation:

All tracheal and cloacal swabs were cultured on embryonated chicken eggs according to OIE (2008) for detection of avian influenza and Newcastle disease virus .

Bacterial isolation:

Samples which include liver, trachea & lung and intestine were cultured on Nutrient agar , MacConkey agar and sheep blood agar (Oxoid) . Isolates were characterized by different cultural and biochemical tests according to Barrow and Feltham (1993) .

RESULT

Serology:

Samples collected from water sewage stations showed the following results HI antibodies against Newcastle disease virus were detected in 4 sera samples from 3 bird species collected from Waddafeea Sewage Station (Table 3) . Antibody titer range from 4log2-6 log2. While HI antibodies aganist AI subtypes were not detected in all sera examined (n=9) .On the other hand sera samples from Soba Station were found negative to HI antibobies against both ND and AI.

Table 1: Distribution of samples according to collection area

Type	Source			Total Number
	Sewage station		Fresh water	
	WadDaffea	Soba	Umshugera/ island	
Serum	9	7	25	41
T. swab	14	9	53	76
C. swab	14	10	53	77
Liver	-	-	29	29
Heart	-	-	29	29
Kidney	-	-	29	29
Spleen	-	-	29	29
Trachea & lung	-	-	29	29
Intestine	-	-	29	29

Table 2: Distribution of Birds samples

No	Bird species	T. No	Sex	Age
1	whiskered Tern	15	un	A
2	curlew sandpiper	12	un	A
3	common snipe	6	un&f	A&J
4	wood sandpiper	2	un	A
5	Ruff	19	un ,f&m	A&J
6	black-tailed godwit	1	f	A
7	common sandpiper	7	un	A
8	little stint	10	un	A
9	Northern pintail	1	F	A
10	Pied kingfisher	3	un &f	A
11	Gerater painted snipe	1	m	A
12	Spur–winged plover	18	Un,m	A
13	yellow wagtail	2	un	A
14	Marsh sandpiper	2	un	A
15	common ringed plover	3	un	A
16	Gull Bill Tern	1	un	A
17	Laughing dove	2	F,un	A
18	common Moorhen	2	un	J&A
19	African mourning dove	2	un	A
20	Common green sandpiper	1	un	A
21	Ruddy Turnstone	1	un	A
22	Glossy Ibi	1	un	A
23	crested lark	1	un	
24	red throat Pipit	3	un	A
	Total No. of birds	**116**		

Un :unknown , F : female , m : male , J : juvenile ,A : Adult

Table 3: Seropervalence of ND in birds from Waddafeea water sewage station

Bird species	No tested	No	(%)Positive	HI titer
Common sandpiper	6	2	(33.3%)	$6\log_2$
African Mourning Dove	2	1	(50%)	$4\log_2$
Laught Dove	1	1	(100%)	$5\log_2$
Total	**9**	**4**	**(44.4%)**	

Table 4: ND Virus isolation from bird species in Waddafeaa and Soba water sewage plants

Area	No positive / T. No	% +ve	Bird species
Waddafeaa	2/14	(14.3%)	Curlew sandpiper African Morning Dove
Soba	1/ 10	(10%)	Common Moorhen

ND: Newcastle disease
Cs: Cloacal swab

Table 5: Bacterial species isolated from samples of UmShugeira island area

	Species	liver	Trachea &lung	Intestine
1	Whiskered Tern	-ve	*Bacillus cereus*	*Staphyloccus aureus*
2	Little stint	-ve	*Bacillus cereus*	-ve
3	Curlew sandpiper	-ve	-ve	*Staphyloccus aureus*
4	Little stint	-ve	-ve	*Enterococcus spp*
5	Northern pintail	-ve	-ve	*Staphyloccus aureus*
6	Pied kingfisher	-ve	-ve	*Enterococus spp*
7	Greater painted snipe	-ve	-ve	*Staphyloccus aureus*
8	Spur –winged plover	-ve	-ve	*Staphyloccus aureus*
9	Curlew sandpiper	-ve	-ve	*Enterococcus spp*
10	Ruff	E Coli	-ve	*Staphyloccus aureus E coli*

No AGID antibodies aganist IBD and AE virus were detected in the sera samples from sewage stations (n=16).

Virus isolation

Newcastle disease virus was isolated from three cloacal swabs ,two fromWaddafeea and one from Soba Sewage Stations (Table 4).All tracheal swabs collected from sewage stations were found negative to ND virus, Tracheal and cloacal swabs were all found negative to AI virus when subjected to virus isolation.

Umshigera island Sera samples (n=25) were all found negative to AI, ND ,IBD and AE antibodies when examined serologically .While all (106) cloacal swabs were found negative to AI, ND by virus isolation.

Bacteriology :

Different bacterial species were isolated from organs collected from Umshigera island .Bacillus cereus were isolated from lung and trachea of whiskered tern (Chlidonias hybrid) and little stint (Calidris minuta) bird while E Coli was isolated from liver and intestine of Ruff (Philomachus pugnax), on the other hand intestine revealed a higher recovery rate of bacterial isolation. Staphylococcus aureus were isolated from 6 species and enterococcus species from three species Table (5).

Discussion

The current study covered three different areas, two water sewage plants and fresh water wetland area. Samples from each area showed different results. Results obtained from waddafea water sewage plant samples were significant from epidemiological point of view, because the area is surrounded by several poultry farms of different husbandry practice. ND viruses have been isolated from 3 species (12.5%) including curlew sandpiper, African morning Dove and common Moorhen. Isolation of the virus from Dove was of great concern because this species was considered as one of the highly susceptible free living birds group (Kaleta, E.F. and Baldauf .,1988) and the duration of virus shedding in this group is quite long (Gilchrist .,2005). Moreover, the study conducted by Otim et al(2007) indicated that other domestic and wild birds are among the risk factors associated with ND outbreaks in free-ranging village in Uganda . Roy et al (1998) reported that birds other than domestic chicken have been known to be sources of ND virus . Detection of HI antibodies in (44.4%) of sample with higher titer suggests a possible circulation of ND virus in wild birds . Similar result were reported by different scientist (Roy et al 1998; Mousa et al.,1988; Al-jumaily et al ;1989 and Ziedler and Hlinak .,1993) . In Sudan . Different results were obtained by Khalafalla. et al (1990) where attempts to isolate NDV and demonstrate NDV antibodies in 160 captured wild birds was done without any success .

Current results revealed that none

of the birds examined showed AI Viruses or HI antibodies to all notifiable subtype , since the outbreak of highly pathogenic avian influenza (HPAI) (2006) in chicken flocks in the country, annual serological and virological surveillances were carried out for both migratory and resident wild birds and the results showed that only nine of the bird examined were found positive to AI virus type A using rapid test kits (annual reports) . IBD antibodies were not detected in all sera examined this result disagree with the finding of Ogawa et al (1998) who stated that Antibodies to IBDV were detected from both sedentary and migratory species In Japan , the authors suggesting that free-living wild birds have an important role in the natural history of IBDV .

In conclusion the present results showed that wild birds might play an important role in the epidemiology of the circulating ND outbreaks in poultry flocks in the country. No evidence that water bird in the study areas are infected with AI, IBD or AE.

Umshigera Island is surrounded by residential areas which make the site highly disturbed with people different activities . Isolation of E coli from wild birds from this area might have a public health significant. Wild birds are considered as an avian reservoir of toxin - producing strains of Escherichia coli that may be transmitted to humans in the context of ringing and migration as noted by Hussein et al (2007). Migratory birds might be involved in dispersal of E. coli (Middleton, J.H. & Ambrose .,2005) . Livermore, et al (2001) stated that wild birds are colonized with various strains of E. coli, including strains such as E. coli O157 that are pathogenic for humans .In addition faecal strains of E. Coli resistant to antibiotics have been found at various prevalences in wild bird populations. Further work was needed on molecular epidemiology of E coil isolates related with those migratory birds. isolation of enterococuss species in this study is similar to pervious studies especially Enterococcus species with a high level of glycopeptides resistance which consider as a major zoonotic infection of wild birds in north America (Sellin .,2005). we concluded that wild birds harbour many pathogenic organisms which are capable to produce disease conditions in human beings.

In conclusion continuous surveillance is very important because wild birds share habitats with both human and domestic chicken.

Acknowledgements

The authors would like to express their thanks to the general Director Animal Resources Research Corporation for his permission to publish .our gratitude is extended to the Director Veterinary Research Institute and Director of wildlife Research centre .thanks are due to Technicians Mohammed Abdel Rahman Medina Bakhiet and Salah Abdalmagid for their technical assistance.

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THE IMMUNE RESPONSE OF MATERNALLY IMMUNE CHICKSTOVACCINATION WITH NEWCASTLE DISEASE VIRUS

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Summary

This study was conducted to determine the persistence of maternally derived antibodies (MDA) to Newcastle disease virus (NDV) in newly hatched chicks and the effect of MDA on vaccination with a very potent vaccine (Avinew® (VG/GA)). Individual variations of chicks in acquiring and maintaining MDA and in their response to vaccination were also investigated.

In order to study the persistence of MDA, 50 one-day-old Hisex breed chicks were divided into five groups based on their age at serum collection (one-day-old, seven days-old, fourteen-days-old, twenty-days-old and twenty eight-days-old), respectively. To assess interference of MDA with vaccination, 30 chicks were divided into three groups based on time of vaccination (days 1, 14, and 28).

Haemagglutination inhibition test was used to measure antibody titer in sera.

Chicks at 1, 7, 14, 21, and 28 days of age showed MDA titers of 9, 8.7, 5.8, 4.2, and 1.6 (log2) respectively. Vaccination at day 1, 14, and 28 of age elicited titers of 2.6, 3.98, and 4.75 respectively. It was noticed that among each group there was variations in titer.

The minimum titer that interfered with vaccination was 4.2 (log2). This titer was obtained in day 18 of age which was considered the optimum time for vaccination with Avinew ®(VG/GA) strain.

LA RÉPONSE IMMUNITAIRE DES POUSSINS AYANT ACQUIS UNE IMMUNITÉ D'ORIGINE MATERNELLE À LA VACCINATION AVEC LE VIRUS DE LA MALADIE DE NEWCASTLE

Résumé

Cette étude a été menée dans le but de déterminer la persistance des anticorps d'origine maternelle (AOM) contre le virus de la maladie de Newcastle (MNC) chez les poussins fraîchement éclos et l'effet des AOM sur la vaccination avec un vaccin très puissant (Avinew® (VG/GA). Les variations individuelles entre poussins au niveau de l'acquisition et du maintien des AOM et de leur réaction à la vaccination ont également été étudiées.

Dans le souci d'étudier la persistance des AOM, 50 poussins d'un jour de race Hisex ont été répartis en cinq groupes en fonction de leur âge au moment du prélèvement du sérum (respectivement 1 jour, sept jours, quatorze jours, vingt jours et vingt-huit jours). Pour évaluer l'interférence des AOM avec la vaccination, 30 poussins ont été répartis en trois groupes en fonction de l'époque de la vaccination (jours 1, 14 et 28).

Le test d'inhibition de l'hémagglutination a été utilisé pour mesurer le titre des anticorps dans les sérums.

Les poussins âgés de 1, 7, 14, 21, et 28 jours ont montré des titres d'AOM respectivement de 9 ; 8,7 ; 5,8 ; 4,2 ; et 1,6 (log2). La vaccination des poussins âgés de 1 ; 14 ; et 28 jours a provoqué des titres respectivement de 2,6 ; 3,98 ; et 4,75. On a remarqué qu'au sein de chaque groupe il y avait des variations au niveau des titres.

Le titre minimum qui a interféré avec la vaccination était de 4,2 (log2). Ce titre a été obtenu au jour 18 d'âge qui a été considéré comme le meilleur moment pour la vaccination avec la souche Avinew ® (VG/GA).

Introduction

Newcastle disease is a highly contagious viral disease that attacks many species of domestic and wild birds and causes devastating losses to the poultry industries. There are many vaccines which are of considerable value in reducing losses (Mozaffor et al., 2010). However, and despite the extensive use of vaccines, outbreaks of ND continue to occur in both vaccinated and unvaccinated flocks (Sa'idu and Abdu, 2008). A crucial unsolved problem for all vaccines is the interference with the maternally derived antibodies (Dhohyung, 2012).

Maternal antibodies to avian paramyxoviruses type-1 (APMV-1) are transferred from vaccinated dams to their chicks, the amount of transferred antibodies varies between individuals to a degree that some chicks may be at risk to (APMV-1) infection in due time before scheduled vaccination (Thomas et al., 1998).

Variation in efficacy of ND vaccines in eliciting high antibody titers to be transferred through eggs to chicks, interference of MDA with vaccination and production of different breeds of chicken necessitate the determination of acquisition and maintenance of MDA against each NDV vaccine.

Materials and methods

Chicks:

For the purpose of determining maintenance and decline of maternally derived antibodies to Newcastle disease virus in newly hatched chicks, a total of 50 one-day-old Hisex breed chicks from parents vaccinated with AVINEW (VG/GA) strain were obtained from Coral Company (Khartoum, Sudan). Chicks were divided into five groups and Serum was collection as in table (1).

Haemagglutination Inhibition (HI) was used for determining antibody levels in sera of these birds.

In order to determine the effect of maternally derived antibodies on vaccination a total number of 30 one-day-old chicks, were concurrently obtained from the same flock of ND vaccinated parents from Coral Company (Khartoum, Sudan). These chicks were divided into three equal groups and were vaccinated with AVINEW® (VG/GA) vaccine as shown in table (2).

ND vaccine:

The AVINEW (VG/GA strain) live vaccine was developed exclusively by MERIAL*. This vaccine was obtained from Coral Company in a form of freeze-dried vaccine stored at controlled temperature environment, between + 2 °C and + 8 °C, and away from light. This vaccine is stable under laboratory condition for a minimum of 2 hour at 25 °C after reconstitution in phosphate buffer saline (PBS) (Merial, 1998).

Vaccination:

The AVINEW (VG/GA strain) live viral vaccine was dissolved in 30 ml (PBS) according to the manufacture instructions, and was used immediately after reconstitution (with in 2 hour). Approximately 100µl was administered intranasally to each chick individually. No post-vaccinal reaction was observed.

* Merial select, INC., (1998). Avinew USA: Gainesville, GA 30503, U.S. vet. Lic. No. 279

Serum collection:

A Hundred microlitter of blood was collected by heart punture of chicks using 1ml syringe containing 0.3ml of normal saline (NS) to make 1:4 dilution, in order to increase the volume for centrifugation purpose; the diluted blood was centrifuged at (1000 rpm for 5 minute), the serum was separated in another tube and preserved at -20°C (Ismail, 1987).

Determination of the effect of maternal antibodies on vaccination: Haemagglutination (HA) test:

Haemagglutination and Haemagglutination inhibition tests were performed according to (Brian and Hiller, 1996). Haemagglutination test was done to determine the four Haemagglutination units (4HAU) of the virus, (AVINEW (VG/GA strain)). Two folds serial dilution along the rows was carried out by adding 100µl of (PBS) into U-shaped wells of a micro titer plate, and then 100µl of virus suspension were added to the first well and mixed, 100µl from this well were transferred

Table 1: Age at which serum was collected:

Group	Age at which serum was collected	Number of samples
A	One day-old	10
B	Seven day-old	10
C	Fourteen day-old	10
D	Twenty one day-old	10
E	Twenty eight day-old	10

Table 2: Schedule of vaccination and serum collection:

Group	Number of samples	Age of vaccination	Age at serum collection
A	10	One day-old	Twenty two day-old
B	10	Fourteen day-old	Thirty five day-old
C	10	Twenty eight day-old	Forty nine day-old

to the second well and the mixing process was repeated, 100µl of (0.5% v/v) washed chicken RBCs were added to all wells and incubated at room temperature for 1 hour. The reciprocal of the highest dilution that produced positive HA was considered as the virus titer (one HAU).

Haemagglutination inhibition (HI) test:

The haemagglutination inhibition test was done to determine the titer of antibodies .100µl of (PBS) were added to each of the 96 U-shaped wells of a micro titer plate; then 100µl of chicks' serum were added to the first well and 100µl from this well were transferred to the second well and two-fold serial dilution was carried out along the row, this serial dilution process was done to each serum sample, then 100µl of 4HAU of the virus preparation were added to each well. The micro titer plate was incubated at room temperature for 1 hour, then 100µl of 0.5% washed chickens' RBCs were added to each well and incubated for 1 hour at room temperature. The result was observed and recorded. The mean titers of sera collected at each date were figured out.

Determination of individual variations:

Individual variations in the immune response among chicks of the same group were assessed using the concept of flock profile adopted by (**Biogal-Galed Labs, 2004. Catalog No: 50PTV203/50PTV230); the results of individual birds were plotted as a bar graph.

**www.biogal.co.il

Results

Maintenance and decline of maternally derived antibodies in chicks of different age:

Maternally derived antibodies were measured weekly starting from day 1 to day 28 and the titers are presented in Table (3) and figure (1).

Individual variations of maternally derived antibodies in chicks:

One day-old chicks showed four different titers; eight, nine, eleven and twelve (log2) given by five, three, one and one chicks respectively figure (2).

Figure (3) summarizes the individual variations of seven days-old chicks that showed four different titers; seven, eight, nine and twelve (log2) given by two, two, five and one chicks respectively with an average of (8.7).

With an average of 5.6, the flock profile of the fourteen days-old chicks showed five different titers; four, five, six, seven and eight (log2) given by two, four, one, two and one chicks respectively figure (4).

Figure (5) summarizes individual variation of chicks at day twenty one when they showed three, four, five and six (log_2) given by two, five, two and one chicks respectively.

The MDA flock profile of twenty eight days-old chicks is shown in figure (6).

The effect of maternal antibodies on vaccination:

The mean of pre-vaccination and post-vaccination titers of chicks at different times are shown in table (4) and Figure (7).

Table 3: Mean titer of MDA of chicks at different ages:

Group	Group Age of chicks	Mean titer (log2)
A	One day-old	9
B	Seven days-old	8.7
C	Fourteen days-old	5.6
D	Twenty one days-old	4.2
E	Twenty eight days-old	1.5

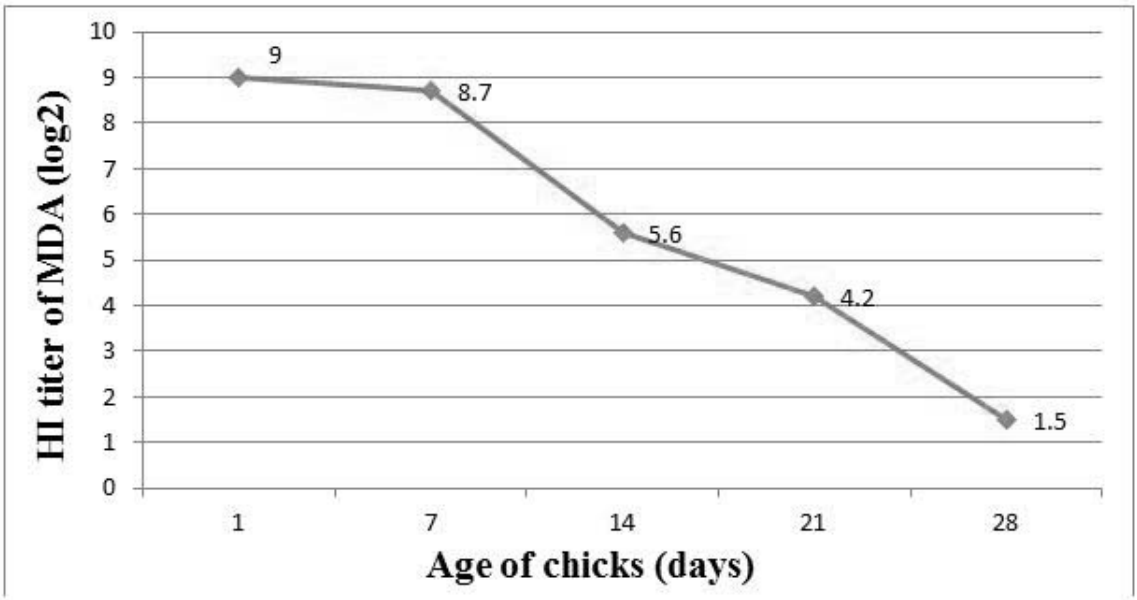


Figure 1: Mean titer of MDA of chicks at different ages

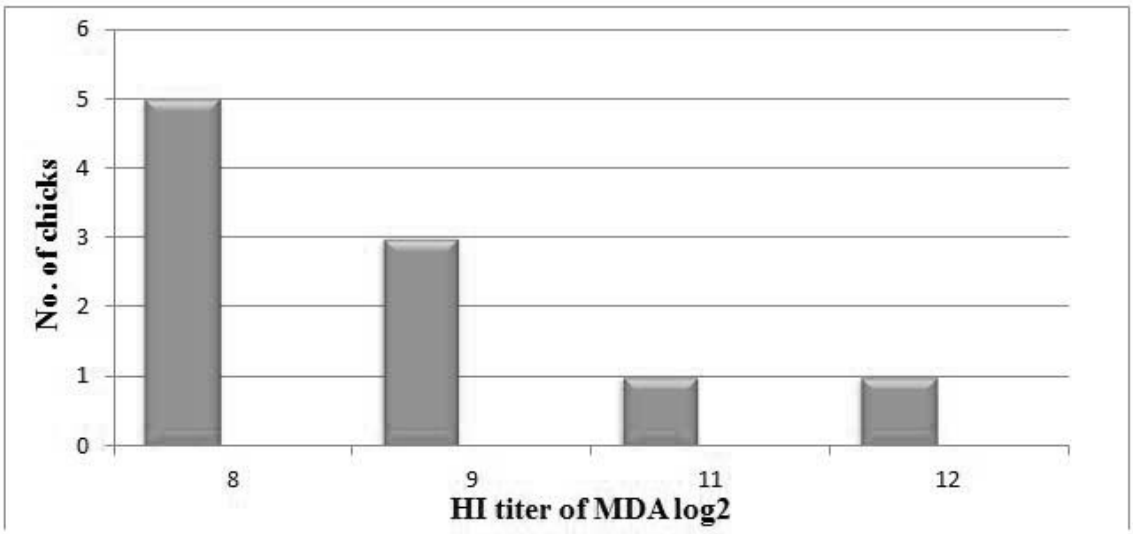


Figure 2: MDA profile of day-old chicks.

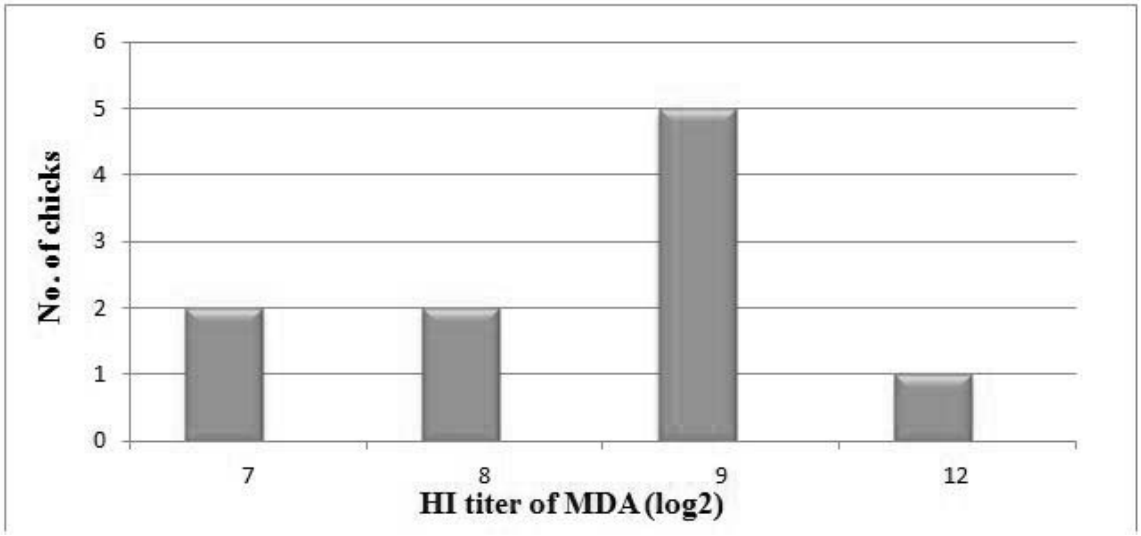


Figure 3: MDA profile of seven days-old chicks.

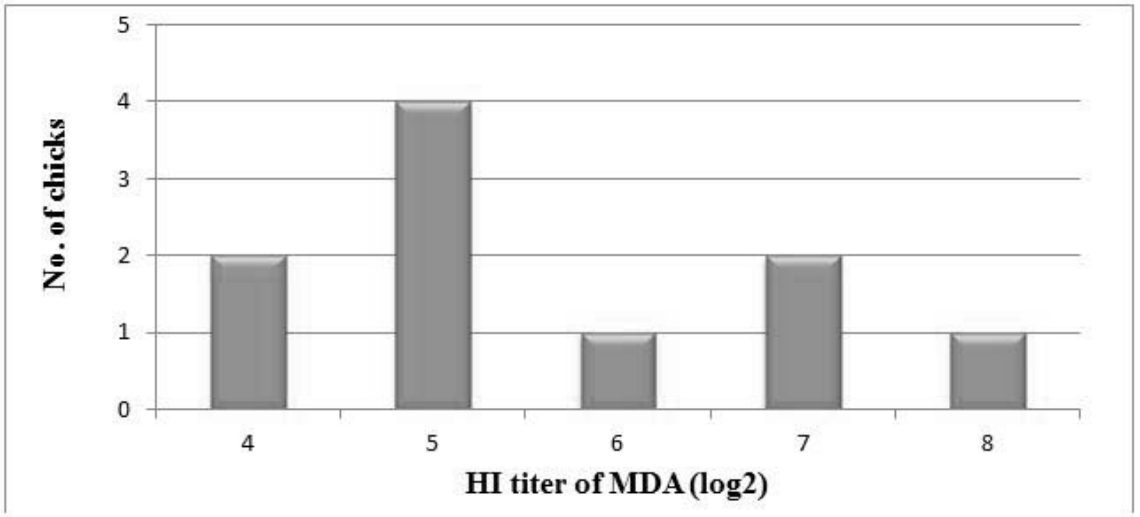


Figure 4: MDA profile of fourteen days-old chicks.

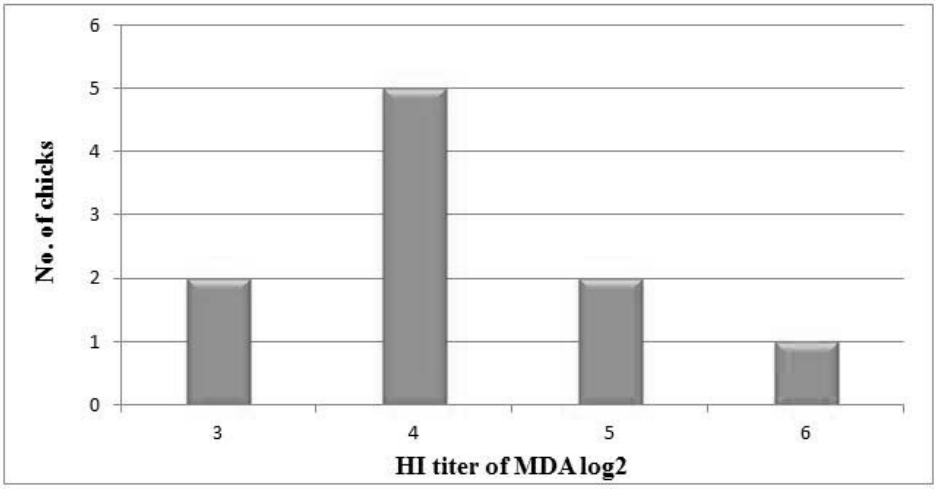


Figure 5: MDA profile of twenty one days-old chicks.

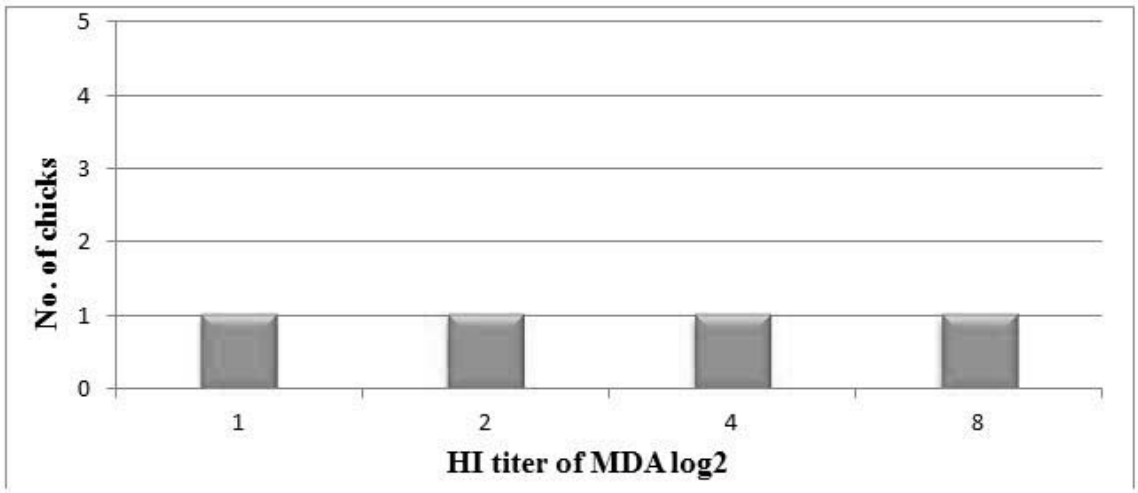


Figure 6: MDA profile of twenty eight days-old chicks.

*Age in graph was the age at which vaccination process was done; the response to vaccination was measured 21 days later.

When the MDA decline curve was overlapped with the vaccination response curve, the point at which they crossed (4.2 log2, day 18) was considered the minimum titer that interfered with vaccination. It was also considered the best day of vaccination because after it almost all chicks gave positive response.

Individual variation of maternally immune chicks to vaccination:

Flock profiles of post-vaccinated chicks are shown in figure 8, 9, and 10.

Discussion

Decline of maternally derived antibodies in chicks with age:

The fact that maternally derived antibodies confirm the transfer of MDA from vaccinated parents to offsprings was stated by many researchers (Gharaibeh et al., 2008; Hamal et al., 2006). It was noticed that the initial titer 9 (log2) at day one, was higher than any previously reported levels by:(Banu et al., 2008; Jalil et al., 2009; Shil et al., 2011). A limited decline of HI titers was recognized from the first week when the titer decreased from 9 (log2) in day-old chicks to 8.7 (log2) in seven days-old chicks. This initial small drop is in agreement with that observed by Ismail, (1987), who reported a limited decrease in titer from 3.52 in day-old chicks to 3.39 at one week of age. This limited decline differed from that reported by Banu et al., (2008) when they detected a decline from 7.5 at day one to 6.5 at day seven, and from that observed by Begum et al (2006) when they measured a higher decline from 8 at day one to 6.6 at day seven. These relative differences in MDA levels may be due to variations in experimental conditions such as; type of vaccine and vaccination procedure. MDA decreased rapidly from day seven reaching 5.4 (log2) at day fourteen. This result reaffirmed the finding of (Ismail, 1987; Begum et al., 2006; Jalil et al., 2009; Msoffe et al., 2006; Shil et al., 2011) when they observed that MDA declined rapidly from day seven to day fourteen. Rao et al., (1987) however, could not detect any antibodies in 15-day-old chicks; this difference may be due to variations in the initial titer at day one or due to experimental conditions. Mean titer decreased from 4.2(log2) at day twenty one to 1.5(log2) at day twenty eight. This low level of titer at day 28 is in agreement with many previous findings (Msoffe et al., 2006; Begum et al., 2006; Mahmud et al., 2007; Jalil et al., 2009; Banu et al., 2009 and Shil et al., 2011). Our results agreed with the general trend that MDA decline starts from the first week of age with a limited drop, it continues to decrease towards day 14, and the decline rate increases dramatically from the 14th day to the 21th day, and undetectable level of MDA was reached by the end of the 4th week of age.

Table 4: Pre-vaccination and post-vaccination titers of chicks at different ages.

Groups	Mean of pre-vaccination titer(log_2)	Mean of 21 days post- vaccination titer
A	9.00	2.67
B	8.7	ND.
C	5.6	3.98
D	4.2	ND.
E	1.5	5.4

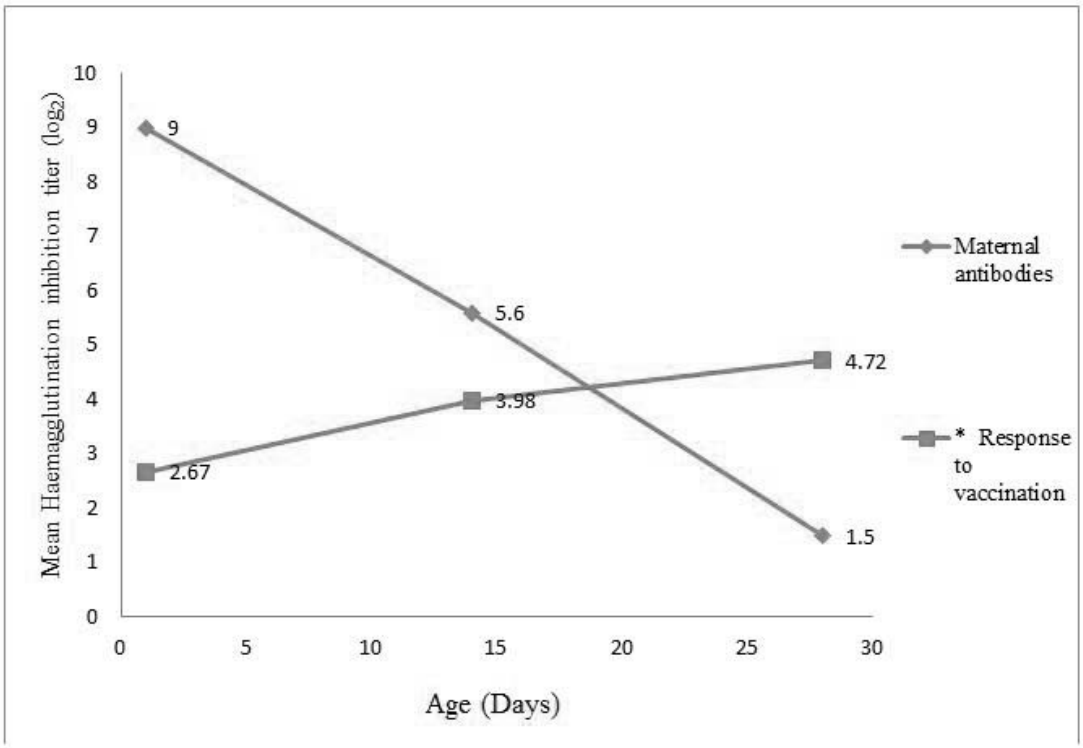


Figure 7: pre-vaccination and post-vaccination titers of chicks at different ages.

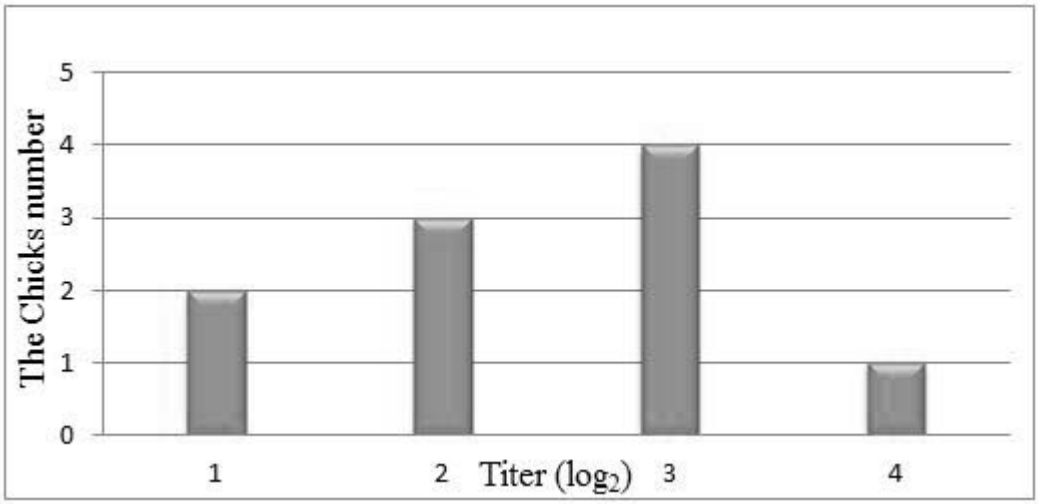


Figure 8: Individual variation in Immune response to vaccination at day

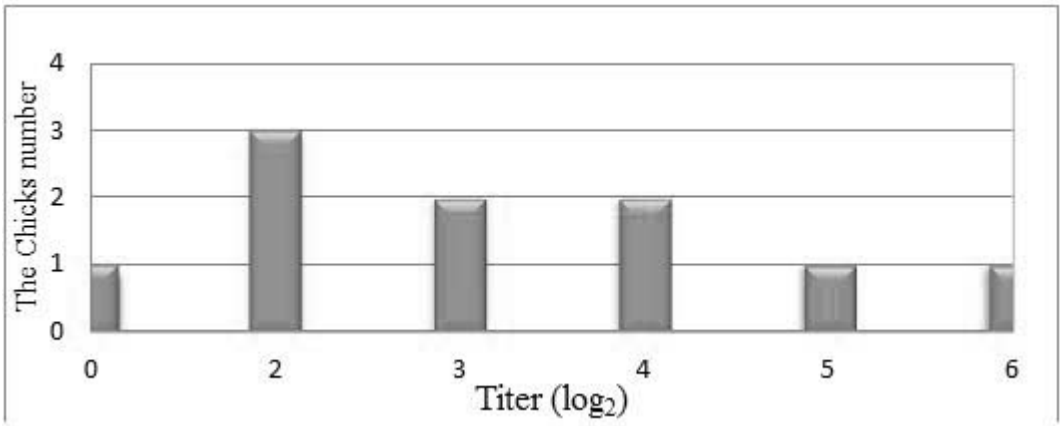


Figure 9: Individual variation in Immune response to vaccination at day 14 of age.

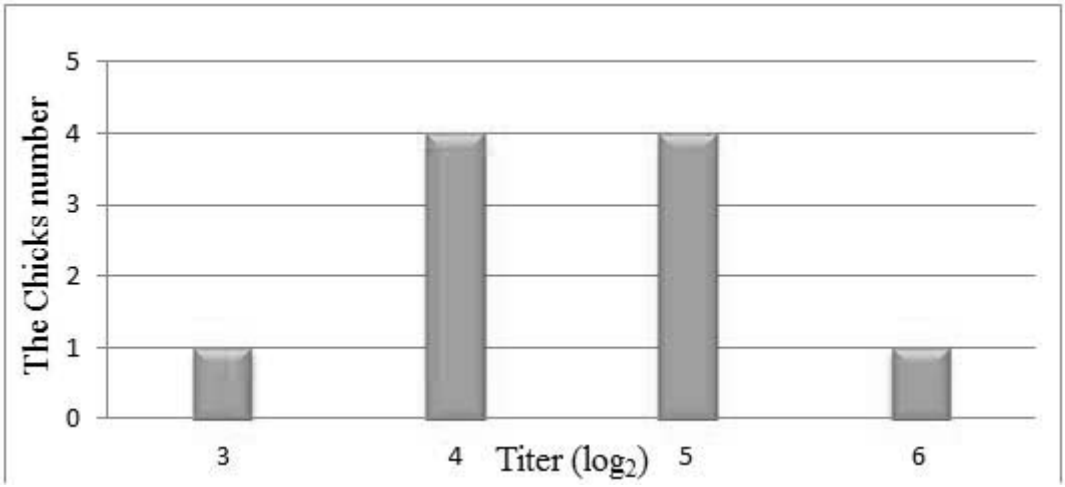


Figure 10: Individual variation in Immune response to vaccination at day 28 of age.

Results of the flock profile showed that there were individual variations among chicks in acquiring MDA specially those of day fourteen when five chicks showed different five titers. These results reaffirmed results of leandro et al., (2011).

In a parallel experiment we studied the effect of MDA on vaccination. We employed chicks from the same flock and used the same vaccine AVINEW® (VG/GA strain).

We found out that the decrease in MDA with time corresponded with an increase in the HI antibodies after vaccination with AVINEW (VG/GA strain) which gave means of 2.67, 3.98, and 4.72 (log2) at days 1, 14, and 28 respectively. This reciprocal relationship between MDA and response to vaccination agreed with Lancaster et al., (1960) who found that congenital passive immunity interfered with the development of active immunity in response to Newcastle disease virus given intramuscularly or subcutaneously A similar result was also obtained by Ismail, (1987) who observed that chicks vaccinated at day 1, 7, and 14 of age and had maternal antibodies above 3 (log2) did not respond to vaccination. That could be due to neutralization of the vaccine virus by these antibodies; similar findings were also obtained by Giamborne and Closser, (1990) when they found that higher antibodies titer in one day-old broilers resulted in fewer vaccine-induced reactions, less vaccine virus shed, and decreased duration of vaccine-induced immunity from coarse-spray vaccination. Jalil et al. (2009) observed that HI antibodies titer decreased significantly after 7 days of single vaccination with Baby Chick Ranikhet Disease Vaccine (BCRDV) and could not protect the birds which were vaccinated at day 1 and day 7 separately. They speculated that was due to the

neutralization of vaccine virus with (MDA).

It could be easily deduced that, in spite of individual variations, the best time of vaccination is the end of the third week particularly at day 18 of age. This result agreed with Begum et al. (2006) when they reported that maternally derived antibody passed over from the parents to progeny chicks remained protective for the chicks hatched from vaccinated parents until 18 days of age, although they found that the minimum titer that interfered with the vaccine was 3.6 (log2). Chicks vaccinated at day 1 and day 14 with MDA above 4.2 (log_2) did not respond to vaccination, this result agreed with Ismail (1987) who reported that chicks vaccinated at day 1, 7, and 14 did not respond to vaccination; although he found that the titer of MDA that interfered with the vaccine was above 3 (log_2),. This could be due to the difference in the NDV vaccine strain on one hand and the poultry breed on the other hand.

The individual variation in the immune response of vaccinated chicks might be due to their initial acquisition of different amounts of MDA from parents, that variably interferes with the vaccine at different ages of chicks; a similar explanation was given by Rhman et al. (2004) who stated that individual variations in the production of HI antibody response might be due to the presence of variable passive immunity in chicks or to varying degree of sensitivity of immune mechanism to antigen.

The wide range of individual variations in acquiring MDA (from 1 to 12 log2), together with the wider range of response of individuals with deferent levels of MDA to vaccination, may explain some of the vaccination failures, encounter with many different NDV vaccine strains and different poultry breeds.

This study shows that the best time of vaccination of chicks from immunized parents should be done no earlier and no later than day 18 of age.

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PATHOLOGIES DOMINANTES IDENTIFIEES EN AVICULTURE SEMI-INDUSTRIELLE AU MALI (ZONES PERIURBAINES DU DISTRICT DE BAMAKO ET DES VILLES DE SIKASSO ET SEGOU)

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Resume

La présente étude, réalisée pendant la période 2003-2007 avait pour but d'identifier les pathologies dominantes en aviculture moderne au Mali. Elle a couvert 22 exploitations avicoles modernes de pondeuses localisées dans les zones périurbaines du District de Bamako et des villes de Sikasso et de Ségou. Les analyses ont porté sur536 prélèvements au total dont 260 sérums, 254 œufs, 149 prélèvements d'organes, 129 échantillons de fientes et 4 écouvillons cloacaux. Les méthodes d'histopathologie, d'isolement en culture et d'identification biochimique ou sérologique ont été utilisées pour la confirmation des maladies infectieuses. Pour l'évaluation des charges parasitaires, la méthode modifiée de Mac Master a été utilisée. En vue d'établir le diagnostic définitif, les données épidémiologiques, les résultats des examens cliniques, lésionnels et de laboratoire ont été pris en compte. Les principales pathologies identifiées sont d'origine virale: maladie de Gumboro (15 cas) , maladie de Newcastle (16 cas), maladie de Marek (6 cas), mycoplasmique (163 sérums positifs à Mycoplasma synoviae et 49 sérums positifs à Mycoplasma gallisepticum sur 260 testés), bactérienne : choléra aviaire à Pasteurella gallinarum (un cas confirmé sur 15), salmonellose à Salmonella pullorum/gallinarum (17 organes positifs sur 83 testés) colibacillose (26 organes positifs sur 66 testés), infection d'œufs par Salmonella pullorum/gallinarum (20 positifs sur 254 testés) et Escherichia coli (14 positifs sur 254 testés) et parasitaire: infestation par Eimeria (36 cas), Ascaridia gallii (15 cas), Raillietina spp. (5 cas), Heterakis spp. (5 cas) et Argas persicus, notamment dans les poulaillers du site de Ségou).

Mots cle: Pathologies, Aviculture moderne, Bamako, Sikasso, Ségou

PREVALENT DISEASES IDENTIFIED IN SEMI-INDUSTRIAL POULTRY FARMING IN MALI (PERI-URBAN AREAS OF BAMAKO DISTRICT AND OF SIKASSO AND SEGOU TOWNS)

Resume

This study was conducted in 2003-2007 with the aim of identifying prevalent diseases in modern poultry farming in Mali. It covered 22 modern layer farms located in the suburban areas of Bamako District and of Sikasso and Segou towns. Analyses focused on a total of 536 samples, 260 sera, 254 eggs, 149 organ samples, 129 fecal samples and 4 cloacal swabs. Methods used in the study to confirm infectious diseases included histopathology, isolation in culture and biochemical or serological identification. To assess parasite loads, the Modified Mac Master Method was used. To establish a final diagnosis, epidemiological data, results of clinical, lesion and laboratory examinations were taken into account. The main diseases identified were of a viral nature : Gumboro Disease (15 cases), Newcastle Disease (16 cases), Marek Disease (6 cases), Mycoplasma (163 Mycoplasma synoviae positive sera and 49 Mycoplasma gallisepticum positive sera out of 260 screened), bacterial : Pasteurella gallinarum avian cholera (15 confirmed cases), pullorum / gallinarum Salmonella (positive results for 17 organs out of 83 screened), colibacillosis (positive results for 26 organs of 66 screened), egg infection by Salmonella pullorum / gallinarum (20 positive cases out of 254 screened) and Escherichia coli (14 positive cases out of 254 screened) and parasitic: infestation by Eimeria (36 cases), Ascaridia gallii (15 cases), Raillietina spp. (5 cases), Heterakis spp. (5 cases) and Argas persicus, including poultry farms in Segou).

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Keywords: Diseases; modern poultry farming; Bamako; Sikasso; Ségou

Introduction

Au Mali, l'aviculture constitue l'une des principales activités génératrices de revenus et de source de protéines pour les populations (Mali, DNSI, 1994). Elle joue un rôle très important dans la réduction du déficit en protéine d'origine animale.

L'effectif du cheptel aviaire au Mali est estimé à 36 750 000 volailles (Mali, DNPIA, 2010). Le secteur moderne représente près de 2 % de cet effectif (Kounta, 1992; Kounta, 1993).

Elle contribue à ce titre à la réduction de la pauvreté et à l'atteinte de la sécurité alimentaire au Mali.

Au cours de ces dernières années l'aviculture moderne a connu un essor considérable dans plusieurs zones périurbaines du Mali. Elle est présentement la principale source d'approvisionnement en œufs de consommation et de poulets de chair.

Cependant, plusieurs contraintes freinent l'amélioration de la productivité de l'aviculture. Parmi elles, les pathologies avec des taux élevés de morbidité et de mortalité occasionnant des pertes économiques considérables occupent une place prépondérante (Kounta, 1992; Kounta, 1993; Mali, DNSI, 2003).

Les poussins sont essentiellement importés d'autres pays, même si des embryons d'unités industrielles de production locale de poussins d'un jour commencent à voir le jour. Des plans de prophylaxie sont aussi mis à la disposition des aviculteurs par les fournisseurs des poussins, malgré l'application desquels, des taux de mortalité élevés sont souvent enregistrés.

L'objet de la présente étude est de contribuer à l'identification des pathologies dominantes en aviculture moderne au Mali.

Materiel et Methodes

Choix des exploitations avicoles

Le choix des aviculteurs test a été basé sur les résultats du recensement des exploitations avicoles modernes localisées dans les zones périurbaines du District de Bamako et des villes de Ségou et Sikasso, des entretien avec les aviculteurs et services d'encadrement, le volontariat et la situation géographique du site qui ne devait excéder un rayon de 50 km autour des villes ci-dessus citées. L'étude a concerné 22 exploitations avicoles au total dont 16 en zone périurbaine du District de Bamako et 3 respectivement dans les zones périurbaines de Sikasso et de Ségou.

Collecte des prélèvements

La collecte des données dans les exploitations a été effectuée par des observateurs à travers l'administration de questionnaires sur la santé de la volaille (évolution pondérale des sujets, évolution hebdomadaire des taux de morbidité et de mortalité, collecte de prélèvements pour le diagnostic des pathologies dominantes, les soins sanitaires appliqués).

Chez les sujets malades et récemment morts, il a été procédé à la collecte de prélèvements d'organes (foie, cœur, intestin, rate) chez les sujets abattus ou récemment morts.

Les différents prélèvements ont été conservés sous glace ou dans du formol à 10% et acheminés au laboratoire Central Vétérinaire pour la réalisation des examens de laboratoire.

Méthodes d'analyse de laboratoire

Les différentes suspicions de maladies virales ont été confirmées sur la base de la spécificité des lésions d'autopsie, des résultats de l'histopathologie (maladie de Gumboro et maladie de Marek), de l'isolement du virus de la maladie de Newcastle sur œufs embryonnés âgés de 9 à 11 jours et de leur identification à l'aide de tests d'hémagglutination et d'inhibition de l'hémagglutination ou d'immunodiffusion sur gélose.

Les principales maladies bactériennes suspectées ont été confirmées après ensemencement des prélèvements sur les principaux milieux classiques d'isolement (bouillons au thioglycollate, au tétrathionate, à base de tryptose du soja, géloses au sang, Mac Conkey, Salmonella Shigella et à base de tryptose du soja). L'identification définitive des

bactéries isolées a été faite sur la base des résultats de divers tests biochimiques, dont ceux du système API (Analytical profile Index).

Dans le cadre du dépistage des infections mycoplasmiques, 260 sérums provenant d'exploitations avicoles dans lesquelles des troubles respiratoires chroniques furent souvent enregistrés ont été testés pour la recherche d'anticorps deMycoplasma synoviae et de Mycoplasma gallisepticum.

Ainsi, les examens bactériologiques ont porté sur 83 prélèvements d'organes. 294 œufs de poules pondeuses ont également été testés dans le cadre de la recherche des salmonelles.

La supervision des activités des observateurs a été effectuée par des chercheurs à travers des missions conjointes à une fréquence trimestrielle.

Resultats

Les pathologies dominantes diagnostiquées en aviculture moderne au Mali sont d'origine virale, mycoplasmique, bactérienne et parasitaire.

Maladies virales

La maladie de Gumboro, la maladie de Newcastle et la maladie de Marek ont été les principales maladies virales diagnostiquées. Les deux premières affections ont souvent évolué avec des infections bactériennes secondaires dues aux salmonelles et à Escherichia coli.

Pendant la période 2003-2007, au total 15 cas de maladie de Gumboro ont été enregistrés dans les trois sites de l'étude. La distribution par année et site d'étude de ces cas est illustrée dans le tableau I. De l'analyse de ce tableau, il ressort que quatre cas (26,66 %) ont été enregistrés en 2003 dont trois dans les élevages du seul site de la zone périurbaine du District de Bamako.

Pendant la même période, 16 cas de maladie de Newcastle ont été diagnostiqués. La fréquence de la maladie par site et année est illustrée dans le tableau II. Leur répartition est la suivante: 11 cas à Bamako (73,3 %), trois cas à Sikasso (18,7 %) et deux cas à Ségou (12,5 %). L'analyse du tableau II montre que la fréquence de la maladie de Newcastle a varié en fonction de l'année: 75 % en 2003, 66,6 % en 2004 et 2006, 100 % en 2005 et 2007,

Dans la zone périurbaine de la ville de Sikasso, un seul cas de maladie de Newcastle a été observé avec la fréquence a été de 33,3 % en 2006. Par contre, seul deux foyers de cette maladie ont été enregistrés dans les fermes de la zone périurbaine de Ségou (deux cas) avec respectivement un cas en 2003 et 2004 (Tableau II).

Il faut noter que dans la plupart des échantillons soumis pour suspicion de maladie de Newcastle, Escherichia coli et Salmonella pullorum/gallinarum ont été isolés.

Pour ce qui concerne la maladie de Marek, elle n'a été diagnostiquée qu'en 2005 (un seul cas à Bamako) et 2006 (cinq cas au total dont quatre à Bamako et un à Sikasso). Par contre, aucun cas de maladie de Marek n'a été enregistré à Ségou (Tableau III).

Mycoplasmoses aviaires

Suite à l'analyse des sérums 260 sérums provenant de certaines exploitations avicoles on été analysés pour la recherche d'anticorps anti-Mycoplasma synoviae et anti-Mycoplasma gallisepticum. Les résultats obtenus montrent que 163 sérums (62,6 %) contenaient des anticorps de Mycoplasma synoviae, 49 étaient positifs aux anticorps de Mycoplasma gallisepticum et 48 sérums étaient négatifs aux anticorps des deux espèces de mycoplasmes (Tableau IV).

Il ressort du tableau IV que les taux d'infection les plus élevés ont été enregistrés dans la région de Ségou avec respectivement 66,6 et 40 % pour Mycoplasma synoviae et Mycoplasma gallisepticum. Les taux les plus bas ont été observés dans le District de Bamako avec respectivement 6,1 et 3,3 %. Des taux d'infection beaucoup plus élevés qu'à Bamako ont été mis en évidence à Sikasso avec 61,1 pour Mycoplasma synoviae et 23,3 % pour Mycoplasma gallisepticum.

Maladies bactériennes

Salmonelloses à Salmonella pullorum/ gallinarum

L'analyse des résultats montre que la prévalence des salmonelloses est assez élevée avec un taux global de 20,4% qui est variable en fonction des régions. Il a été respectivement de 18,9 %

Tableau I : Fréquence par site de la Maladie de Gumboro chez les poules pondeuses

Sites	Cas par année de maladie de Gumboro										Total
	2003		2004		2005		2006		2007		
	Nbre	%	Nbre	%	Nbre	%	Nbre	%	Nbre	%	
Bamako	3	75	2	66,6	3	100	2	66,6	2	100	12
Sikasso	0	0	0	0	0	0	1	33,3	0	0	1
Ségou	1	25	1	33,3	0	0	0	0	0	0	2
Total	**4**		**3**		**3**		**3**		**2**		**15**

Tableau II : Fréquence par site de la Maladie de Newcastle chez les poules pondeuses

Sites	Cas par année de maladie de Gumboro										Total
	2003		2004		2005		2006		2007		
	Nbre	%	Nbre	%	Nbre	%	Nbre	%	Nbre	%	
Bamako	3	60	2	66,6	3	100	2	66,6	1	50	11
Sikasso	1	20	0	0	0	0	1	33,3	1	50	3
Ségou	1	20	1	33,3	0	0	0	0	0	0	2
Total	**5**		**3**	**3**	**3**		**3**		**2**		**16**

Tableau III : Fréquence par site de la Maladie de Marek chez les poules pondeuses

Sites	Cas par année de maladie de Gumboro										Total
	2003		2004		2005		2006		2007		
	Nbre	%	Nbre	%	Nbre	%	Nbre	%	Nbre	%	
Bamako	0	0	0	0	1	100	4	80	0	0	5
Sikasso	0	0	0	0	0	0	1	20	0	0	1
Ségou	0	0	0	0	0	0	0	0	0	0	0
Total	**0**		**0**		**1**		**5**		**0**		**6**

Tableau IV: Prévalence sérologique des mycoplasmoses chez les poules pondeuses dans les sites de l'étude

Régions	Nombre de Sérums testés	Sérums positifs aux anticorps de Mycoplasma synoviae		Sérums positifs aux anticorps de Mycoplasma gallisepticum	
		Nombre	%	Nombre	%
Sikasso	90	55	61,1	21	23,3
Ségou	60	40	66,6	24	40
Bamako	110	68	6,1	4	3,3
TOTAL	**260**	**163**	**62,6**	**49**	**18,8**

à Bamako, 50 % à Sikasso et 20 % à Ségou. Pour ce qui concerne les œufs, un taux moyen d'infection de 7,8 % a été enregistré et tous les échantillons testés provenaient des élevages du site de Bamako (Tableau V). L'examen bactériologique de 4 écouvillons cloacaux (3 à Ségou et 1 à Sikasso) a permis d'isoler Salmonella pullorum/gallinarum à partir des échantillons provenant de Ségou.

Colibacillose aviaire

Au cours de la même période, sur 66 échantillons d'organes envoyés au laboratoire pour suspicion de colibacillose aviaire, 26 (39,39%) ont été positifs à l'isolement d'Escherichia coli (Tableau VI).

Par contre, suite à la mise en culture de 254 œufs de poules pondeuses, Escherichia coli n'a été isolé que dans 14 cas (5,5 %). Tous les œufs testés provenaient également du District de Bamako.

Tableau V: Prévalence bactériologique de Salmonella pullorum /gallinarum à partir de prélèvements d'organes de poules pondeuses

Sites	Echantillons testés		
	Nombre	Positif	Pourcentage (%)
Bamako	74	14	18,9
Sikasso	4	2	50
Ségou	5	1	20
Total	**83**	**17**	**20,4**

Tableau VI: Prévalence bactériologique de la colibacillose chez les poules pondeuses

Sites	Echantillons testés		
	Nombre	Positif	Pourcentage (%)
Bamako	55	19	34,5
Sikasso	4	3	75
Ségou	5	4	80
Total	**66**	**26**	**39,3**

Tableau VII: Principales espèces de parasites internes identifiées chez les poules pondeuses

Provenance	Nombre total d'échantillons examinés	Nombre de positifs	%	Valeurs OPG et fréquence des parasites identifiés
Bamako	88	41	46,5	50 *Ookystes*(30), 50 *Raillietina sp*.(1), 50 *Heterakis*(4), 50 *Ascaridia gallii* (6),100 *ascaridia gallii*(1)
Sikasso	17	6	35,2	50 *Ookystes*(2), 50 *Ascaridia gallii*(3), 50 *Raillietina sp*.(1)
Ségou	24	11	45,8	50 *Ookystes*(2),1600 *Heterakis spp*.(1), 1300 *Ascaridia gallii* (5),50 *Raillietina sp*.(3)
Total	**129**	**58**	**44,9**	

Par ailleurs, sur 15 échantillons d'organes provenant d'exploitations avicoles du District de Bamako, seul un (6,6 %) a donné une culture positive de pasteurelles (agent étiologique du choléra aviaire).

Maladies parasitaires

Les infestations par les coccidies (50 Ookystes), ascaris (50-100 œufs/g de fèces), heterakis (50 – 1600) œufs /g de fèces) et Raillietina (50 œufs/g de fèces) ont été les principales affections parasitaires dépistées (Tableau VII). Argas persicus a été le principal parasite externe identifié. Il a été fréquemment observé à l'intérieur des bâtiments des fermes localisées dans la zone périurbaine de Ségou (présence d'argas dans un élevage sur trois visités).

Discussion

La présente étude a permis d'établir que la maladie de Gumboro, la maladie de Newcastle, les mycoplasmoses constituent les contraintes majeures à l'amélioration de la productivité de l'aviculture moderne au Mali. Ce constat est semblable à ceux qui ont été rapportées par Couacy-Hymann et al. en Côte d'Ivoire (1991), Arbelot et al. (1997) et Cardinale (1998) au Sénégal. Constantin et al. (1988)) ont également montré également que la maladie de Gumboro constitue une préoccupation dans les pays développés, notamment en France.

Le taux de fréquence est assez élevé pour cette maladie (26,66 %) dans les fermes de la zone périurbaine du District de Bamako. Ce

phénomène est certainement lié à la présence de populations réceptives de poules pondeuses très importantes (22 exploitations avec des tailles variant entre 1000 et 30 000 sujets) avec de nombreux cas d'échec de vaccination propres à cette maladie), par rapport aux sites de Sikasso et de Ségou avec seulement trois fermes ayant des effectifs moyens de 500 pondeuses.

Les taux de prévalence bactériologique de Salmonella pullorum/gallinarum chez les poules pondeuses sont relativement élevés au Mali (20,8 % pour la salmonellose à Salmonella pullorum/gallinarum à partir d'organes et 19,53 % à partir d'œufs de pondeuses).

Ce taux élevé d'infection des œufs de pondeuses par Salmonella pullorum/gallinarum confirme que leur transmission se fait surtout par la voie transovarienne.

Des taux d'infection par Salmonella pullorum/gallinarum nettement plus élevés ont été obtenus par Arbelot et al. (1997) chez des pondeuses de la zone de Niayes au sénégal(38 %). En Somali, un taux de prévalence sérologique de 54,8 % a été établi pour l'infection à Salmonella gallinarum en aviculture intensive (Prosperi et al., 1981). Les auteurs indiquent par ailleurs dans le même secteur un taux de prévalence bactériologique de 74,2 %.

Des investigations sérologiques menées par Arbelot et al. (1997) montrent également que les mycoplasmoses aviaires, la maladie de Gumboro et la salmonellose figurent parmi les pathologies dominantes en aviculture industrielle dans la Zone du Cap Vert au Sénégal. Les résultats desdites études montrent qu'en saison des pluies, 4 à 5 % des lots de pondeuses étaient infectés par Mycoplasma gallisepticum, 20 à 28 % par Mycoplasma synoviae et 41 à 45 % par Salmonella gallinarum pullorum.

Il faut noter que les taux d'infection des poulets par Mycoplasma synoviae et Mycoplasma gallisepticum ont varié selon les régions. Qu'il s'agisse des anticorps de Mycoplasma synoviae ou de ceux de Mycoplasma gallisepticum, les taux d'infection les plus élevés ont été enregistrés dans la région de Ségou et Sikasso Ceci s'expliquerait probablement par certains facteurs épidémiologiques favorisants comme la cohabitation souvent fréquente de poules pondeuses avec des volailles locales reconnues comme réservoirs potentiels des mycoplasmes.

La mise en évidence de cas d'infestation par les coccidies, ascaris, Heterakis et Raillietina montre que les plans de déparasitage appliqués dans les exploitations concernées ne sont pas adaptés. La présence des argas dans certains poulaillers du site de Ségou est également la preuve de l'inefficacité des mesures de lutte anti-argas employées par les aviculteurs.

Conclusion

Les pathologies dominantes identifiées en aviculture moderne au Mali sont d'origine virale (maladie de Newcastle, maladie de Gumboro, maladie de Marek, etc), , mycoplasmique (infections par Mycoplasma synoviae et Mycoplasma gallisepticum), bactérienne (typhose/pullorose, colibacillose, choléra aviaire) et parasitaires (infestations par les heterakis, ascaris, coccidies et argas). En zone sahélienne (Ségou), une attention particulière doit être accordée aux argas.

Des stratégies mieux adaptées de lutte devront être mises au point et vulgarisées pour améliorer la santé et la productivité du cheptel aviaire en aviculture moderne (vaccinations systématiques contre la maladie de Gumboro, la maladie de Newcastle, la maladie de Newcastle, les salmonelloses et le choléra en tenant compte de la spécificité des souches impliquées dans l'étiologie de ces pathologies).

Remerciements

Les auteurs adressent leurs sincères remerciements au Service d'Aide et Coopération de l'Ambassade de France au Mali, à travers le Fonds de Solidarité Prioritaire, pour son appui financier qui a permis la réalisation de la présente étude, aux observateurs et techniciens, respectivement pour leur participation à la collecte des données dans les élevages et la réalisation des analyses de laboratoire.

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PERFORMANCE AND CARCASS YIELD OF SEXED BROILER CHICKENS REARED ON TWO HOUSING TYPES

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Abstract

In spite of availability of specially formulated feeds and other aids to intensive poultry production, the provision of appropriate housing remains the most basic requirement for successful poultry production. This study thereby determined the performance, carcass yield and meat composition of 300 sexed Arbor Acre broiler chickens reared on deep-litter and deep-litter with a run housing types. The birds were brooded for 2 weeks, differentiated into male and female by feather sexing and balanced for weight. Thereafter, 150 male and female chicks each were confined separately in deep litter and deep litter with a run. Weekly live weights and physiological parameters were taken. At the end of the study, 2 birds which were similar to the average weight of each replicate were selected for carcass analysis. Serum cholesterol and calcium were also determined at the end of the experiment. The data obtained were arranged in a 2×2 factorial experimental layout in a Completely Randomized Design. Male birds had higher final weights, weight gain and cost of feed per day of 2208.33g/b,44.41g/b/d and N21.96, respectively compared to female birds. Birds on deep litter had higher live weight and plucked weight of 2216.67 and 1985.00g, respectively. Female birds had highest percentage breast of 22.81. Serum cholesterol and calcium of birds on deep litter with run was higher. It was concluded that both male and female broiler chickens had higher carcass yield on deep litter housing type. However, for higher live weight gain female broiler chickens should be reared on deep litter while male broiler chickens could be reared conveniently on any of the housing types.

Keywords: Performance, carcass yield, female broiler, male broiler, serum cholesterol, calcium

PERFORMANCE ET RENDEMENT ENVIANDE DES POULETS DE CHAIR ÉLEVÉS SÉPARÉMENT PAR SEXES DANS DEUX TYPES DE LOGEMENT

Résumé

En dépit de la disponibilité d'aliments spécialement préparés et d'autres aides à la production intensive de volailles, la fourniture de logements adéquats reste la condition élémentaire pour une production de volailles réussie. Ainsi, la présente étude a déterminé la performance, le rendement en viande et la composition de viande de 300 poulets de chair Arbor Acre regroupés par sexe, élevés en système de litière accumulée et en système de litière accumulée avec une cour. Les oiseaux ont été couvés pendant 2 semaines, différenciés en mâles et femelles par sexage de plumes et équilibrés pour le poids. Par la suite, 150 poussins mâles et femelles ont été confinés séparément en stabulation à litière accumulée et en stabulation à litière accumulée avec une cour. Les poids vifs et les paramètres physiologiques ont été enregistrés sur une base hebdomadaire. A la fin de l'étude, 2 oiseaux dont le poids était similaire au poids moyen de chaque répétition ont été choisis pour l'analyse de carcasse. Le cholestérol sérique et le calcium ont également été déterminés à la fin de l'expérience. Les données obtenues ont été organisées dans un dispositif expérimental factoriel 2 × 2 selon un schéma complètement aléatoire. Les oiseaux mâles avaient un poids final, un gain de poids et un coût alimentaire par jour plus élevés, respectivement de 2208,33g/b ; 44,41g/b/d et N21.96, par rapport aux oiseaux femelles. Les oiseaux en stabulation avec litière accumulée

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avaient un poids vif et un poids plumé élevés, respectivement de 2216,67 et 1985,00g. Les femelles avaient le plus haut rendement filet de 22,81. Le cholestérol sérique et le calcium des oiseaux sur litière accumulée avec cour étaient plus élevés. Il a été conclu que les poulets de chair mâles et femelles avaient un rendement en viande plus élevé en stabulation avec litière accumulée. Cependant, pour augmenter le gain de poids vif, les poulets de chair femelles devraient être élevés en système de litière accumulée tandis que les poulets de chair mâles peuvent être élevés facilement dans l'un ou l'autre système de logement.

Mots-clés : Performance, rendement en viande, poulet de chair femelle, poulet de chair mâle, cholestérol sérique, calcium

Introduction

Broiler birds have been a source of quality protein supplies and other nutrients needed for growth and development of man particularly in the tropical environment (Gwaza and Egahi, 2009). They are also potential means of income generation and employment either in a small scale or large scale production (Aphunu and Akpobasa, 2009). Besides, other considerations for broiler production bother on their short generation intervals, faster growth rate, lower age at maturity, high meat yield at slaughter and absence of cultural barrier or taboos to consumption (Ehebha et al., 2010).

There has evolved several different methods or systems in poultry production. Attempts are made to approach animal agriculture with the aim of creating integrated, humane, environmentally and economically sustainable housing types to produce acceptable livestock for human nutrition. The natural environment should thereby be enhanced or protected and product quality be maintained or enhanced in any housing type adopted. In the production chain, carcass yields provide useful information to guide farmers as to strain, sex, and slaughter age options that would supply consumers' demands. Consumers prefer chickens with high yield of economic parts, such as breast, drumsticks, and thighs. Female broilers have more flesh than the male of similar weights because the male have relatively bigger or heavier bones which could be attributed to hormonal differences between the two sexes. These differences may be related to growth and muscle development potential between males and females (Toldrá, 2003; Le Bihan-Duval, 2004). An adequate adjustment of the nutritional level for broiler chickens requires the knowledge on body composition and bird growth potential for enhanced performance and profits. This on the other hands, may be related to the housing types which may mar or make the bird's ability to exhibit its natural behaviour as previously studied by Sogunle et al. (2013) for cockerel chickens. This study thereby explored the differences in the body conformation of female and male broiler chickens as influenced by housing types. The aim of this research is therefore to determine the effects of housing types (deep litter and deep litter with a run) on the performance and carcass yield of sexed (female and male) broiler chickens.

Materials and methods

Experimental Site

The experiment took place at the poultry unit of the Teaching and Research Farm Directorate, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. This area is situated in the rainforest vegetation zone of South-western Nigeria on Latitude 7°13'49.46"N, Longitude 3° 26' 11.98" E and altitude of 98m above sea level. The climate is humid with a mean annual rainfall of 1003mm annual mean temperature and humidity ranges from 31.9 to 34.8^{0}C and 79.7 to 90.1%, respectively.

Experimental animal and design

A total number of 300 Arbor Acre broiler breed of birds were purchased from a reputable hatchery and brooded for two weeks. Vaccination schedule and medications for broiler chicks were strictly adhered to. The birds were sexed after the second week of age using feather sexing and weights were balanced. The birds were subdivided into two sub-groups of Housing type (deep-litter and deep-litter with a run) of 75 birds each thus making the

study a 2x2 factorial experimental layout with four (4) treatment groups. Each sub-group was replicated three times with 25 birds each. The treatment groups were as follow; Male birds on deep-litter, Male birds on deep litter with run, Female birds on deep litter and Female on deep litter with run.

Housing

The birds were brooded on deep-litter for 2 weeks in confinement and fed ad-libitum on starter diet. Thereafter, 150 male and female chicks each were confined separately in deep-litter and deep-litter with a run (an outside run with a provision for perching). The deep litter housing system was a concrete floor with dwarf wooden wall of about (0.7m) from floor level with chicken mesh at the upper side for cross ventilation. The roof was made of corrugated zinc sheet. Birds on the deep litter were stocked at 0.08m2/bird. The same stoking density was used for the birds in deep-litter with a run but with an additional open space (outside run) of 0.16m2/bird. The birds were fed ad-libitum on both systems with the same quality and quantity of feed.

Experimental diet

The birds were fed commercial starter and finisher diets formulated to meet the nutrient requirement of the birds (NRC, 1994).

Data collection

Performance

Daily records of average body weight gain and mortality of birds at the different Housing types were taken using the following formulae:

Feed intake (g) =
Total feed given (g) – feed left-over (g)

Average feed intake (g/bird/day) =

$$\frac{\text{Feed intake}}{\text{Number of birds}} \Big/ \text{Number of days}$$

Weight gain (g) =
Final weight (g) – Initial weight (g)

Average body weight gain (g/bird/day)=

$$\frac{\text{Weight gain}}{\text{Number of birds}} \Big/ \text{Number of days}$$

Feed conversion ratio =

$$\frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

Percentage mortality =

$$\frac{\text{Number of birds that died}}{\text{Total number of stocked birds}} * 100$$

The birds were observed regularly to detect any occurrence of diseases such as foot pad lesion (i.e. a sore on the foot pad), breast blisters, skin burns and bruising. The daily body temperatures of the birds were taken via their rectum. The values were between 41-42^{0}C which fell within the range for optimum performance of birds in the tropical environment (Sogunle et al., 2010, 2012).

Carcass Yield

At the end of the eighth week of the experiment, two birds which were of average live weight for each replicate were selected and sacrificed humanely by cervical dislocation. They were properly bled for two minutes and then scalded using water at temperature, 60 oC. After de-feathering, the plucked weight was recorded. The heads and shanks were removed and weighed.

After evisceration, the carcass yield was recorded. The weight of the cut-up parts (head, shanks, thighs, breast, neck, back, wings and drumsticks) and organs (heart, kidneys, lungs, livers, spleen, gizzard, proventriculus and spleen) and abdominal fat were determined.

Cost Benefit

The prevailing market costs at the time of study (N158.70 = 1$) were used to calculate the total cost of feed consumed per bird (N/day) and the economy of feed per weight gain (N/g/bird).

The following were determined under the cost benefit:

i. Cost of feed intake per bird per day =
Cost of 1 kg feed*feed intake

ii. Cost of feed intake per weight gain =

$$\frac{\text{Cost of feed intake per bird per day}}{\text{Weight gain per bird}}$$

Serum Cholesterol level determination

At the end of the experiment, 2 ml of blood samples were drawn from the wing (bronchial vein) of a bird per replicate group into a sample bottle. The cholesterol of the serum was determined using enzymatic endpoint method. The absorbance of the sample was measured against the blank reagent within 60 minutes with the reading taken at wavelength 520 nm.

Serum Calcium level determination

Also, at end of the experiment, 2 ml blood samples were drawn from the wing (bronchial vein) of a bird per replicate group into a sample bottle. Blood Calcium (Ca+) was determined by flame emission spectrometry as described by Cheesbrough (1991).

Statistical Design and Analysis

Data obtained were arranged in a 2×2 factorial experimental layout and then subjected to Completely Randomized Design. Significantly ($P<0.05$) different means were separated using Duncan's multiple range test as contained in Statistical Analysts Software (SAS, 2003) package. The model in the factorial experimental layout is shown below:

$$\gamma ijk = \mu + Ai + Bj + (AB)ij + \varepsilon ijk$$

Where:

γijk = individual observation

μ = general mean

Ai = effect of factor A (Sex; male and female)

Bj = effect of factor B (Housing type; deep litter and deep litter with run)

$(AB)ij$ = effect of interaction AB (Sex*Housing type)

εijk = experimental error

The data obtained for serum cholesterol and serum calcium were compared using Bar Charts.

Results

Effect of sex and housing type on growth performance and cost benefit of broiler chicken

The main effect of sex and housing type on growth performance and cost benefit of broiler chickens is presented in Table 1. Significant ($P<0.05$) differences were found between sexes in final weight, weight gain, feed intake and cost of feed intake with male birds recording higher values of 2208.33g/bird, 44.41, 120.48g/bird/day and N11.33/bird/day, respectively than those obtained in female birds. In the housing type, significant ($P<0.05$) differences were found in final weight, weight gain, mortality and cost of feed intake per weight gain. Birds managed on deep litter had higher final weight and weight gain than birds on deep litter with run while birds on deep litter with run recorded a higher (poorer) mortality and cost of feed intake per weight gain than those on deep litter.

Table 2 shows the effect of interaction between sex and housing type on growth performance and cost benefit of broiler chicken. There were significant ($P<0.05$) differences in final weight, weight gain, feed intake, feed conversion ratio, cost of feed intake and cost of feed intake per weight gain. Male birds on deep litter recorded the highest weight gain which was comparable to value obtained for male birds on deep litter with a run. This same trend was obtained in feed intake and cost of feed intake. The best feed conversion ratio ($P<0.05$) was recorded in female birds on deep litter and it was similar ($P>0.05$) to the values obtained in male birds on deep litter and deep litter with a run. Female birds on deep litter with a run recorded the poorest feed conversion ratio and cost of feed intake per weight gain.

Effects of sex and housing type on carcass yield of broiler chicken

In the main effect of sex and housing type on carcass yield of broiler chicken (Table 3), significant ($P<0.05$) differences were obtained in the eviscerated weight, head, shank and breast for the sex. Female birds had a higher

Table 1: Main Effect of sex and housing type on growth performance and cost benefit of broiler chicken

	Sex		Housing type	
Parameter	**Female**	**Male**	**DL**	**DLR**
Initial weight (g/bird)	333.33 ±4.53	343.33 ±5.02	342.00 ±6.10	334.67 ±3.62
Final weight (g/bird)	1965.83±44.77[b]	2208.33 ±25.62[a]	2140.83±46.63[a]	2033.33±72.19[b]
Weight gain (g/bird/day)	38.87 ±1.11[b]	44.41 ±0.58[a]	42.83 ±1.02[a]	40.44 ±1.74[b]
Feed intake (g/bird/day)	108.92 ±0.52[b]	120.48 ±0.75[a]	114.36 ± 2.84	115.04 ±2.47
Feed conversion ratio	2.82 ±0.09	2.72 ±0.04	2.67 ±0.04	2.86 ±0.07
Mortality (%)	2.00 ±1.37	1.33 ±0.84	0.00 ±0.00[b]	3.33 ±1.23[a]
Cost of feed intake (N/bird/day)	10.24±0.07[b]	11.33±0.13[a]	10.75±0.48	10.81±0.42
Cost of feed intake per weight gain (N/g/bird)	0.26±0.02	0.26±0.01	0.25±0.01b	0.27±0.01[a]

[a,b] Means in the same row by factor with different superscripts differ significantly (P<0.05)
DL – Deep litter, DLR – Deep litter with run Average cost of 1 kg feed = N94.00

Table 2: Effect of interaction between sex and housing type on growth performance and cost benefit of broiler chicken

Sex	**Female**		**Male**	
Housing type	**DL**	**DLR**	**DL**	**DLR**
Parameter				
Initial weight (g/bird)	331.33 ±8.17	335.67 ±5.61	352.67 ±2.40	334.00 ±5.77
Final weight (g/bird)	2048.33 ±11.67[b]	1883.33 ±55.48[c]	2233.33 ±46.67[a]	2183.33 ±21.86[a]
Weight gain (g/bird/day)	40.88 ±0.41[b]	36.85 ±1.33[c]	44.78 ±1.13[a]	44.03 ±0.52[a]
Feed intake (g/bird/day)	108.16 ±0.40[b]	109.68 ±0.78[b]	120.55 ±1.28[a]	120.39 ±1.06[a]
Feed conversion ratio	2.65 ±0.03[b]	2.98 ±0.11[a]	2.69 ±0.09[b]	2.74 ±0.16[b]
Mortality (%)	0.00 ±0.00	4.00 ±2.31	0.00 ±0.00	2.67 ±1.33
Cost of feed intake (N/bird/day)	10.16±0.05[b]	10.30±0.12[b]	11.33±0.24[a]	11.32±0.16[a]
Cost of feed intake per weigh gain (N/g/bird)	0.25±0.01[b]	0.28±0.02[a]	0.25±0.02[b]	0.26±0.00[b]

[a,b,c] Means in the same row with different superscripts differ significantly (P<0.05)
DL – Deep litter, DLR – Deep litter with run Average cost of 1 kg feed = N94.00

(P<0.05) value for the eviscerated weight and breast while male birds higher percentage head and shanks. In the housing type, significant (P<0.05) differences were obtained only in the plucked weight and percent head. While birds on deep litter had a higher plucked weight, the birds on deep litter with a run had a higher percentage head.

The effect of interaction between sex and housing type on carcass yield of broiler chicken is presented in Table 4. There were significant (P<0.05) differences on plucked weight, percentage head, shanks and breast. The highest plucked weight and percentage breast were obtained in female birds on deep litter than other treatment groups whereas male birds on deep litter with run recorded highest percentage head and shanks which were similar to those recorded for male birds on deep litter housing type.

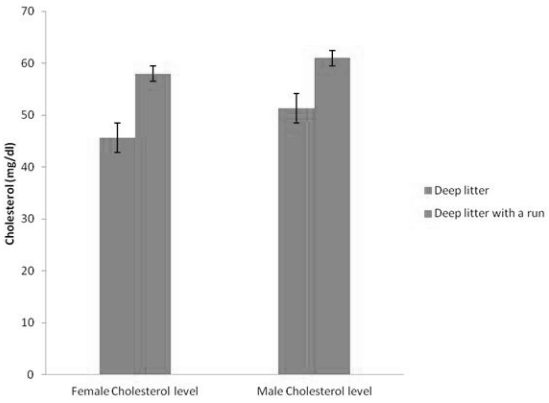


Figure 1: Serum Cholesterol of female and male broiler chickens on deep litter and deep litter with run Housing types at week 8

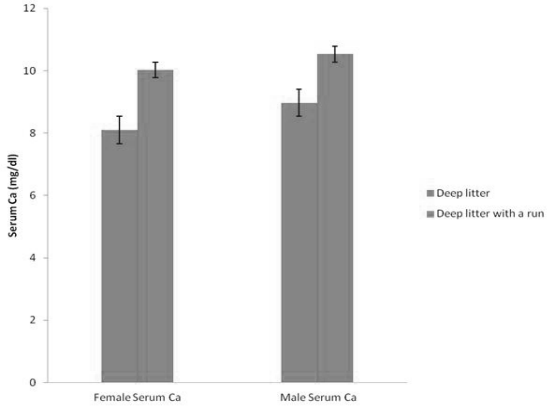


Figure 2: Serum Ca of female and male broiler chickens on deep litter and deep litter with run Housing types at week 8

Table 3: Main effect of sex and housing type on carcass yield of broiler chicken

	Sex		Housing type	
Parameter	**Female**	**Male**	**DL**	**DLR**
Live weight (g)	2133.33 ± 33.89	2154.17 ± 40.57	2216.67 ± 38.59	2170.83 ±18.93
Carcass yield				
Plucked weight (g)	1950.00 ± 41.74	1864.17 ± 67.05	1985.00 ± 59.01[a]	1829.17 ±44.58[b]
Dressing percentage	83.36 ± 0.76	79.16 ± 1.86	81.43 ± 1.92	81.09 ±1.07
Eviscerated weight (g)	1540.83 ± 31.42[a]	1450.00 ± 28.87[b]	1497.50 ±32.43	1493.33 ±33.81
Cut-up parts[1]				
Head	2.16 ± 0.069[b]	2.51 ± 0.07[a]	2.23 ± 0.07[b]	2.44 ±0.09[a]
Shanks	3.77 ± 0.074[b]	4.92 ± 0.09[a]	4.36 ± 0.18	4.34 ± 0.21
Thighs	10.48 ± 0.26	14.14 ± 4.04	10.30 ±0.41	14.33 ±4.02
Breast	22.81± 0.42[a]	18.74 ± 0.40[b]	20.92 ±0.82	20.64 ±0.64
Neck	4.05 ± 0.19	3.79 ± 0.17	3.67 ±0.23	3.98 ±0.14
Back	12.34 ± 0.41	13.56 ± 0.43	12.88 ±0.53	13.02 ±0.36
Wings	9.71 ± 0.25	9.98 ± 0.38	10.03 ±0.38	9.66 ±0.26
Drumsticks	10.31 ± 0.16	10.78 ± 0.22	10.52 ±0.21	10.58 ±0.20
Organs[2]				
Heart	0.38 ± 0.02	0.41 ± 0.02	0.39 ±0.02	0.40 ±0.02
Kidneys	0.41 ± 0.05	0.37 ± 0.03	0.37 ±0.04	0.41 ±0.03
Lungs	0.46 ± 0.04	0.51 ± 0.03	0.45 ±0.04	0.53 ±0.03
Liver	1.49 ± 0.08	1.49 ± 0.15	1.48 ±0.12	1.50 ±0.12
Gizzard	1.72 ± 0.06	1.71 ± 0.06	1.65 ±0.05	1.78 ±0.06
Proventriculus	0.34 ± 0.02	0.29 ± 0.01	0.31 ±0.02	0.33 ±0.02
Spleen	0.09 ± 0.01	0.09 ± 0.01	0.09 ±0.01	0.09 ±0.01
Abdominal fat	0.90 ± 0.13	0.73 ± 0.09	0.77 ±0.12	0.86 ±0.11

[a,b] Means in the same row by factor with different superscripts differ significantly (P<0.05)
[1, 2]: Percentages of the live weight
DL – Deep litter, DLR – Deep litter with run

Table 4: Effect of interaction between sex and housing type on carcass yield of broiler chicken

	Sex		Housing type	
Parameter	**Female**	**Male**	**DL**	**DLR**
Live weight (g)	2200.00 ±21.64	2166.67 ±24.72	2233.33 ±31.46	2175.00 ±30.96
Carcass yield				
Plucked weight (g)	2025.00 ±64.23[a]	1875.00 ±35.94[ab]	1945.00 ±102.75[ab]	1883.30 ±81.31[b]
Dressing percentage	83.66 ±1.03	83.07 ±1.99	79.21 ±3.62	79.12 ±1.44
Eviscerated weight (g)	1536.67 ±44.09	1545.00 ±48.91	1458.33 ±45.49	1441.67 ±39.62
Cut-up parts[1]				
Head	2.05 ±0.05[c]	2.26 ±0.12[bc]	2.41±0.06[ab]	2.61 ±0.12[a]
Shanks	3.83 ±0.10[b]	3.71 ±0.11[b]	4.89 ±0.14[a]	4.96 ±0.13[a]
Thighs	10.51 ±0.46	10.45 ±0.29	10.09 ±0.70	18.19 ±8.05
Breast	23.19 ±0.68[a]	22.43 ±0.49[a]	18.64 ±0.67[b]	18.84 ±0.51[b]
Neck	4.02 ±0.36	4.08 ±0.19	3.72 ±0.29	3.87 ±0.21
Back	12.38 ±0.70	12.29 ±0.48	13.37 ±0.79	13.74 ±0.38
Wings	9.64 ±0.44	9.77 ±0.29	10.42±0.61	9.54 ±0.45
Drumsticks	10.23 ±0.14	10.39 ±0.31	10.81 ±0.38	10.76 ±0.27
Organs[2]				
Heart	0.39 ±0.02	0.38 ±0.03	0.40 ±0.03	0.43 ±0.01
Kidneys	0.34 ±0.05	0.48 ±0.05	0.39 ±0.05	0.35 ±0.03
Lungs	0.41 ±0.05	0.52 ±0.06	0.49 ±0.05	0.53 ±0.03
Liver	1.56 ±0.12	1.41 ±0.12	1.39 ±0.21	1.59 ±0.21
Gizzard	1.71 ±0.07	1.72 ±0.10	1.59 ±0.08	1.84 ±0.05
Proventriculus	0.33 ±0.03	0.36 ±0.04	0.29 ±0.01	0.31 ±0.01
Spleen	0.10 ±0.01	0.09 ±0.01	0.09 ±0.01	0.09 ±0.01
Abdominal fat	0.94 ±0.20	0.86 ±0.17	0.59 ±0.10	0.85 ±0.15

[a,b] Means in the same row by factor with different superscripts differ significantly (P<0.05)
[1, 2]: Percentages of the live weight
DL – Deep litter, DLR – Deep litter with run

Serum cholesterol and calcium of sexed broiler chickens on Deep Litter and Deep Litter with Run Housing types

Figure 1 shows the serum cholesterol of female and male broiler chickens on deep litter and deep litter with run housing systems at week 8. The serum cholesterol levels of male birds on deep litter and male birds on deep litter with run were numerically higher than those obtained for female birds on deep litter and female birds on deep litter with run, respectively. It could be observed that in both sexes, birds on deep litter with a run housing type had increased serum cholesterol.

Figure 2 shows that the serum calcium of the male birds was higher than in female birds on both housing types. The birds (both male and female) on deep litter with run had increased serum calcium than those on deep litter housing type.

Discussion

The higher final weight, weight gain and feed intake of the birds with respect to deep litter housing type was in agreement with the findings of Sogunle et al. (2013) that chickens reared in confinement achieved considerably higher body weight compared to those on other housing type or management system. However, contrary to the submissions of

Castellini et al. (2008), the male birds on deep-litter with run housing type had the highest weight gain and feed intake in the interaction. The higher feed intake of the males group than females of similar ages had also been reported by Laseinde and Oluyemi (1996). This could be attributed to the higher activity of the males than the females. Fischer (1985) reported that male birds are behaviorally more active than the females; hence consume more feeds and gain weight faster than the females. The result of this study showed that deep-litter with run significantly decreased body weight and feed intake of female broiler chickens compared to the deep-litter housing type. This is in agreement with Castellini et al. (2002) which reported that outdoor rearing of birds reduced growth rate but with no particular reference to the sexes. This was expected because the chickens dispensed a lot of energy as they move freely on run. The housing types influenced the mortality rate as birds managed on deep litter with run had a higher mortality than those managed on deep litter. This further confirmed the findings of Olaniyi et al. (2012) who reported higher mortality in birds managed on free range as an alternative to total confinement.

Male broiler chicken on deep litter had the higher cost of feed intake per day than female broiler chicken which could be partly due to the higher feed intake recorded for the male broiler chickens. On the other hands, the female birds on deep-litter with run had the highest cost of feed intake per weight gain. The relatively reduced cost by female birds on deep litter is supported by the reports of Turkylmaz et al. (2002) that broilers in floor management were more profitable than cage system.

The variations in the cut-up parts and organs of the broiler chickens due to different housing types agree with the earlier reports of Verapeen and Driver (2000). However, Swain et al. (2002) reported no significant influence for system of rearing on live weight gain, feed intake and carcass. The relatively bigger male's cut-up parts than obtainable in female's cut-up parts could be attributed to higher growth rate of the males. The higher weight of the head of the males than the females could be due to the presence of bigger comb and wattles in the males. Also the higher weight of the shank of the males is probably due to the greater activities of the male broiler chickens in the different housing types. The higher percentage breast found in the female broiler chicken was in consonance with the reports of Merkley et al. (1980) who found significant differences between sexes with heavier breast weight but smaller leg in female birds when compared to the male birds. However, this contrasted with the findings of Goliomytis et al. (2003) who reported that chickens with heavier body weight produce a greater percentage of breast meat per carcass weight.

The serum cholesterol of male and female broiler chickens managed on deep litter with a run was higher than those managed on deep litter due largely to increased activity. On the contrary, Deshaies et al. (1983) reported that increased activity did not affect serum total cholesterol values. In female broiler chickens, the values obtained was numerically lower than those recorded for the male broiler chickens. This same trend was observed for the broiler chickens on the different housing types for serum calcium.

Conclusion

It could be concluded from this study that:

- Female broiler chickens on deep litter performed better than the males though the male broiler chickens consumed more feed than the females of similar age.
- Birds reared on deep litter had a higher carcass yield compared to those reared on deep litter with a run.
- The serum cholesterol and serum calcium of the broiler chickens were higher in the deep litter with a run housing type

Impact

The study was carried out for poultry farmers to further explore acceptable housing types/management systems that will engender profitable production of broiler chickens (sexed or unsexed) in order to bridge the ever widening protein consumption gap in developing countries. The study thereby confirmed profitable production of female broiler chickens in total confinement while the

male broiler chickens could survive and attain the expected market weight of 2 kg in either of the housing types studied.

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EVALUATION DES PLANS DE PROPHYLALAXIE APPLIQUES EN AVICULTURE MODERNE AU MALI : CAS DE LA MALADIE DE GUMBORO, DE LA MALADIE DE NEWCASTLE ET DES PARASITOSES INTERNES

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Resume

Cette étude a été réalisée pendant la période 2003-2007 et a concerné 22 exploitations avicoles semi-industrielles modernes (poules pondeuses) localisées dans les zones périurbaines du District de Bamako et des villes de Sikasso et Ségou. Elle a permis d'établir que chez 1163 poules pondeuses le taux d'immunité post-vaccinale global était de 93,9 % pour la maladie de Newcastle avec respectivement 89, 6 % à Ségou, 92,3 % à Sikasso et 96,3 % à Bamako. Cependant, pour la maladie de Gumboro, un taux de couverture immunitaire global de 50,5 % a été obtenu chez les poules pondeuses avec des variations en fonction des sites (40,7 % à Sikasso, 64,0 % à Ségou et 49,9 % à Bamako). L'examen de 89 échantillons de fientes collectés dans les élevages avicoles de la de la zone périurbaine du District de Bamako a permis de détecter des parasites internes dans 23 cas (25,8 %) et les espèces identifiées ont été Ascaridia gallii (Valeurs de l'OPG variant entre 50 et 100) et Eimeria spp. (Valeur OPG égale à 50) En outre, l'analyse de 28 échantillons de fientes collectés dans les élevages de Ségou a permis d'obtenir 5 cas d'infestation (17,8 %) avec des charges parasitaires très élevées pour les Heterakis avec une valeur del'OPG de1600 œufs/g de fèces et les Ascaris (1300 œufs/g de fèces). Sur 34 prélèvements de fientes collectés dans les élevages de poules pondeuses de Sikasso, 3 (8,8 %) contenaient des coccidies (50 œufs/g de fèces). L'étude a montré que dans les exploitations avicoles semi-industriellescouvertes, les poules pondeuses sont insuffisamment immunisées contre la maladie de Gumboro et nettement mieux contre la maladie de Newcastle .Elle montre également que les schémas de déparasitage dans la plupart des exploitations ne sont pas adaptés.

Mots clés: Aviculture, Plans de Prophylaxie, Pathologies, Mali

EVALUATION OF PROPHYLAXIS SCHEMES AS APPLIED TO MODERN POULTRY FARMING IN MALI: THE CASE OF GUMBORO DISEASE, NEWCASTLE DISEASE AND INTERNAL PARASITIC INFECTIONS

Resume

This study was conducted in 2003-2007 and involved 22 modern semi-industrial poultry farms (layers) located in the suburban areas of Bamako District and of Sikasso and Segou towns. It was found out that in 1163 layers, the global post- vaccination immunity level was 93.9 % for Newcastle Disease with 89, 6 %, 92.3% and 96.3 % in Segou, Sikasso and Bamako respectively. However, for Gumboro Disease, an overall immunization coverage of 50.5 % was obtained in layers with variations depending on the sites (40.7% in Sikasso, 64.0% in Segou and 49 9% in Bamako). Examination of 89 fecal samples collected in poultry farms in Bamako District peri-urban area showed internal parasites in 23 cases (25.8%), and species identified were Ascaridia gallii (EPG values ranging from 50 to 100) and Eimeria spp. (EPG value equal to 50). In addition, analysis of 28 fecal samples collected from farms in Segou identified 5 cases of infection (17.8%) with very high parasite loads for Heterakis with an EPG value of 1600 eggs/g of faeces and Ascaris (1300 eggs/g of feces). Out of 34 fecal samples collected in layer farms in Sikasso, 3 (8.8 %) contained coccidia (50 eggs/g of faeces).The study showed that in semi-industrial poultry farms, layers are inadequately immunized against Gumboro disease and markedly better immunised against Newcastle Disease. It also shows that deworming patterns in most farms are inadequate.
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Key words: Poultry farming; Prophylaxis schemes; Diseases; Mali

Introduction

L'aviculture occupe une place importante dans l'économie malienne. Le secteur moderne représente près de 2 % de l'effectif national (Kounta, 1992, 1993; Mali, DNSI, 2003; Mali, DNPIA, 2009).

Cette activité constitue une source précieuse de revenus et de protéines pour les populations.

Au cours de ces dernières années l'aviculture moderne a connu un essor considérable dans plusieurs zones périurbaines du Mali. Elle est présentement la principale source d'approvisionnement en œufs de consommation et de poulets de chair.

Les poussins sont essentiellement importés d'autres pays, même si des embryons d'unités industrielles de production locale de poussins d'un jour commencent à voir le jour. Des plans de prophylaxie sont aussi mis à la disposition des aviculteurs par les fournisseurs des poussins, malgré l'application desquels, des taux de mortalité élevés sont souvent enregistrés.

En aviculture moderne, les volailles sont vaccinées contre la maladie de Newcastle, la maladie de Gumboro, la bronchite infectieuse, la maladie de Marek et la variole aviaire.

En raison de nombreux cas de mortalité qui sont régulièrement enregistrés suite à la maladie de Newcastle et la maladie de Gumboro en aviculture moderne, la présente étude s'est fixée comme objectif d'évaluer l'efficacité des plans de prophylaxie mis en œuvre contre ces deux maladies infectieuses majeures.

Materiel et Methodes

Choix des exploitations

Les exploitations-test ont été choisies sur la base des résultats du recensement des exploitations avicoles modernes localisées dans les zones périurbaines du District de Bamako et des villes de Ségou et Sikasso, des résultats des entretiens avec les aviculteurs et services d'encadrement et de critères objectifs dont l'accord du promoteur pour collaborer et la localisation de l'exploitation dans un rayon de 50 km au maximum. L'étude a couvert 22 exploitations avicoles au total dont 16 en zone périurbaine du District de Bamako, trois en zone périurbaine de Sikasso et trois à Ségou.

Plans de prophylaxie

Les plans de prophylaxie comprennent entre autres, la vaccination des volailles contre la maladie de Newcastle en administrant des vaccins vivants par l'eau de boisson au 5e jour en primo-vaccination contre la maladie de Newcastle et la bronchite infectieuse avec un rappel avec un vaccin inactivé injectable, une semaine après la primo-vaccination. Le schéma de prophylaxie en vigueur prévoit par ailleurs l'administration de vaccin vivant en rappel pour booster l'immunité contre la maladie de Gumboro. Deux semaines après cette vaccination, un vaccin inactivé est administré afin de booster l'immunité contre la maladie de Newcastle.

Un anticoccidien était couramment utilisé à la deuxième semaine d'âge avec un rappel 1 à à 3 mois plus tard. Un vermifuge était aussi utilisé à deux mois d'âge avec un rappel une semaine plus tard dans le cadre du déparasitage interne.

Quant aux fientes, elles ont été récoltées à l'état frais à raison de cinq échantillons par poulailler. La fréquence du déparasitage interne dans les exploitations semi-industrielles de de Bamako a varié en selon l'exploitation (trois mois pour trois exploitations (21,4 %), quatre mois pour une exploitation (7,1 %), et elle était inconnue pour 8 fermes (57,1 %).

Le délai entre les opérations de déparasitage et de collecte des fientes a été de un mois pour une ferme (7,1 %), deux mois pour une exploitation (7,1 %), trois mois pour une exploitation (7,1 %), six mois pour une exploitation (7,1 %) et inconnu pour six exploitations (42,8 %).

La fréquence des opérations de déparasitage interne dans les exploitations semi-industrielles de Ségou a aussi varié d'une exploitation à l'autre. Parmi les six exploitations

visitées, une procédait chaque mois au déparasitage interne (16,6 %), une d'entre elles le faisait trimestriellement (16,6 %) et dans l'une des fermes, l'opération était réalisé avec une fréquence semestrielle (16,6). En outre, dans trois des exploitations concernées, les délais qui s'écoulent entre l'opération de déparasitage et la collecte de prélèvement ont varié entre un et six mois ; dans les trois autres exploitations, ce temps n'était pas connu.

Collecte de prélèvements et méthodes de diagnostic de laboratoire

L'évaluation de l'efficacité des programmes de prophylaxie a porté sur le dosage des anticorps post-vaccinaux dans les échantillons de sérums prélevés chez les volailles vaccinées et l'évaluation des charges parasitaires dans les fientes.

En vue d'évaluer l'efficacité des vaccinations réalisées contre la maladie de Gumboro et la maladie de Newcastle, 1189 et 1163 échantillons de sérums ont été collectés respectivement chez des pondeuses ayant été vaccinées contre ces maladies. Le sang a été prélevé à la veine alaire de l'oiseau sous un volume de 2 à 3 ml par sujet sur 10% de l'effectif total.

Le test d'inhibition de l'hémagglutination a été utilisé pour la détection des anticorps post- vaccinaux de la maladie de Newcastle selon la technique utilisée par Picault (1993). Par contre, un Kit Elisa commercialisé par IDEXX a été utilisé dans le cadre de la recherche des anticorps post-vaccinaux de la maladie de Gumboro.

La méthode quantitative de Mac Master a été utilisée afin de déterminer les charges des parasites internes.

Chaque prélèvement a été identifié individuellement (date de prélèvement, Code de l'exploitation, dates de la vaccination et du déparasitage interne).

Un enquêteur avait en charge le suivi clinico-épidémiologique et était tenu d'effectuer au moins deux passages par semaine dans les élevages au cours desquels il procédait à la collecte des prélèvements requis. Les enquêteurs ont été au préalable formés en techniques de prélèvement et à la collecte des données.

La supervision de leurs activités a été effectuée par des chercheurs de notre équipe de recherche à travers des missions ponctuelles trimestrielles.

Resultats

Les résultats obtenus portent sur l'évaluation des taux d'immunité chez des pondeuses vaccinées contre la maladie de Gumboro et la maladie de Newcastle.

Taux d'immunité chez des pondeuses vaccinées contre la maladie de Newcastle et la maladie de Gumboro

L'évaluation de l'immunité chez les poules pondeuses vaccinées contre la maladie de Newcastle a permis d'obtenir un taux d'immunisation global de 93,9 % (1093 sérums positifs sur 1163 testés). Ce taux a varié selon le site (92,3 % à Sikasso, 89,6 % à Ségou et 96,3 à Bamako) (Tableau I).

Par contre, suite à l'analyse de 1189 sérums au total, 601 sérums se sont révélés positifs (50,5 %) aux anticorps du virus de la maladie de Gumboro (Tableau II). Comme en maladie de Newcastle, ce taux a varié en fonction du site (64,0 % à Ségou, 49,9 % à Bamako et 40,7 % à Sikasso).

Charges parasitaires chez des sujets ayant reçu des vermifuges

Au cours de cette étude, 151 échantillons de fientes ont été examinés (89 à Bamako, 28 à Ségou et 34 à Sikasso).

La réalisation de 89 analyses coprologiques a révélé 23 cas positifs (25,8 %) au niveau du site de Bamako et les espèces d'helminthes identifiées ont été Ascaridia gallii, Heterakis. Eimeria spp. a été la seule espèce de protozoaire mise en évidence (Tableau III).

De l'analyse du tableau III, il ressort que sur 14 exploitations dans lesquelles des échantillons de fientes ont été collectés, Ascaridia gallii a été identifié dans cinq exploitations (35,7 %), les coccidies un cas (7,1 %).

Ainsi, sur 89 échantillons de fientes prélevés dans lesdites exploitations, 23 (25,8 %) se sont révélés positifs. Les valeurs des OPG pour Ascaridia gallii ont varié entre 500 et 100

Tableau I: Résultats du dosage d'anticorps post vaccinaux de la Maladie de Newcastle

Sites	Sérums testés	Age des oiseaux	Sérums testés	
			Nombre	%
Sikasso	302	7-12 mois	279	92,38
Ségou	232	7-17 mois	208	89,65
District de Bamako	629	3-17 mois	606	96, 34
Total	**1163**		**1093**	**93,98**

Tableau II: Résultats du dépistage d'anticorps post vaccinaux de la Maladie de Gumboro

Sites	Sérums testés	Age des oiseaux	Sérums testés	
			Nombre	%
Sikasso	302	7-12 mois	123	40,72
Ségou	250	7-17 mois	160	64
District de Bamako	637	3-17 mois	318	49, 92
Total	**1189**		**601**	**50,54**

Tableau III: Résultats des examens de fèces prélevés chez des poules pondeuses ayant reçu des traitements anthelminthiques et anticoccidiens (Site de Bamako)

Exploi-Tations	Echantillons de fientes			Charges parasitaires	Fréquence de déparasitage
	Total testé	Positifs	% des positifs		
BK/01	4	0	0	Absence de parasite	4 mois
BK/02	14	0	0	Absence de parasite	inconnue
BK/03	6	5	83,33	50 Ascaridia gallii	semestrielle
BK/04	5	0	0	Absence de parasite	Inconnue
BK/05	7	0	0	Absence de parasite	Inconnue
BK/06	9	0	0	Absence de parasites	Inconnue
BK/07	6	4	66,66	100 Ascaridia gallii	Inconnue
BK/08	10	3		100 Ascaridia gallii	Trimestrielle
BK/09	2	0	0	Absence de parasite	Inconnue
BK/010	2	1	50	50 Ascaridia galli	Inconnue
BK/011	11	10	90,90	100 Ascaridia gallii 500 Heterakis 50 Eimeria spp.	Trimestrielle
BK/012	3	0	0	Absence de parasite	Trimestrielle
BK/013	5	0	0	Absence de parasite	Inconnue
BK/014	5	0	0	Absence de parasite	Inconnue
Total	**89**	**23**	**25,8**		

œufs /gram de fèces. Par ailleurs, pour Eimeria spp. et Heterakis, les valeurs dudit paramètre obtenues étaient de 50 et 500 œufs/gram de fèces respectivement.

La fréquence des déparasitages était connue pour cinq exploitations sur 14 visitées (35,7 %) et se situait entre trois et six mois.

L'examen de 28 échantillons de fientes collectés à Ségou a permis d'obtenir 5 cas d'infestation avec un taux global de 17,8 % avec des charges parasitaires très élevées pour les Heterakis (1600 œufs/g de fèces) et ascaris (1300 œufs/g de fèces) (Tableau IV). Au niveau de ce site, Heterakis gallinarum a été mis en évidence dans l'exploitation SG/02 dont les pondeuses n'avaient reçu que l'anticoccidien

Tableau IV: Résultats des examens de fèces prélevés chez des poules pondeuses ayant reçu des traitements anthelminthiques et anticoccidiens (site de Ségou)

Exploi-tations	Echant. examinés	Echant. Positifs	% des positifs	Charges parasitaires	Fréquence de déparasitage
SG/01	16	0	0	Absence de parasite	Trimestrielle
SG/02	9	1	22,22	1600 Heterakis 1300 Ascaridia gallii	Semestrielle
SG/03	9	0	0	Absence de parasite	Mensuelle
SG/06	3	2		200 Ascaridia gallii	Inconnue
SG/08	1	0	0	Absence de parasite	Inconnue
SG/07	5	2	40	250 Ascaridia gallii	Inconnue
Total	**28**	**5**	**17,85**		

Tableau V. Résultats des examens de fèces prélevés chez des poules pondeuses ayant reçu des traitements anthelminthiques et anticoccidiens (Site de Sikasso)

Exploi-tations	Echant. examinés	Echant. Positifs	% des positifs	Charges parasitaires	Fréquence de déparasitage
SK/01	9	0	0	Absence de parasite	Trimestrielle
SK/02	9	0	0	Absence de parasite	Trimestrielle
SK/03	9	0	0	Absence de parasite	Trimestrielle
SK/04	6	3	50	50 Eimeria spp	Semestrielle
SK/05	1	0	0	Absence de parasite	Trimestrielle
Total	**34**	**3**	**8,82**		

(1600 OPG). Par contre, Ascaridia gallii a été identifié avec une charge parasitaire variant entre 250 et 1300 œufs par gramme de fientes dans la plupart des élevages (83,3 %).

Dans ce site, la fréquence du déparasitage n'était connue que dans 50,0 % des exploitations.

Parmi les exploitations avicoles semi-industrielles de Sikasso dans lesquelles des échantillons de fientes ont été collectés, seule une (SK/04) s'est révélée infestée par les coccidies (20,0 %) avec trois fientes infestées sur six examinées (50,0 %). Ceci dénote de l'efficacité des opérations de déparasitage réalisées dans les exploitations SK/01, SK/02, SK/03, SK/05 (Tableau 5). De l'examen du tableau 5, il ressort que sur 34 échantillons de fientes collectés, trois se sont avérés positifs aux coccidies (8,8 %). La charge parasitaire de ces coccidies a été de 50 œufs par gramme de fiente. Dans les exploitations avicoles de Sikasso, la fréquence du déparasitage a varié entre trois mois (80,0 %) et six mois (20,0 %).

Discussion

La qualité de la protection vaccinale contre la maladie de Newcastle et la maladie de Gumboro a été estimée par le titrage des anticorps sériques respectivement aux tests d'inhibition de l'hémagglutination selon la technique utilisée par Picault (1993) et

d'immunodiffusion sur gélose.

Si la résistance à l'épreuve virulente est le seul moyen de mesurer la protection globale contre les infections, la recherche des anticorps permet de mettre en évidence indirectement une infection ou une prise vaccinale (Fournier et al, 1995). En raison des moyens assez limités, la réalisation des examens sérologiques constitue un excellent moyen de contrôle de la réussite de la vaccination au Mali.

L'évaluation des plans de prophylaxie appliqués en aviculture moderne a montré que les poules pondeuses sont insuffisamment immunisées contre la maladie de Gumboro (50,5 %).

En revanche, un taux nettement plus élevé a été obtenu pour la maladie de Newcastle (93,9 %). Arbelot et al. (1997) ont établi le même constat au Sénégal. Ils ont en effet montré que sur 53 bandes de poulets de chair et 34 bandes de poulettes, à 45 jours d'âge, la majorité des lots de poulets de chair n'étaient pas protégés contre la maladie de Gumboro (89,0 % des lots). Arbelot et al (1995) ont fait l'évaluation quand les sujets avaient 30 - 45 jours, âge auquel le risque d'éclosion de la maladie de Gumboro est très élevé. Pour la maladie de Newcastle, ce taux était de 83,0 % des lots. Les auteurs ont par ailleurs montré que 62,0 % des élevages de pondeuses à l'entrée en ponte étaient insuffisamment protégés contre la maladie de Newcastle et la couverture vaccinale des poulettes d'un mois contre la maladie de Gumboro était insuffisante pour 95,0 % des lots.

Par ailleurs, Sagna (1977) montre que la première semaine d'âge est indiquée pour une vaccination efficace contre la maladie de Gumboro, l'immunité devenant solide 2 à 4 jours après l'administration du vaccin. Fabienne et al. (2009) expliquent les échecs des vaccinations contre la maladie de Newcastle par les variations phylogénétiques existant entre les souches vaccinales et les souches virulentes circulantes et estiment que de nouveaux candidats vaccins, moins sensibles aux anticorps maternels et plus proches génétiquement des souches circulantes sont nécessaires pour améliorer l'efficacité des vaccins commerciaux conventionnels.

La présente étude montre qu'il existe des contraintes liées à l'administration de certains vaccins dans l'eau de boisson. Plusieurs phénomènes peuvent expliquer les échecs observés : mauvaise conservation du vaccin, mauvaise concentration vaccinale, présence de désinfectants dans l'eau utilisée, abreuvoirs mal nettoyés, utilisation de vaccins reconditionnés. Par conséquent, il est indispensable d'initier les aviculteurs aux bonnes pratiques de vaccination.

A la différence de certains pays, la vaccination contre les coccidioses ne se pratique pas en aviculture moderne au Mali, les aviculteurs privilégiant le traitement anticoccidien et contre les autres parasitoses internes avec une fréquence variant entre 1 à 6 mois, même si cette fréquence n'était pas connue dans la majorité des exploitations localisées en zone péri-urbaine du District de Bamako et 50,0 % de celles de Ségou. L'immunité naturelle contre les coccidioses est souvent insuffisante et toute agression par un agent pathogène ou par un stress d'élevage contribue à la baisse la résistance des volailles (Maho et Ndobale, 1997).

Conclusion

Cette étude a permis d'établir qu'en aviculture moderne, les poules pondeuses sont insuffisamment immunisées contre la maladie de Gumboro et nettement mieux contre la maladie de Newcastle. Elle a également montré que les schémas de déparasitage étaient inadaptés dans la plupart des exploitations avicoles visitées. D'autres études devront être menées en vue de déterminer les principales causes des échecs de vaccination contre la maladie de Gumboro en aviculture moderne au Mali.

Remerciements

Les auteurs adressent leurs sincères remerciements au Service d'Aide et Coopération de l'Ambassade de France au Mali pour son appui financier qui a permis la réalisation de la présente étude, aux techniciens des différents laboratoires impliqués dans l'exécution des examens de laboratoire et aux agents des services d'encadrement pour leur contribution de qualité.

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PRACTICES AND CONCERNS OF CASTRATION OF DOGS IN NIGERIA

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Abstract

This study evaluated the practices and concerns regarding castration, and level of awareness of Nigerian Veterinarians on alternatives to surgical castration in dogs. Questionnaires were distributed to one hundred veterinarians during the 2012 annual Veterinary Association Conference. The questionnaire comprised of the demography of respondents, record of castration performed, practice of castration, complications associated with castration and awareness on alternative castration methods. Descriptive statistics comprising frequency table, cross tabs and chi square test were used to analyze data. Ninety five (95) of the questionnaires were completed and returned. Majority of respondents (52.1 percent) had postgraduate qualifications, worked in mixed practices (58.7 percent) and have been in practice for less than ten years (64.2 percent). Castration is often done in mixed breeds of dogs (42.7 percent) and for elective reasons (84.3 percent). Surgical method (95.6 percent) is the major method used for castration. Surgical castrations are done using sedation and local anaesthesia (91.0 percent), while majority of respondent do administer analgesics (54.5 percent) and antibiotics (89.9 percent) after castration. Scrotal swelling (27. 6 percent) and scrotal mutilation (11.5 percent) are the most frequently reported complications following castration. Majority of respondents (67.4 percent) are aware of alternative castration methods, however only few (9.7 percent) have used alternative methods before. In addition, majority of respondent (68.4 percent) are willing to adopt non-surgical castration. Level of education, number of years in practice and type of practice did not significantly (P> 0.05) influence castration method, frequency of complication and awareness of alternative castration method. The results of this study showed that most veterinarians prefer surgical castration and complications associated with surgical castration is minimal.

Key words: Concern, Practice, Castration, Veterinarians, Alternative, Dogs

PRATIQUES ET PREOCCUPATIONS CONCERNANT LA CASTRATION DES CHIENS AU NIGERIA

Résumé

Cette étude a évalué les pratiques et les préoccupations concernant la castration, et le niveau de connaissances des vétérinaires nigérians sur les alternatives à la castration chirurgicale des chiens. Des questionnaires ont été distribués à cent vétérinaires au cours de la conférence annuelle de l'Association vétérinaire en 2012. Le questionnaire portait sur la démographie des répondants, les informations sur les castrations effectuées, la pratique de castration, les complications liées à la castration et la connaissance d'autres méthodes de castration. Des statistiques descriptives comprenant la table de fréquence, les tables croisées et le test du chi carré ont été utilisées pour l'analyse des données. Quatre-vingt quinze (95) des questionnaires ont été remplis et retournés. La majorité des répondants (52,1 pour cent) avaient un diplôme post-universitaire, travaillaient dans des pratiques mixtes (58,7 pour cent) et étaient dans la profession depuis moins de dix ans (64,2 pour cent). La castration se fait souvent pour les races mixtes de chiens (42,7 pour cent) et pour des raisons d'urgence (84,3 pour cent). La méthode chirurgicale (95,6 pour cent) est la principale technique utilisée pour la castration. Les castrations chirurgicales sont effectuées sous sédation et anesthésie locale (91,0 pour cent), tandis que la majorité des répondants administrent des analgésiques (54,5 pour cent) et des antibiotiques (89,9 pour cent) après la castration. Le gonflement du scrotum (27, 6 pour cent) et la mutilation du scrotum (11,5 pour cent) sont les complications les plus fréquemment rapportées à la suite de la castration. La majorité des répondants (67,4 pour cent) étaient au courant des méthodes de castration alternatives, mais seulement quelques-uns (9,7 pour cent) d'entre

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eux les avaient pratiquées. En outre, la majorité des répondants (68,4 pour cent) sont disposés à adopter la castration non-chirurgicale. Le niveau de formation, le nombre d'années de pratique et le type de pratique n'ont pas significativement (P> 0,05) influencé la méthode de castration, la fréquence des complications et la connaissance des méthodes de castration alternatives. Les résultats de cette étude ont montré que la plupart des vétérinaires préfèrent la castration chirurgicale, et que les complications associées à la castration chirurgicale sont minimes.

Mots-clés : éoccupation ; Pratique ; Castration ;Vétérinaires ;Alternative ; Chiens

Introduction

Populations of dogs vary between different habitats, different cultures and different social strata of the human populations (Amber, 2008). In developing countries, a large number of free roaming dogs exist. The presence and abundance of stray dogs depends on the attitudes towards dogs of the humans in these areas. Stray dogs have a great impact on human public health and the ecosystems. Due to the close contact with humans, dogs are responsible for the spread of several zoonotic diseases. Additionally, domestic dogs are potentially effective predators of the native fauna and can have competitive interactions with endemic wild carnivores (Butler et al., 2004). Preying of small mammals and birds and transmission of diseases can have disastrous consequences for ecosystems in which feral dogs become established.

Pet overpopulation is a serious problem that results in animal abandonment and relinquishment. It places strain on animal shelters and shelter personnel, and leads to the euthanasia of unwanted pets (Bartlett et al., 2005, McNeil and Constandy, 2006). Population control of dogs and cats is thus recognized by the American Veterinary Medical Association as a primary welfare concern of American society.

Castration can be defined as the direct or indirect removal or destruction of the testes in male animals in order to render them impotent and prevent the production of testosterone (Spain et al., 2004). Castration is widely accepted for population control and helps to prevent high population densities, animal suffering and the spread of zoonosis. In addition, castration helps to prevent behavioral problems such as control of aggression towards other animals or the handlers. Castration has also been shown to reduce the risk of certain tumors and diseases (Kustritz, 2007).

There has been an exponential growth in the number of pet dogs being kept in Nigeria. In addition, the number of stray dogs or abandoned dogs is increasing in Nigeria with attendant risk of rabies and other dog related injuries to humans. However, it appeared that the frequency of request for gonadectomy is very small compared with the number of dogs being kept. There appeared to be no record on the frequency of castration in dogs in Nigeria and the poor attitude of owners towards castrating their dogs might have contributed to the high prevalence of stray or abandoneed dogs. This study aimed at generating data on the actual rate of castration in dogs in Nigeria, the techniques of castration being employed and the concerns of veterinarians over this technique.

Materials and Methods

This study made use of structured questionnaire which was pretested for accuracy and ease of administration. Questionnaires were distributed to one hundred Veterinarians during the 2012 annual Veterinary Association Conference. The questionnaire comprised of the demography of respondents, record of castration performed, practice of castration, complications associated with castration and awareness on alternative castration methods. Questions on demography included sex, age and academic and professional qualifications of the respondent. Other areas covered include the location and nature of the practice. Questions regarding the practice of castration included the method of castration, technique of surgical castration, the anaesthetic technique, administration of analgesics and antibiotics after castration. Questions regarding complications associated with castration included the nature of complication recorded and frequency of occurrence. Questions regarding the

Veterinarians' awareness of alternatives to surgical castration included type of non-surgical castration they are aware of, previous use of non-surgical castration methods and willingness to adopt non-surgical method of castration Descriptive statistics comprising frequency table, cross tabs and chi square test were used to analyze data. Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) version 19.0 (IBM Corporation, USA). A P- value less than 0.05 was considered significant in all cases.

Results

Ninety five (95) of the questionnaires were completed and returned given a response rate of ninety five percent (95%). Majority of the respondents were male (Fig 1) and age ranged between 21-50 years (Fig 2). The respondents worked either in Veterinary Teaching Hospital, State Veterinary Hospital, Private Veterinary Clinics and other Veterinary agencies (Fig. 3). Majority of the respondents had mixed type of practice (Fig 4). The question on record of

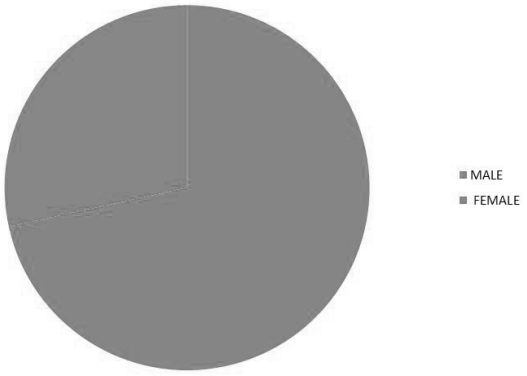


Figure I: Sex Distribution of the Respondents

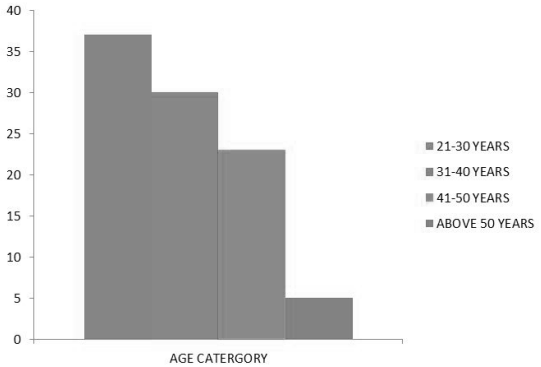


Figure 2: Age Distribution of the Respondentsents

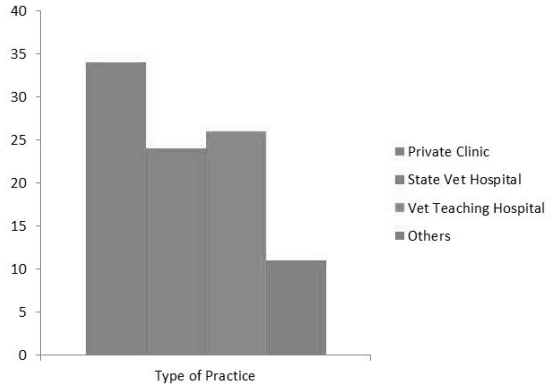


Figure 3: Distribution of type of Practice

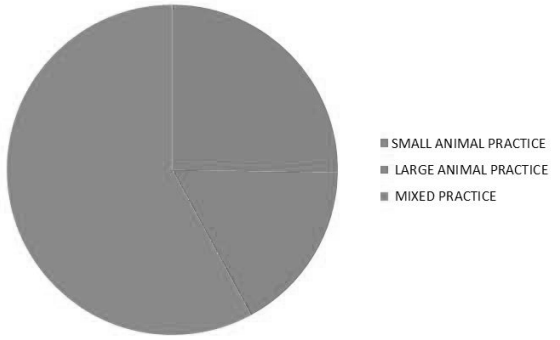


Figure 4: Distribution of Nature of Practice

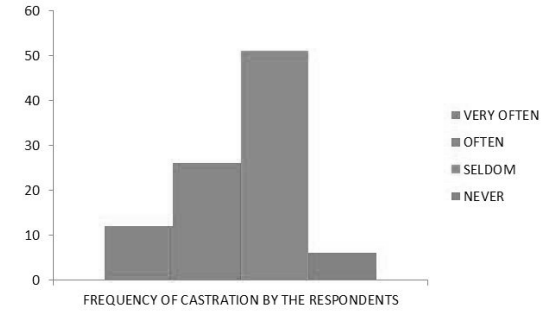


Figure 5: Frequency of Castration by Respondents

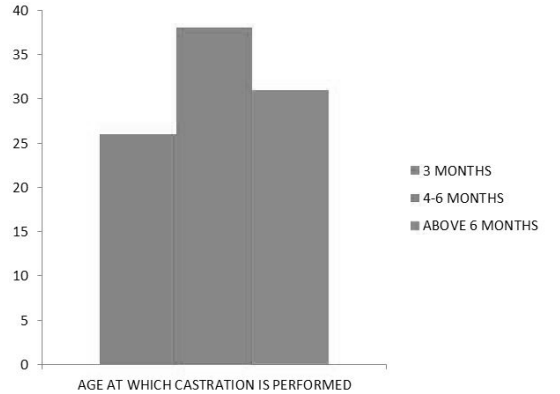


Figure 6: Distribution of Age at which castration is performed

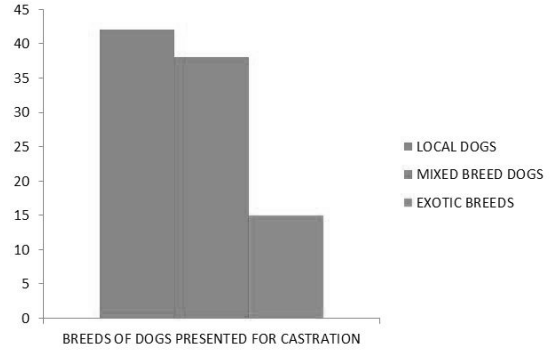


Figure 7: Distribution of breeds of dogs often castrated

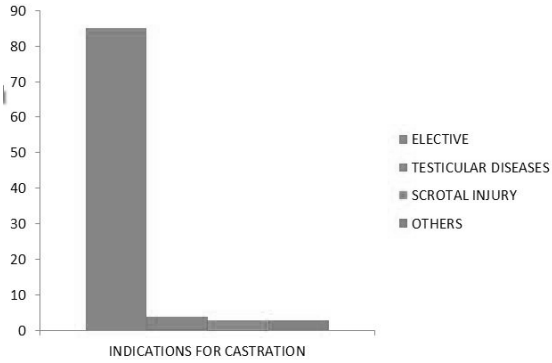


Figure 8: Distribution of indications for castration

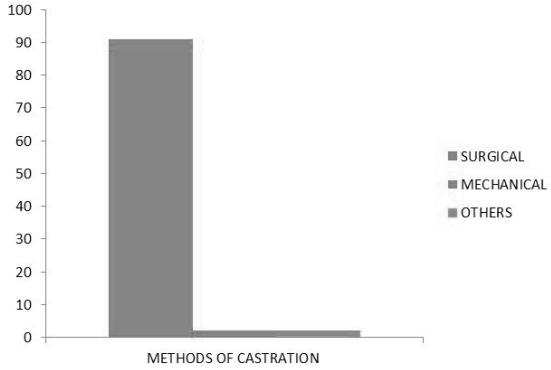


Figure 9: Distribution of methods of castration

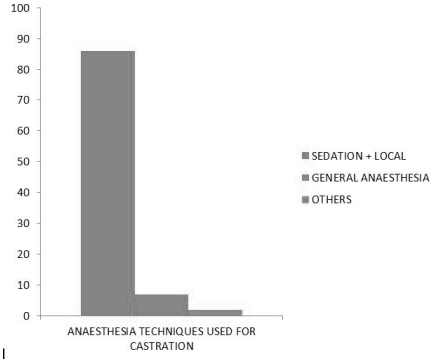


Figure 10: Distribution of techniques of anaesthesia used for castration

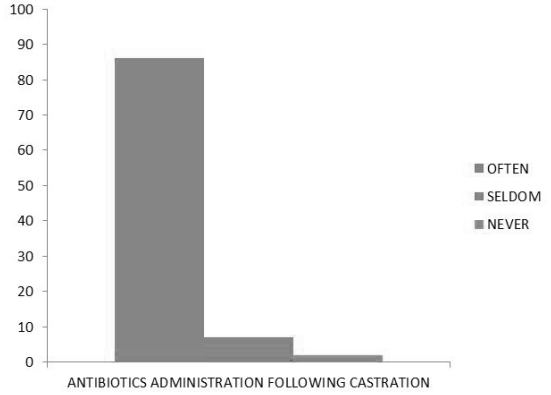


Figure 11: Distribution of antibiotic usage during castration in dogs

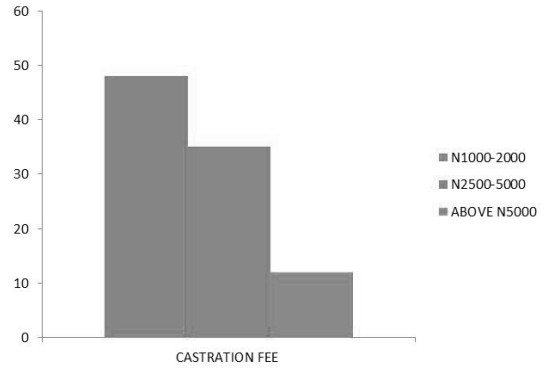


Figure 12: Distribution of castration fee in dogs

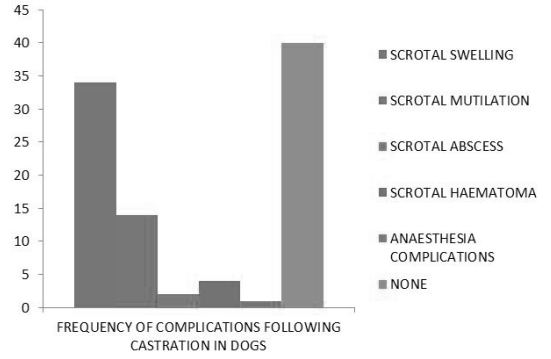


Figure 13: Distribution of complications during castration in dogs

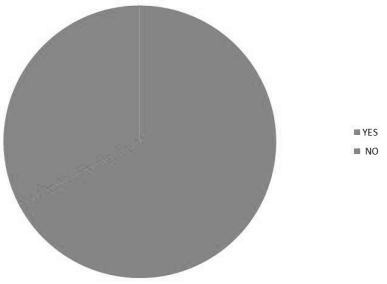


Figure 14: Awareness of alternative methods of castration in dogs

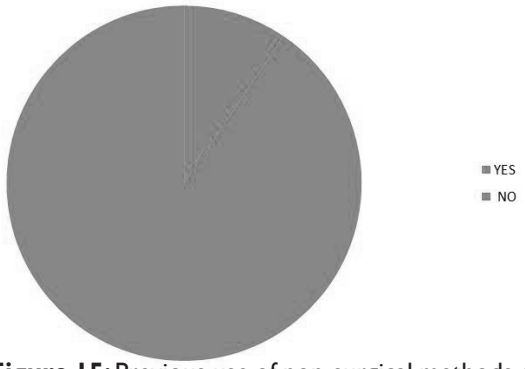


Figure 15: Previous use of non-surgical methods of castration in dogs

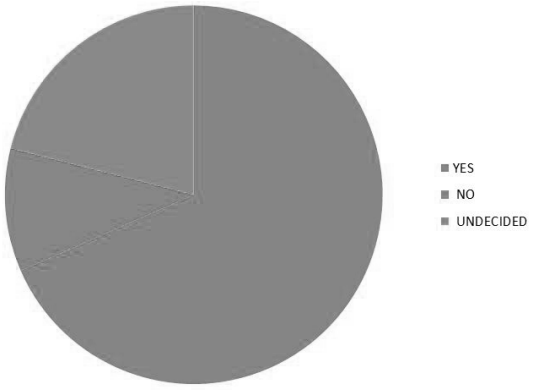


Figure 16: Willingness to adopt non-surgical methods of castration in dogs

castration performed showed that most of the respondents seldomly perform castration (Fig. 5) and most of the castrations are done between 4-6 months of age (Fig 6). Castration is often done in local dogs and mixed breeds of dogs (Fig. 7) mainly for elective reasons (Fig. 8). Surgical method (95.6 percent) is the major method used for castration (Fig. 9), while surgical castrations are done using sedation and local anaesthesia (Fig. 10). Majority of respondent do administer analgesics (54.5 percent) or antibiotics after castration (Fig. 11). Fees charged for castration ranged between N1, 000 and N5, 000 (Fig. 12). Complications often experienced after surgical castration included scrotal swelling (27. 6 percent), scrotal mutilation (11.5 percent) and haematoma (Fig. 13). Majority of respondents (67.4 percent) are aware of alternative castration methods (Fig. 14), however only few (9.7 percent) have previously used non-surgical castration methods before. In addition, majority of respondent (68.4 percent) are willing to adopt non-surgical methods of castration. Level of education, number of years in practice and type of practice did not significantly ($P > 0.05$) influence castration method, frequency of complication and awareness of alternative castration method. The results of this study showed that most Veterinarians prefer surgical castration, although most of the respondent reported complication following surgical castration.

Discussion

The result of this study showed that surgical castration is still the main method of gonadectomy in male dogs even though the procedure is seldomly carried out. In addition, dog castration in Nigeria is often carried out between 4-6 months of age and for elective reasons and that sedation with local anaesthesia is the main anaesthetic technique used for castration.

The prevalence of castration among dogs has been shown to be related to the economic status of the country (Amber, 2008). For instance, the overall prevalence of castration in dogs in the United States of America was reported to be about 90 percent (Trevejo et al., 2011). Although the exact prevalence of dog castration in Nigeria was not determined, the results suggested that castration is seldomly performed. This is in agreement with earlier report which stated that people in Nigeria are against castration as they want more animals to sell for financial gain (Amber, 2008). Considering the rate of dog abandonment and free roaming dogs in Nigeria and the implication this has on the spread of rabies, it is thus suggested that more awareness is required on the benefits of castration in dogs.

There is controversy on the appropriate age for castration in dogs. Early castration in dogs has been attributed to result in poor development of the secondary sexual characteristics in dogs (Root et al., 1997). Other adverse effect of early age castration was delayed closure of the distal radial physis (Bloomberg, 1996). Results of this study showed that castration is often done early in dogs in Nigeria. A study in the United States of America showed that 55 percent of dogs are castrated between 6-12 months of age (Trevejo et al.,

2011). The exact reason for early castration in dogs in Nigeria was not established in this study, neither did we evaluate the adverse effect associated with this practice. However, it may be that early castration might be the tradition of surgical castration in Nigeria.

There are welfare concerns regarding surgical castration. Such concerns are that surgery is painful and places the animal at risk because it requires general anaesthesia (Soto et al., 2005). This study showed that sedation with local anaesthesia is majorly used for surgical castration in Nigeria thus implying that the risk associated with anaesthesia is negligible. The preference for this anaesthetic technique may be related to the nature of most practices in Nigeria in which most practices are lone operators, while majority of the facilities do not have trained anaesthetist. The welfare issue with the use of sedation and local anaesthesia may be that adequate analgesia may not be provided during castration. However, the practice of administering analgesics agent after castration might be an advantage.

A study of Canadian Veterinary Private Practitioners found complication rates of 19% for castrating male dogs (Pollari & Bonnet, 1996). Serious complications such as infections, scrotal abscesses, rupture of the surgical wound, and chewed out sutures were reported at a 1- 4% frequency, with castration surgeries accounting for 10% of these complications (Lund et al., 2006). The results of this study showed that scrotal swelling and scrotal mutilation are the major complications associated with surgical castration in dogs in Nigeria, even though the exact prevalence of the these complications were not determined in this study.

Owing to growing welfare concerns regarding surgical castration in dogs, several non-surgical methods of castration have been reported in dogs. These methods include chemical castration using Zinc gluconate or Calcium chloride (Jama & Samantha, 2007; Levy et al., 2008) and immunocastration using gonadotrophin releasing hormone vaccines (Kutzler & Wood, 2006). The advantages associated with non- surgical castration include minimal complications and the lack of need for general anaesthesia. In addition, it holds the promise that many animals can be castrated within a short period even in areas where trained surgical personnel and facilities are not available. This study showed that even though most Nigerian veterinarians are aware of non-surgical castration, surgical castration is still the traditional method in Nigeria. This may be due to the fact that the drugs required for the non-surgical castration is not available or the low level of the awareness of the benefits of non-surgical castration.

In conclusion, the large number of roaming dogs in Nigeria supports the findings that castration is seldomly done in Nigeria. However, owing to the endemic nature of animal and human rabies in Nigeria and the role that dogs play in the epidemiology of rabies, it will thus be very important to increase advocacy on the need for people to embrace castration of dogs as well as increasing awareness on non-surgical castration in dogs.

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RESPONSE OF PRIMIPAROUS UDA EWE TO CONCENTRATE SUPPLEMENTATION

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Abstract

Eight Uda weighing between 34-39kg, and aged 2-3years were used for this study. They were fed with 2kg of concentrate supplement (per day) divided into two parts (morning and evening). The weight all the ewes were taken before the commencement of feed supplementation, and for 14 weeks after at 2 weeks interval. Blood samples were collected for haematology and serum chemistry before the commencement of feeding. A significant increase ($P<0.05$) in weight of the Uda ewe was observed after the commencement of feed supplementation.

A microcytic hypochromic anaemia which is believed to be nutritionally induced was observed. Certain nutrients needed for haemopoesis in a large sheep breed were believed to be inadequate. Furthermore reduction of Blood glucose, Blood Urea Nitrogen (BUN) and Total Protein following supplementation corroborates the inadequacies in the constituents of the ration used. It was concluded that this ration may only be beneficial to support physiological functions in a large breed of sheep like the Uda breed provided extensive mineral supplementation as well as crude protein and energy level are increased.

Key Words: Primiparous Uda Ewe, Concentrate Supplement, Heamatology, Serum Chemistry.

REPONSE DES BREBIS UDA PRIMIPARES A UNE SUPPLEMENTATION EN ALIMENTS CONCENTRES

Resume

Huit brebis Uda pesant entre 34 et 39 kg et âgés de 2 ans à 3 ans ont été utilisées pour cette étude. Elles ont reçu 2 kg de supplément concentré (par jour) divisé en deux parties (matin et soir). Le poids de toutes les brebis a été enregistré avant le début de la supplémentation alimentaire, et ensuite pendant 14 semaines, à intervalle de 2 semaines. Des échantillons de sang ont été prélevés pour l'hématologie et la chimie sérique avant le début de l'alimentation. Une augmentation significative ($P < 0,05$) du poids des brebis Uda a été observée après le début de la supplémentation alimentaire.

Une anémie microcytaire hypochrome qui semblerait être nutritionnellement induite a été observée. Certains nutriments nécessaires pour l'hématopoïèse chez une grande race de moutons ont été jugés insuffisants. En outre, la réduction de la glycémie, de l'azote uréique du sang (BUN) et de la protéine totale à la suite de la supplémentation corrobore les insuffisances des constituants de la ration utilisée. Il a été conclu que cette ration ne peut être bénéfique que pour soutenir les fonctions physiologiques chez une grande race de moutons comme la race Uda, à condition qu'une supplémentation extensive en minéraux et en protéines brutes et le niveau d'énergie soient augmentés.

Mots-clés : Brebis Uda primipares ; Supplément concentré ; Hématologie ; Chimie sérique.

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Introduction

Sheep is widely distributed throughout the world, having a capacity of adaptation which has permitted it to survive in a great variety of environment from arid zones and semi-deserts to hard mountainous regions (Anonymous, 1980).

20% of the world sheep population is located in the tropical and sub-tropical regions with the production of meat being the major objectives and to a lesser extent, production of skin, milk, manure and wool (Anonymous, 1980).

The Uda breed of sheep is found in Northern Nigeria, Southern Niger, Central Chad, Northern Cameroun and Western Sudan. Also known as Oudah biclore (French), Bali-Bali, Bororo, Fellata, Foulbe, Houda, Louda, North Nigerian Fulani, Ouda, Pied. (Rim, 1989).

The Uda is one of the hair sheep breeds of the Sahel type. It is a meat breed. It is a long –legged breed of sheep with distinctive coat colour of brown or black anterior and white posterior. They are large with straight and long face. The Rams of the Uda are horned and the ewes are usually polled. The Uda is slightly smaller bodied than the Balami. Milk yield per lactation ranges between 32 and 36kg for an average lactation length of 91 days. (Rim, 1989).

Large size up to 85cm (male 75-85cm, female 65-75cm). Weight: male 65kg, female 45kg. Head long and heavy with flat forehead, often with a central depression, and slightly converse profile.

Horns are almost universally present in males, long and spirally twisted growing horizontally out from head. When present in females, it is short and fine, bars pendent, thin, long to very long about 22cm (Wilson, 1991).

The essential nutrients are proteins, carbohydrates, fats, minerals and vitamins all of which are provided by the forage feed and may be supplemented where early maturity is required. Water is also essential to diet. The highly variable nature of the food normally consumed by tropical sheep makes it difficult to be precise as regards their nutritive requirements (Davendra and Mcleroy, 1982).

Supplements are foods which are fed to sheep only in small quantities in addition to grasses and basal diet and which supply essential nutrients (Charry et al., 1992). Most of the foods eaten by sheep are roughages, which have low level of metabolisable energy and crude protein. Supplements of energy (energy concentrates or molasses) and protein (protein concentrates or non-protein nitrogen) therefore increase productivity (Charry et al., 1992).

Supplements are therefore given only to categories of sheep in which there is a large response in production. (Essential for ewes in late pregnancy and early lactation and finishing lambs or at times when the natural vegetation is of very poor quality i.e towards the end of the dry season or in drought) (Charry et al., 1992).

Feed supplementation is advised to be given to does and ewes to improve mothering ability, prevent abortion and to be able to sustain multiple birth since the weight of the ewes and does determine the litter size and birth weight (Oyeyemi et al., 2001).

This category of feedstuffs is the cereals, grains, numerous other seeds, oil cake meals, and agro-industrial bye products. They are highly digestible, possess low fibre content and are rich in protein. On the basis of protein content, concentrates may be further divided into carbonaceous feed with relatively low protein content such as the cereal grain and nitrogenous feed that are rich in protein such as the various oil cakes and animal by-products (Gatenby and Rim, 1991). Sheep are often allowed to eat as much food as they want (although not always offered the type of food they like best). The intake of nutrients depends on the type of food available and amount eaten. Sheep will eat more of fine food than coarse food for the reason that straw is sometimes chopped. Intake also depends on the energy density of the diet. (Gatenby and Rim, 1991).

There is dearth of information on effects of concentrate supplementation on the hematology and serum chemistry of Uda ewe. Hematology and serum chemistry of Uda ewe is pivotal to the reproductive potential of the animal.

The outcome of this project could provide important baseline data needed for

livestock farmers and scientists in providing necessary supplementation for maximum production.

Materials and Method

Location

The study was carried out at the Small Ruminant Unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan. The average temperature of this location ranged from 24oc in the rainy season to 33oc in the dry season. The relative humidity ranged from 46.3% in the dry season to 81.0% in the rainy season.

The Experimental Animals

This consists of eight clinically healthy primiparous Uda ewes obtained from a local market in Ibadan, Nigeria aged between 2 and 3 years.. The weight of the animals ranged between 34kg and 39kg. The animals which were free from obvious reproductive diseases were kept under the same condition in a washed and disinfected pen for a period of 10days to acclimatize.

During this period, animals were treated against helminthes parasite with Albendazole. Rectal temperature, heart rate, pulse rate and respiratory rates were monitored before the start of the experiment.

Management of Experimental Animals

The semi-intensive system of management was adopted. The house was designed with concrete floor and allowed for cross ventilation with a side paddock for grazing. The pen was routinely cleaned, washed and disinfected with disinfectant (Lysol). Pen measures 4.70x3.14m in size.

The animals were zero fed in the morning, while they were released to graze on guinea grass (Panicum maximum) and elephant grass (Pennisetum pueperium) between 0700 and 1200 hours each day. Animals were given 2kg of concentrate daily and water ad libitum.

Routine medication include deworming using Albendazole bolus at a dosage rate of one bolus per 25kg body weight (as a broad spectrum anthelmintic) against all classes of worms in small ruminants. Prophylactic antibiotic was administered using oxytetracycline hydrochloride (oxytetra) at a dose rate of 50mg/10kg for 4days.

Experimental Protocol

Feed Supplementation

Each of the eight ewes, in addition to grazing on guinea grass (Panicum maximum) and elephant grass (Penisetum pupureum), were given supplement feed of 2 kilograms twice daily in divided parts of 1 kilogram in the morning and evening. Fresh clean drinking water was provided ad-libitum.

Proximate Analysis of Experimental Feeds

The proximate analysis of the experimental diets and the various constituents made up of corn meal, wheat offal, palm kernel cake, dry brewer's grain and ground nut cake was done using the Association of Official Analytical Chemists (AOAC, 1990) method of analysis. This is shown in tables 1 a and b.

Body Weight and Blood Sample Collection

Prior to the commencement of the experiment, the ewes were weighed and their weight recorded while blood samples were collected through the jugular vein. About 3ml of blood samples were collected at each instance. The samples were placed in sample bottles with EDTA (anticoagulant) for hematological analysis.

This was done to identify any differences in their weighst and hematological parameters during the experiment.

The weights of the ewes were taken on weekly basis. Blood samples were also collected from the jugular vein of the ewes into EDTA sample bottles at interval of 3weeks.

Statistical Analysis

The mean and standard deviation of mean of collected data were calculated. The results of these studies were subjected to statistical analysis using one way Analysis Of Variance (ANOVA) and values less than 0.05 were taken as being significant.

Table 1: Composition and Proximate Analysis of the ration used in the study

a) Composition

Ingredients	Percentage component
Corn meal	20
Wheat offal	20
Palm kernel cake	16
Brewer's grain (dry)	40
Groundnut cake	3.5
Salt	0.4
Mineral/premise	0.1

Source: Akusu and Oyeyemi (1998).

b) Proximate Analysis

Chemical composition	Percentage component
Crude protein	23.50
Fat	6.16
Crude fibre	10.6
Ash	13.42
Moisture content	2.69
Energy	2,271.45 cal/kg
Mineral/premise	0.1

Source: Akusu and Oyeyemi (1998).

Result

Haematology

Table 2: Mean + standard deviation of haematological parameter of ewes during experiment.

Hematological Parameter	Control	3rd Week	6th Week
Pack Cell Volume (PCV)	38.50±.51^{a}	26.88±7.04^{b}	22.00±1.07^{b}
Haemoglobin (HB)	12.80±1.69^{a}	7.10±0.72^{b}	7.30±0.43^{b}
Red Blood Cell (RBC)	11.64±1.18^{a}	8.84±2.15^{b}	10.035±0.66^{b}
White Blood Cell (WBC)	9.15±2.61^{b}	7.00±1.34^{c}	12.10±1.39^{a}
PLATELETS	13.00±1.07^{a}	9.75±2.49^{b}	6.00±00c
Mean Corpuscular Volume (MCV)	32.50±2.33^{a}	30.88±8.82^{a}	21.50±0.54^{b}
Mean Corpuscular haemoglobin (MCH)	10.50±0.93^{a}	8.376±1.69^{b}	6.50±0.54^{c}
Mean Corpuscular Haemoglobin concentration (MCHC)	33.00±00^{a}	3.00±00	33.00±00
Lymphocytes (LYM)	55.00±2.39	59.25±4.65	56.00±4.28
Neutrophils (NEUT)	44.50±2.20	40.63±4.17	43.00±4.28
Eosinophils (EOS)	00±00	00±00	00±00
Monocytes (MONO)	0.50±0.54^{b}	0.63±0.52^{ab}	1.00±0.00^{a}

Means with the same superscripts are not significantly different at (P> 0.05) level of significance along rows.

Serum chemistry analysis of weight changes (kg) during the experiment

Table 3: Mean + standard deviation of mean of serum chemistry of ewes

Serum Chemistry Parameters	Control	3rd Week	6th Week
Total protein	3.90 ± 0.29[a]	3.38 ± 0.25[b]	3.150 ± 0.16[b]
Albumin	1.13 ± 0.89	1.11 ± 0.84	1.050 ± 0.54
Globulin	2.78 ± 0.36[a]	2.26 ± 0.21[b]	2.100 ± 11[b]
Potassium (K++	28.75 ± 2.78[a]	24.250± 1.17[b]	21.00 ± 1.07[c]
Sodium (Na++)	48.75 ± 6.48[a]	37.63 ± 2.13[b]	34.00 ± 4.28[b]
Creatinine	1.63 ± 0.56[a]	1.047 ± 0.44[b]	1.001 ± 0.01[b]
Alanineaminov Tranferase (ALT)	30.50 ± 4.93[a]	28.25 ± 1.67[a]	25.00 ± 1.07[b]
Aspartateamino Transferase (AST)	53.75 ± 9.81[a]	38.50 ± 3.34[b]	33.500±1.60[b]
Blood Urea Nitrogen (BUN)	1.18 ± 0.16[a]	1.08 ± 0.46a[b]	1,050 ± 0.54[b]
Glucose	52.25 ± 10.92[a]	43.00 ± 2.07[b]	40.00 ± 2.14[b]

Means with the same superscripts are not significantly different at (P> 0.05) level of significance along rows.

Analysis of weight changes (kg) during the experiment

Table 4: Mean and standard deviation of mean of weight parameters of ewe.

Parameter	Mean ± Standard deviation of mean
1st Week	28.45 ± 15.51[b]
2nd Week	36.28 ± 1.99[a]
3rd Week	36.40 ± 1.92[a]
4th Week	36.23 ± 2.04[a]
5th Week	36.40 ± 2.06[a]
6th Week	36.76 ± 2.36[a]

Means with the same superscripts are not significantly different at (P> 0.05) level of significance along rows.

Discussion and Conclusion

The hematological results show a significant decreased level of PCV, Hb, RBC count, Platelets, MCV, and MCH suggest a microcytic hypochromic anemia.This is believed to be nutritionally induced.

Although, the ration used had been found to be effective in supporting the physiological functions of the goat including their reproductive activities (Oyeyemi and Akusu, 2002), nutritional requirements and utilization are known to vary with Age, breed, species and the size of the animal (Hassan, 1987). While the WAD goat (to which this feeds had been extensively fed) rarely exceed 25kg body weight, the Uda sheep which is a large breed to which this feed was experimented weighs up to 50-60kg.

Increasing the quantity of feeds intake may be effective in supplying some feed nutrients needed by the Uda sheep in quantity but such quantitative feeding without supplementation with vital minerals needed for normal physiological functions including hemopoiesis may be the cause of the observed microcytic normochromic anemia.

The gradual reduction of the haematological parameters in this study can be associated with progressive depletion of essential minerals required for hemopoiesis as reported by Anosa, and Ogbogu, (1978).

The unimpressive weight gain over the duration of this experiment further suggests that this ration was hardly sufficient for maintenance purposes in a large breed of sheep like Uda.

From the study, it could be deduced that the composition of the experimental ration is inadequate to supply the essential nutrients required for the normal physiological function in a large breed of sheep like Uda.

Recommendation

Therefore, it is advisable that when this ration is to be used in large breed of sheep, higher levels of crude protein and energy as well as extensive mineral supplementation will

be required for optimal physiological function.

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RIFT VALLEY FEVER IN CAMELS IN NORTHERN BURKINA FASO

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Abstract

This study was done in three provinces located in Northern Burkina Faso, home of about 15705 camels. To investigate Rift Valley Fever (RVF) in these animals, serological examinations including Seroneutralisation Test (SNT) were performed on 270 camel serum samples. Positive results were obtained in 140 (51.85%) camels thus tested. Seventy five percent of seropositive camels were adult ≥ than 4 years old and the remaining 25% were young ranging from 8 months to 4 years. The results of the questionnaires administrated during the sampling to the shepherds and owners showed that association of abortion and mortalities in young animals were often observed.

The survey revealed that high prevalence of RVFV is observed in camels in the sahelian desert zone of Burkina Faso which is not routinely diagnosed. Recommendations for systematic RVF investigation in camels and others domestic ruminants were made in order to improve the animal productivity. Habitual consumption of raw milk and close contact with infected animals signify possible zoonotic importance of RVF in the studied area. A risk assessment of the disease should be also undertaken in order to understanding the epidemiology and knowledge of the disease in the country and the sahelian region.

Keywords: Serology, Camel, Rift Valley Fever, IgG, IgM, Seroneutralisation, Public health, Northern Burkina Faso.

FIEVRE DE LA VALLEE DU RIFT CHEZ LES CHAMEAUX DANS LE NORD DU BURKINA FASO

Resume

Cette étude a été réalisée dans trois provinces situées dans le nord du Burkina Faso qui abrite environ 15.705 chameaux. Pour étudier la fièvre de la Vallée du Rift (FVR) chez ces animaux, les examens sérologiques dont le test de séroneutralisation (SNT) ont été effectués sur 270 échantillons de sérum des chameaux. Des résultats positifs ont été obtenus chez 140 (51,85%) chameaux ainsi examinés. Soixante-quinze pour cent des chameaux séropositifs étaient des adultes ≥ 4 ans et les 25% restants étaient des juvéniles dont l'âge variait entre 8 mois et 4 ans. Les résultats des questionnaires administrés aux bergers et aux propriétaires lors de l'échantillonnage ont révélé une association entre l'avortement et les mortalités observés chez les jeunes animaux.

L'enquête a révélé qu'une forte prévalence du virus de la FVR a été observée chez les chameaux de la zone désertique sahélienne du Burkina Faso qui n'est pas régulièrement soumise au diagnostic. Des recommandations pour le dépistage systématique de la FVR chez les chameaux et autres ruminants domestiques ont été formulées, en vue d'améliorer la productivité des animaux. La consommation habituelle de lait cru et le contact étroit avec des animaux infectés signifient une importance zoonotique possible de la FVR dans la zone étudiée. Une évaluation des risques de la maladie doit être également menée afin de

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mieux comprendre l'épidémiologie et connaître la maladie dans le pays et dans la région sahélienne.

Mots-clés : Sérologie ; Chameau ; Fièvre de la Vallée du Rift ; IgG ; IgM ; Séroneutralisation ; Santé publique ; Nord duBurkina Faso.

Introduction

Camels (camelus dromedarius) are vital domestic animal species that are known to be best adapted to harsh environments and fluctuating nutritional conditions of arid and extreme arid zones (Bekele, 2004). In Burkina Faso, camels are mainly reared in the sahelian region, the desert part of the country. Camel population was estimated at 15,401 heads with annual growth and operating rates of 2% and 8% respectively (MRA, 2010). Camel husbandry is mainly practiced by specific socio-ethnic groups, Touareg and Sonrai. Its plays a paramount role in the life of the populations of this dry zone. Subsistence camel production is practiced in the sahelian region where cattle and small ruminants (sheep and goats) are also intensively reared. The region is also known to be the heartland of camel production as around 84% of population is produced in this region.

The increasing demand of animal products (milk and meat including hides) inherent to human population growth, camels' meat and milk are also consumed not only by Touareg and Sonrai in sahelian region but also all over the country mainly in urban areas. Despite the low production capacity, the camel milk has the potential to permanently change the livelihoods of poor communities living in arid and semi-arid lands (Musiga et al., 2008). In addition, they also serve as a draught animal for agriculture and most intensively for transport of people as well as goods (Swartz and Dioli, 1992). Indeed, despite the arrival of motorized transport, camels are the sole means of transport in this arid and semi-arid zone as well as water lifting.

In spite of its vital importance particularly to the marginalized communities in these dry zones of the country, studies about camels are almost nonexistent. Due to the fact that camel production is usually a migratory system in remote areas with harsh living conditions and poor infrastructures, the animals are presumed to be inaccessible to research (Bekele). In many African countries, camels are considered as neglected animals, because, from global perspective, their economic production seems minimal. In Burkina Faso, they are subset of huge livestock resource when considered from national economic point of view (MRA; 2010) and no development project features camels. Generally there is negligence towards the promotion of camel health and production in most African countries (Kane et al; 2002). It is only recently that camels became the subject of more intensive and systematic interest (Baumann et al, 1992) because of the susceptibility to many diseases both parasitic (trypanosomiasis) (Demeke, 1998) and infectious (poxvirus) (Kane et al.; 2002) and most recently Peste des petits ruminants (PPR) (Abraham et al. 2005; Khalafalla et al. 2010) as well as Rift valley fever (Ould El Mamy et al. 2011; El-Harrak et al. 2011) RVF is one the infectious diseases that affect both camels and camels owners (Eisa, 1977; Iman et al., 1979; Eisa, 1981). Although, the prevalence of RVFV in domestic ruminant is known (Some, 1989), the status of the disease in camels has not been investigated in Burkina Faso. The aim of the present study was to investigate the presence of RVF virus in camels in Burkina Faso and its impacts in livestock rearing system and public health.

Materials and Methods

Study areas

The study was conducted in two provinces (Seno and Oudalan) of the sahelian region of Burkina Faso bordering with Niger and Mali. The region has high (84%) concentrated populations of camels of the country. It has several dams, swamps and ponds. The climate is arid with average rainfall ranging from 300 to 500 mm per annum. Surface water is a paramount serious problem in the region. Traditional deep wells, sinking, natural and artificial damps, swamps, ephemeral ponds and shallow wells are water sources for both human and livestock.

Livestock rearing is mainly characterized by extensive pastoral production system and seasonal mobility. Cattle are dominating livestock species 20.6% followed by goats16.78%, and sheep 14.02% in addition to camels 84%. Cattle and camel herd are divided into seasonal migration (transhumance) and home-based as a strategy to mitigate forage and water scarcity (Desta, 2000) but also as provide milk to the family.

Sampling and analysis methods

Serum samples were collected from camels throughout the sahelian region of Burkina Faso mainly in Seno and Oudalan provinces. Herd, age, sex, clinical story and date and place of sampling were systematically recorded in a structured data collection form. Blood samples were drawn from 270 camels and three serological tests were performed. These tests included indirect ELSA for IgG, competitive ELISA (cELISA) for IgM and virus neutralization test (VNT) (Paweska et al., 2005).

Results

Positive results were recorded in 80 (29.62%) camels for IgG and 140 (51.85%) camels for VNT. All the samples were found negative for IgM. RFV virus IgG antibodies were found positive in 25 (19.23%) and 45 (32.14%) camels in Oudalan and Seno provinces while respectively 50 (38.46%) and 90 (64.28%) camel samples were positive to VNT test. The distribution of RVFV antibodies varies significantly to the province Oudalan and Seno were there natural seasonal swamps favourable the multiplication of the disease vector. Seventy per cent of the positive camels (56) were adults older than 5 years and the remaining 30% were younger (24) from 9 months to 5 years for IgG. One hundred (78.57%) positive camels were adults and 30 (21.43%) were young for SNT test. Sex has not influence the RVFV antibodies distribution. Sixty five (28.88%) positive camels were females and 15 (33.33%) males for IgG and 25 (55.55%) males and 115 females (51.11%) were positive to SNT test.

In infected herds, abortion cases, stillborn and stillbirths were reported by herders and camels owners.

Discussions

The results of the study indicated the serological evidence of RFV virus in camels in the Sahelian region of Burkina Faso. These results corroborate with the previous study conducted 30 years ago that RVFV was circulating among the domestic ruminants (Some, 1988; Akakpo et al., 1994) despite the absence of clinical disease (swanepoel et al., 2004). High seroprevalence of RVFV antibodies have been described in different sub-Saharan countries and sahelian countries (Davies et al, 1985; Formenty et al., 1992Mariner et al, 1995; Olaleye et al., 1996, Abd el-rahim et al, 1997), either by epidemics outbreaks (Senegal and Mauritania), virus isolation or serological evidence in Cote-Ivoire, Mali, Niger, Nigeria, Burkina Faso and Central African region (Zeller et al. 1995; Ringot et al., 2003; Lebreton et al., 2006; Martin et al, 2008). Camels have been involved in the spread of the disease in some instances (Scott et al. 1963; Abd el-rahim et al, 1997, Labeaud et al., 2008; Linthicum et al.1999; Ould El Mamy et al., 2011; Schwartz et al., 1992).

The results clearly indicate high seroprevalence of RVFV antibodies in older animals than younger camels, showing that could have occurred some years ago. Previous study also support that camels moving across the Sahara have contact with RVFV (eisa et al., 1977; Mehdi al-Haralk et al, 2011). In particular semi-arid and arid areas such as the Sahel and desert such as the Sahelian region of Burkina where the study was conducted, particular attention should be paid to singular wet areas such as the oases (Chevalier et al., 2003). Indeed, in our study, the highest seroprevalence of RFVV antibodies was found in Seno province where there semi-permanent large swamp which is the grazing and water points of almost 80% of the province livestock population during the dry season. The presence of water and pasture makes this environment favourable to the multiplication of the disease vectors and hosts and could be considered as breeding site for mosquitoes (Chevalier et al. 2003; Sissoko et al 2009, El-Harrak et al, 2011). In addition, local climatic parameters can play a

central role in determining the distribution and abundance of these vector organisms, either directly or indirectly, through the effects of such parameters on the host animals (Martin et al., 2008). The population of camels of the region should be monitored for RFVF and others vectors borne diseases. The impact both artificial ponds and temporary natural swamps on human and animal health should be investigated to identify advantages and drawbacks of a possible alternative to their use for livestock in the region and the country (Thiongane et al. 2000; Chevalier et al., 2003).

In most of the Saharan region of Africa, camels are valuable livestock appreciated as source of meat, milk and as main means for transportation for both human and goods (Desta. 2000; Bekele, 2004; Medhi Al-Harrak, 2011). The movement of camels across the Sahara desert could carry the disease from North Africa to West Africa desert where the study was carried out. It should be noted that RFVV was previously isolated from blood samples from healthy, naturally infected camels in Egypt and Sudan (Iman et al., 1979; Eisa et al., 1981;El-Harrak et al. 2011)). Furthermore, camels are suspected of playing major role in RFV propagation from northern Sudan to southern Egypt (Eisa et al., 1977). This could explain the seroprevalence of camels in Oudalan province bordering Niger and Mali desert with same populations living across the countries and also sharing the same desert areas from western desert to northern desert in Sudan and Egypt (Meegan et al. 1977).

The results of the SNT test showed high seroprevalence of RFV-specific antibodies of 51.85% in camels in the region with 38.46% and 64.28% respectively for Oudalan and Seno province. This confirmed the evidence of RFVV circulation in the region without any clinical outbreaks (Zeller et al. 1995; Thiongane et al. 2000; Martin et al., 2008). The presence of RVFV-specific antibodies indicates that the infection starts in early life probably through sucking and the younger animals may have inherited through maternal immunity (Medhi El-Harrak et al., 2011). Similar patterns were reported in cattle, sheep, goats in Chad (Ringot et al., 2003) in Senegal (Chevalier et al. 2005) and camels in Kenya (Labeaud et al. 2008) that positive animals were younger 3 years.

The recent unexpected occurrence of RVF outbreak in camels in 2010 (Ould El Mamy et al., 2011) has introduced a new dimension to the epidemiology of the disease. The national veterinary authorities need to consider camels as risk species for the disease and undertake RVF risk assessment in the country (Consultative group for RVF Decision-Support; 2010). An active laboratory-based surveillance system for RFV should be implemented for early detection the occurrence of the disease and early response in order to undertake preventive activities as well as the use of existing early warning systems and risk assessment tools for better forecasting of RVF in the sahelian region (Linthicum et al. 1999; Martin et al. 2008).

Conclusion

This survey confirmed the serological evidence of RVF virus specific antibodies in camels in the sahelian region of Burkina Faso showing a significant prevalence of 29.62% for IgG and 51.85% to SNT. The results of this study support that camels moving across the Sahara desert have contact with RVFV.

Intervention strategies should include safe breeding systems, lab-based surveillance system with regular serological testing in order to alert veterinary services, health care providers, epidemiologists as well as policy makers. Furthermore, particular attention should be paid to singular wet areas such as oases, natural swamps and artificial ponds. Thus, it is recommended that regular serosurveillance study to be carried out through sentinel herds of both camels and domestic ruminants. Further studies are also needed to assess the influence of ecologic factors on Aedes abundance and their relationships to the risk for RVF transmission around the temporary ponds and swamps. It is also recommended that the use of existing knowledge and generate new data to develop systems for anticipating, preventing and controlling changes in the occurrence and distribution of certain climate-associated transboundary animal diseases and zoonoses mainly RFV and its potential outbreaks in the sahelian region.

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SERO-PREVALENCE OF PESTES DES PETITS RUMINANT (PPR) IN SHEEP, GOATS AND CATTLE IN GHANA.

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Abstract

Peste des Petits ruminants (PPR) is an acute highly contagious, highly fatal viral disease of small ruminants, characterized by pyrexia, occulo-nasal discharge, stomatitis, pneumonia and diarrhoea. The disease is endemic in many regions of world and responsible for significant economic losses in goats and sheep due to high morbidity and mortality rates. The present study was carried out to determine the sero-prevalence of antibodies against PPRV in unvaccinated sheep, goats and cattle in Ghana. A total of 3,455 blood samples were collected from 1,617 sheep, 1,534 goats and 304 cattle, randomly sampled in 74 villages in 31 districts in the 10 regions of the country over a period of 38 months from July 2009 to September 2012. Competitive Enzyme Linked ImmunoSorbent Assay (c-ELISA) was used to detect the antibodies in sera against PPR virus. The prevalence in of PPR antibodies in sheep was 50.34% (814/1617) while the prevalence in goats and cattle were 45.50% (689/1534) and 6.90% (21/304) respectively. The overall prevalence was found to be 44.37%.

Key words: Peste des Petits Ruminants, sero-prevalence, antibodies, sheep and goats.

SÉROPRÉVALENCE DE LA PESTE DES PETITS RUMINANTS (PPR) CHEZ LES OVINS, LES CAPRINS ET LES BOVINS AU GHANA

Resume

La peste des petits ruminants (PPR) est une maladie aiguë très contagieuse, mortelle hautement virale des petits ruminants, caractérisée par l'hyperthermie, l'écoulement oculo-nasal, la stomatite, la pneumonie et la diarrhée. La maladie est endémique dans de nombreuses régions du monde et est responsable de pertes économiques importantes chez les caprins et ovins en raison des taux élevés de morbidité et de mortalité. La présente étude a été réalisée pour déterminer la séroprévalence des anticorps contre le virus de la peste des petits ruminants (VPPR) chez les ovins et bovins non vaccinés au Ghana. Au total, 3.455 échantillons de sang ont été prélevés sur 1.617 moutons, 1.534 chèvres et 304 bovins choisis de manière aléatoire dans 74 villages des 31 districts des 10 régions du pays sur une période de 38 mois, de juillet 2009 à septembre 2012. Le test d'immuno-absorption enzymatique de compétition (c-ELISA) a été utilisé pour détecter les anticorps dirigés contre le virus de la PPR dans le sérum. La prévalence des anticorps de la PPR était de 50,34% (814/1617) ; 45,50% (689/1534) ; et 6,90% (21/304) respectivement chez les ovins, les caprins et les bovins. La prévalence globale a été établie à 44,37 %.

Mots-clés : Peste des petits ruminants ; Séroprévalence ; Anticorps ; ovins et caprins.

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Introduction

Peste des petits ruminants (PPR) is an acute, highly contagious, notifiable and economically important, transboundary, fatal viral disease of domestic and wild small ruminants (Roeder et al., 1999; Ozmen et al., 2009). The mortality usually ranges from 50% to 90%, although it sometimes can be zero, and morbidity varies from 10% to 100%, or sometimes lower than 10%, depending on prevailing circumstances (Anderson et al., 1990). The disease was first reported in Côte d'Ivoire in West Africa (Gargadennec L. & Lalanne A. (1942), and later in other parts of the world, namely sub-Saharan Africa, the Arabian Peninsula, the Middle East and the Indian subcontinent (Shaila et al., 1996). Goats are said to be severely affected but sheep undergo a mild form of the disease (Lefevre and Diallo, 1990), while cattle have a sub-clinical infection (Anderson and McKay, 1994). The disease is a major constraint on small ruminant production, causing great economic losses because of morbidity, mortality, and losses of productivity (Singh et al., 2004). Small ruminants are important livestock species, both numerically and economically, in many developing countries including Ghana. The husbandry of small ruminants generates self-employment, raises income, improves household nutrition and plays an important role in sustainable agriculture and generation of employment (Tuah et al., 1990; Peacock, 2005).

The population of sheep and goats in Ghana is about 13.3 million while that of cattle is about 8 million (Veterinary Services Department, 2012). The ratio of sheep to goats and the population density vary greatly under different agro-ecological conditions (Oppong-Anane, 2011). The disease in Ghana is associated with the onset of rains in the northern part of the country and the onset of the dry season in the South. Seasonal outbreaks of the disease occur on regular basis in the country and large populations of animals are lost to the disease each year.

Information on the prevalence of antibodies to PPRV in small ruminants is available in various African countries where the disease has been reported to be endemic (Taylor et al., 1990; Lefèvre et al., 1991; Shaila et al., 1996; Ozkul et al., 2002;). However, the pattern of PPRV infection and its sero-prevalence in small ruminants and cattle in Ghana has not been studied. In the present study, efforts have been made to collect information on the prevalence of antibodies to PPRV in the sheep, goat and cattle populations to provide baseline information on the sero-epidemiology of PPRV infection in the small ruminant and cattle populations. This information will be very useful in the development of an effective control programme for the PPR disease in small ruminants in Ghana.

Materials and Methods

Sample collection

The study was conducted in all the 10 regions of Ghana. A total of 74 villages in 31 districts in the country were covered. From purposively random selected villages of each district, a total of 3,455 blood samples were collected from 1,617 sheep, 1,534 goats and 304 cattle over a period of 38 months from July 2009 to September 2012. The study population was identified to include small ruminants older than 6 months that had not been vaccinated against PPR. Blood (about 5.0ml/animal) was collected by jugular veno-puncture using vacutainers, transported to the laboratory on ice and left to clot at 4°C overnight. The sera were centrifuged at 5000rpm for 10 minutes and harvested in to screw-capped serum tubes and preserved at -20oC until used.

Competitive enzyme immunosorbent assay for detection of antibodies to PPRV

Competitive enzyme-linked immunosorbent assay (c- ELISA) was used to test 3455 serum samples collected from small ruminants from all study sites to determine PPR sero-prevalence.

The samples were analyzed using the c-ELISA developed at CIRAD (France). Briefly, the ELISA plates were coated with (1:3000) recombinant N-PPRV antigen (50 µl/wells) and the plates were incubated at 37°C for 1 h with constant shaking. Unbound antigen was washed 3 times with diluted PBS (0.002M pH 7.4) followed by adding 45µl blocking buffer

(PBS+0.05v/v Tween 20 +05% negative lamb serum) to all wells of plate. Later 5µl blocking buffer to monoclonal control well, 55µl blocking buffer to conjugate control wells, 5µl of test serum (1:20), 5µl of strong positive, 5µl weak positive and negative serum was applied to the wells followed by 50µl of monoclonal antibody dilution (1:150) (except conjugate control). After incubating for 1 h with shaking, the plates were washed 3 times; 50µl of anti-mouse conjugate (1:1000) was added and incubated for another 1 hour. Plates were again washed as above, 50µl of chromogen solution was applied and color development was stopped by adding 50µl of 1MH2so4 after 10 min. Optical Density (OD) values were read at 492nm with an ELISA plate reader (Ledetect96, Biomed, Germany). Percentage Inhibition (PI) values were calculated from the OD using the formula below with the aid of ELISA Data Interchanges (EDI) software (FAO/IAEA).

$$PI = \text{Absorbance of the test wells} \times 100$$

Absorbance of the mAb control wells

Test sera showing mean PI values of 50% or greater were considered as positive for PPR antibodies, while the test sera demonstrating mean PI values less than 50% were considered as negative. The test was applied at the Council for Scientific and Industrial Research- Animal Research Institute (CSIR-ARI) Laboratory (Accra, Ghana). Data generated were analyzed by Statistical Analytical System software (SAS, Institute Inc., Cary, NC, USA). Test for significance was done using the Chi Square ($\chi 2$) statistic at $p<0.05$ significance level.

Results

In the present study, the overall antibody prevalence recorded in sheep, goats and cattle was 44.37% (Figure – 1). The prevalence of PPR antibodies in sheep was 50.34% (814/1617) while the prevalence in goats and cattle were 45.50% (689/1534) and 6.90% (21/304) respectively. Sero-prevalence showed variations across agro-ecological zones (55.3% to 34.8%) form the rain forest to the Sudan savanna zones respectively (Table 1). These variations were more evident across districts with prevalence estimates ranging from 22.0% in the East Gonja district to 90.6% in South Nanumba district (Table 3)

Discussion

An overall prevalence of 47.98 % antibodies to PPRV among unvaccinated small ruminants meant that the animals could have been exposed to the field virus naturally or got in contact with vaccinated animals or cattle that shed the vaccine virus.

Findings also suggested that the sero-prevalence between sheep and goats was not significantly different (50.3% versus 45.5%, P<



Figure1: Agro-ecological zones of Ghana.

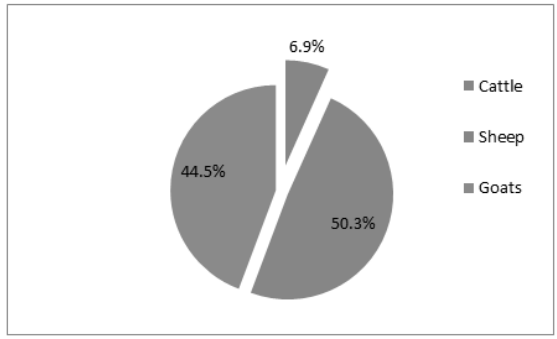


Figure 2: Overall prevalence of PPR antibodies in cattle, sheep and goats in Ghana

Table 1: Sero-prevalence of PPR in sheep and goats tested with an indirect c-ELISA in the various agroecological zones of Ghana.

Agro-ecological zone	Sheep serum	Goat serum	Total
Coastal Savanna	69/161 (42.9%)	138/227 (49.8%)	235/438 (53.7%)
Rain forest	143/242 (33.7%)	85/157 (54.1%)	228/412 (55.3%)
Deciduous Forest	181/357 (50.7%)	137/384 (39.4%)	318/705 (45.1%)
Transitional Zone	148/273 (54.2%)	99/201 (49.3%)	247/474 (52.1%)
Guinea savanna	235/487 (48.2%)	221/487 (45.4%)	428/961 (44.5%)
Sudan Savanna	38/97 (39.2%)	18/64 (28.1%)	56/161 (34.8%)
Totals	**814/1617 (50.3%)**	**698/1543 (45.5%)**	**1512/3151 (48.0%)**
95% CI	**47.9 – 52.8**	**43.01 – 47.99**	**46.24 - 49.72**

Table 2: Sero-prevalence of PPR in cattle tested with an indirect c-ELISA in 13 districts of Ghana.

District	Tested	+Ve	-Ve	% Positive
Savelugu Nanton	29	2	27	6.90
Karaga	30	0	30	0.00
East Gonja	30	2	28	0.67
East Sunyani	16	0	16	0.00
Sunyani West	25	0	25	0.00
Nsuta	25	0	25	0.00
Kintampo	25	0	25	0.00
Dangbe West	25	0	25	0.00
Ejura	15	1	14	0.67
Tolon	28	4	24	14.29
Yendi	31	1	30	3.23
Ga East	25	0	25	0.00
Totals	**304**	**21**	**283**	**6.91**

95% Confidence Interval (CI) 4.06- 9.76

0.05). This is in contrast with Abdalla et al., 2012; Abubakar et al., 2008 and Al-Afaleg et al., 2004) who reported that sheep were more affected than goats. Our findings also, contrasts those of Khan et al., 2007; Swai et al., 2009; Luka et al., 2011; Balamurugan et al., 2011 who reported a higher sero-prevalence in goats rather than sheep. Our findings indicated heterogeneity and variations in the various communities, this may be correlated with variations in the sheep and goat husbandry practices within the different agro- ecological zones, the uncontrolled movement of animals, the levels of natural immunity and the sharing of grazing field amongst agro and pastoralists. Prevalence rates were higher in forest and coastal savanna zones in the southern parts of the country than the drier savanna zones of the North. This might be due to the practice of keeping animals in doors for most part of the day in the southern humid parts of the country which provide ideal conditions for the transfer of the virus among animals and between flocks. Climatic conditions and seasonal availability of forage determine husbandry patterns in the northern Guinea and Sudan savanna zones. Here, small ruminants are left to roam in the dry season when there is limited availability of forage and tethered on trees around the homestead in the wet season. The practice of allowing animals to roam in the dry season may favour dispersal of the virus due to greater contact between animals from different flocks. However, the high temperatures may be responsible for the

Table 3: Sero-prevalence of PPR in sheep and goats tested with an indirect c-ELISA in 31 districts of Ghana.

District	Sheep tested	No +ve	Goats tested	No +ve	Total	No +ve	Percentage (%)
North Tongor	12	12	66	26	78	38	48.72
Awutu/Senya	51	18	79	42	130	60	46.15
Dangbe West	39	17	67	20	106	37	34.91
Dangbe East	30	2	44	8	74	10	13.51
Lower Manya	30	29	50	27	80	56	70.00
Upper Manya	11	3	37	19	48	21	43.75
Asuogyaman	63	40	25	12	88	52	59.09
West Akim	43	28	66	41	109	69	63.30
Shama	73	37	79	42	152	79	51.97
Ahanta west	69	43	30	20	99	63	63.64
Tarkwa	100	63	48	23	148	86	58.11
Ejisu	50	11	84	18	134	29	21.64
Atwima/Kwahoma	65	47	45	14	110	61	55.45
Tano South	57	21	42	27	99	48	48.48
Sunyani	76	47	36	22	112	69	61.67
Techiman	84	57	39	18	123	75	60.96
Kintampo North	56	23	84	32	140	55	35.29
Ejura	60	23	20	12	80	35	68.75
Sekyere Central	64	20	42	21	106	41	38.68
Wa Minicipal	51	11	53	18	104	29	27.88
Kasena Nankana	40	15	28	13	68	28	41.78
Bolgatanga	97	38	64	18	161	56	34.78
Tolon/Kumbungu	26	7	15	5	41	12	29.27
Savlugu/Nanton	47	21	66	15	113	36	31.86
East Gonja	3	0	102	33	105	33	22.00
Nanumba North	47	31	39	29	86	60	69.77
Nanumba South	0	0	43	39	43	39	90.67
Zabzugu/Tatale	57	42	26	18	83	60	69.77
Yendi	54	28	24	14	78	42	53.85
Karaga	89	48	32	24	121	72	59.51
Tamale South	71	32	59	28	130	60	46.15
TOTALS	**1617**	**814**	**1534**	**698**	**3151**	**1512**	
% positive		**50.34**		**45.50**			**47.98**

95% CI for sheep = 47.9 – 52.8, CI for goats = 43.01 – 47.99, CI for overall population = 46.24 -49.72

low infection rate observed in the dry season in the Guinea and Sudan savanna zones.

From the results of this study, PPR antibody sero-prevalence in cattle was 6.90%. This is in agreement with Ozkul et al., (2002), Abraham et al., (2005),) and Rashid et al., (2008) who reported sero-prevalence of 15.57%, 9.0% and 8.0% respectively in cattle. This high sero-prevalence in cattle may have been due to high population density and mixed grazing resulting in increased contacts between small ruminants and cattle or the small sample size used in this

study.

Conclusion

In conclusion, antibody sero-prevalence in sheep, goats and cattle suggested natural transmission of PPR virus under field conditions and c- ELISA is an effective tool for determining the sero-prevalence of the disease in the laboratory. The presence of PPRV-specific antibodies in cattle demonstrates that cattle are exposed to PPR infection naturally, and the mode of transmission may be direct or indirect. Further, it implies the importance of bovines as subclinical hosts for the virus besides widespread presence of the disease in sheep and goats in the country. The importance of bovines in transmission of the disease should be considered in establishing PPR control strategies in Ghana.

Acknowledgment

We are sincerely grateful to the IAEA for funding this work, to Dr. Adama Diallo for providing technical support for the PPR-CRP work. We also thank the technical team of CSIR-Animal Research Institute for their support.

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ANTITHEILERIAL CHEMICAL DRUGS: A REVIEW

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Abstract

Synthetic or semi synthetic chemical drugs were used for treatment of Theileria species. These drugs include antimalarial, trypanocides and antibiotics, antiviral, etc. The aim of this study was to overview chemical drugs tested for treatment of theileriosis.

Keywords: Theileria, treatment, chemical drug

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Introduction

Parasites of Theileria species infect wild and domestic animals in the tropical and subtropical regions of the world. Bovine theileriosis in cattle worldwide has been extensively studied, but a paucity of information exists concerning ovine theileriosis. Recently, interest has arisen in sheep infected with Theileria parasites. Of these, Theileria lestoquardi (formerly T. hirci) and the newly described Theileria species (China) are considered to be highly pathogenic (Hooshmand-Rad and Hawa, 1973; Jianxung and Yin, 1997). The other species, such as T. ovis and T. separata are less pathogenic and have lower economic importance than T. lestoquardi (Uilenberg, 1981; Alani and Herbert, 1988).

T. lestoquardi (Morel and Uilenberg, 1981) is a tick-borne protozoan parasite of sheep which occurs in South Eastern Europe, Northern Africa, Western and Central Asia (Uilenberg, 1981) and in India (Sisodia, 1981). Theileria lestoquardi causes malignant theileriosis in sheep and goats which may be acute, subacute or chronic. Theileria lestoquardi is transmitted by Hyalomma anatolicum anatolicum (Hooshmand-Rad and Hawa, 1973a). Recently malignant theileriosis has been considered to cause great losses in sheep flocks in Khartoum State (Nagwa, 1986; Tageldin et al., 1992). The prevalence rates of T. lestoquardi antibodies in Sudanese sheep from nine geographical areas in Sudan ranged from 23.4% in River Nile state to 10% in Kasala and Darfur provinces with an overall prevalence of 16.2% indicating widespread distribution of infection (Salih et al., 2003).

Theileriosis constitutes a real hazard to livestock and development plans. It would be a waste of effort and resources to import exotic breeds or implement ambitious cross-breeding programs without control of ticks (Shomein and Obeid, 1973). However, tick control has become less reliable because of the acaricide resistant.

Antitheilerial chemical drugs

Hawking (1958) used in vitro screening to test forty compounds including antimalarial, trypanocides and antibiotics in the concentration of (0.01-10mg/100ml), (0.1-5mg/100ml) and (3-10mg/100ml), respectively for chemotherapeutic activity against T. annulata. No activity was discovered in these compounds. Neitz (1959) reported that many trials have been made involving testing the efficacy of about fifty protozoacidal and bactericidal compounds. The results were often inconsistent and contradicting.

Barnett (1968) reported that since the erythrocytic piroplasm and schizont stages respond differently to different drugs, suitable compounds or combination of compounds having effects on both stages (Piroplasm and schizont) are needed.

Diminazene aceturate (Berenil):

Berenil is an active trypanocide, babesiocide and bactericide, acting directly on the parasite. Its chemical structure is shown in Fig. 1

Mahmoud et al. (1956) reported that T. annulata-infected cattle and buffalo- were cured of the disease by Berenil at doses of 7-10 mg/kg bodyweight and 10-12mg/kg bodyweight, respectively. Schmulevich and Evplov (1958) described the usefulness of Berenil for the treatment of T. annulata. However, Lavrent'ev (1960) and Marutyan (1960) did not obtain encouraging results with Berenil. Never the less, Gautam et al. (1970) reported that deep intramuscular administration of Berenil at 10-15 mg/kg bodyweight proved to be effective against T. annulata. Two to three injections had to be given in each case to eliminate the infection.

Tetracyclines

Mode of action of tetracyclines

Tetracyclines are bacteriostatic acting by inhibiting the protein synthesis by the microorganisms. This is by chelating the metallic ions of the enzymes of the bacteria. They inhibit a wider range of G +ve and G-ve organisms than penicillin and streptomycin and certain protozoa as theileria, as well as rickettsia and mycoplasma. Chemical structures of Tetracyclines are shown in Fig. 2.

Neitz (1957) used considerable numbers of drugs for treatment of T. parva. Aureomycin (Chlortetracycline) was the

only effective one at a dose of 100 mg/kg bodyweight. Tetracycline possessed the same prophylactic properties as aureomycin. Terramycine (oxytetracycline) or aureomycin and primaquine when administered together at a dose of 10mg/kg/bodyweight and 1ml/kg/bodyweight, respectively exerted some effects. Treatment of cattle with tetracycline hydrochloride on the first day of experimental infection at a dose of 15mg/kg/bodyweight suppressed the development of a febrile state but failed to cure established cases when treatment was commenced on the first day of fever (Brocklesby and Bailey, 1962). Wilde et al. (1966) tested 170 chemotherapeutic agents in East Africa, the therapeutic effects of tetracycline administered during the incubation period were confirmed, although Wilde (1967) concluded that neither oxytetracycline nor chlortetracycline had any significant effect on the course of the disease when given parentrally at 10 mg/kg body weight. Mugera et al. (1973) found that 43% of cattle treated with chlortetracycline recovered from East Coast fever at 12.5 -15 mg/kg bodyweight.Also Brown et al. (1977) reported that Oxytetracycline at a dose of 15 mg/kg bodyweight for five days was effective when administered either on the day when the parasites were first detected or administered at onset of fever.

Schmulevich and Evplov (1958) found that oxytetracycline was effective against Therileria anaulata infection. Hashemi-Fesharki and Shad-Del (1974) reported that oxytetracycline had no therapeutic value when administered at a dose of 12 mg/kg bodyweight, but Singh et al. (1980) found it effective at a dose of 10-15mg/kg bodyweight for 4-6 days. Treatment with long-acting oxytetracycline at 20 mg/kg bodyweight had no effect when administered either at a single or triple dose, but Bansal and Sharma (1986) found it effective at a single dose of 20 mg/kg bodyweight. Also Bhattacharyulu (1988) found that treatment of calves- infected experimentally with T. annulata-with oxytetracycline at a dose of 20 mg/kg bodyweight reduced the severity of the symptoms of the disease. Spooner (1990) found that oxytetracyline at a dose of 5µg/ml, or higher, for 8 days (in vitro), completely inhibited the establishment of schizonts and their ability to transform host cells infected with T. parva.

Primaquine phosphate, Sulphamethoxypyrazine, Trimethoprim, Suramin (Naganin) and Quinuronium sulphate (Acaprin)

The following structural diagrams (Fig. 3) represent Primaquine phosphate, Sulphamethoxypyrazine, Trimethoprim, Suramin and Quinuronium sulphate

Sulphamethoxypyrazine and trimethoprim, Naganin,Acaprin were previously used as effective agents against malaria, trypanosomiasis and babesiosis, respectively (Zhang, 1987).

Quinuronium sulphate is a compound of quinoline and urea. It is effective against all Babesia, but does not completely eradicate the parasites. It has potent anticholinesterase activity, releases histamine and depresses cellular oxidation.

Zhang (1987) reported that Primaquine phosphate (C15 H21 O N3.2 H3 PO4) was an effective parasiticide against the gametocytes of T. annulata at a dose of 0.75 mg/kg bodyweight, but Naganin, Acaprin, and a combination of Sulphamethoxypyrazine and Tryimethoprim at doses of 30, 1.37, 100 and 50 mg/kg bodyweight, respectively was not effective. Moreover, all five drugs were not effective in eliminating the schizont of T. annulata. Earlier reports, such as those of (Antipin et al. 1956) showed that Naganin at a dose of 15-20 mg/kg bodyweight (10%) intravenously inhibited the growth of schizont and formation of gametocytes; Hutyra et al. (1959) found that Acaprin (5%) was an efficient drug causing disappearance of the schizonts in liver and spleen at a dose of 5ml/100 kg bodyweight subcutaneously.

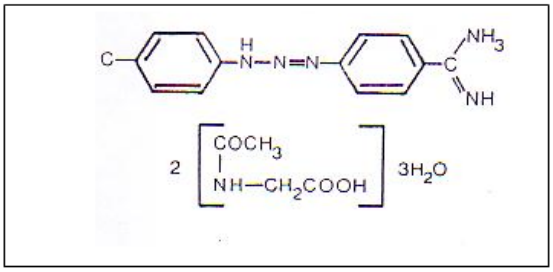


Figure 1: Chemical structure of Diminazene aceturate

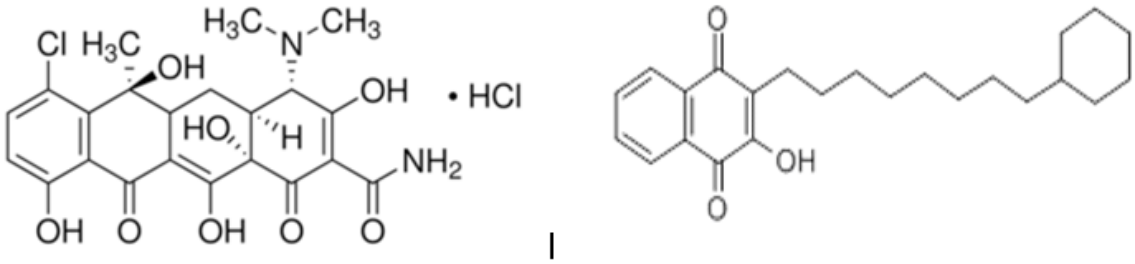
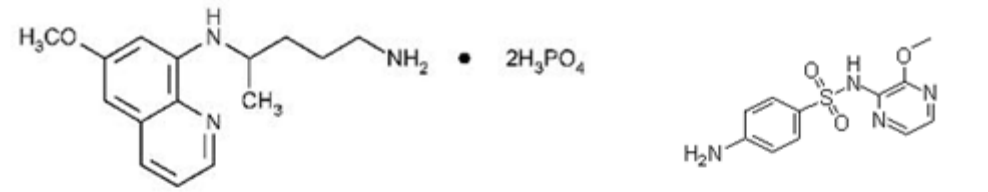
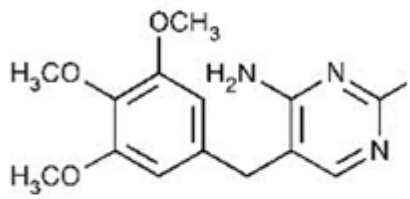


Figure 2: Chemical structure of tetracycline

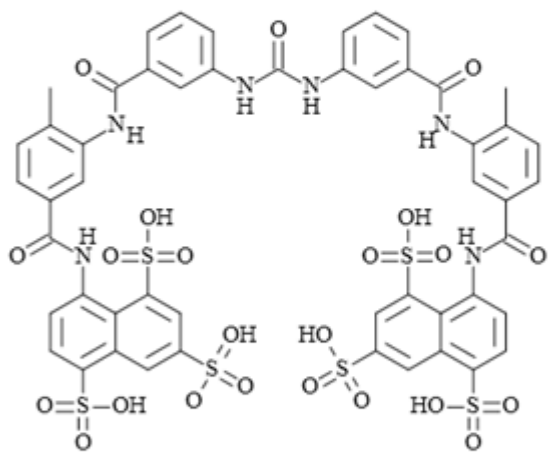


Primaquine phosphate

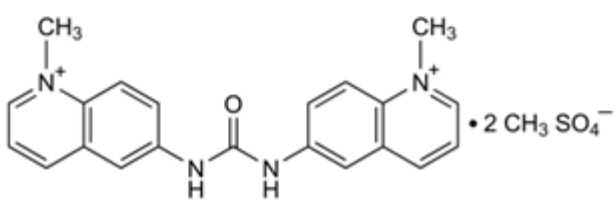
Sulphamethoxypyrazine



Trimethoprim



Suramin



Quinuronium sulphate

Figure 3: Chemical structures of Primaquine phosphate, Sulphamethoxypyrazine, Trimethoprim, Suramin and Quinuronium sulphate :

Halofuginone (Terit, Hoechst AG)

Chemical structure of Halofuginone is shown in Fig. 4

Halofuginone bromide (di-trans-7-bromo-6-chloro-3-[3-(hydroxyl-2-piperidinyl)-2-oxopropyl]-4(3H)-quinazolinone), was identified as an effective treatment against clinical and experimental infections of T. parva and T. annulata when administered orally at 1.2 mg/kg bodyweight, and 1.2-2mg/kg bodyweight, respectively (Schein and Voigt, 1979; Voigt and Heydorn, 1981). Halofuginone lactate was also effective in the therapy of both T. parva and T. annulata infections at doses of 1-2 mg/kg bodyweight. Doses above 3mg/kg bodyweight were toxic to cattle (Schein and Voigt, 1981). Njau et al. (1985) found earlier field cases of East Coast fever were successfully treated with Halofuginone lactate as a single dose of 1.2 mg/kg bodyweight orally while cases at advanced stage required two doses of 1.2 mg/kg bodyweight given at about two days intervals. Piroplasms were not affected by the drug (Njau et al., 1985). Stewart et al. (1990a) reported that splenectomised calves naturally infected with T. buffeli were treated with Primaquinone phosphate and Halofuginone lactate either separately or in combination. When used alone neither of the drugs eliminates infection. The most successful results were obtained when two treatments of Halofuginone lactate, at a dose rate of 1mg/kg bodyweight orally and six treatments of Primaquine phosphate at a dose rate of 2 mg/kg bodyweight intramuscularly were administered concurrently. Singh et al. (1993) reported that Halofuginone lactate given orally to calves infected experimentally with T. annulata at a dose of 1.2 mg/kg bodyweight was effective but caused anorexia, diarrhoea and debility.

Imidocarb

Figure 5 represents the chemical structure of Imidocarb

Imidocarb dihydrochloride administered subcutaneously at a dose of 1.2mg/kg bodyweight to Friesian cattle infected with T. annulata resulted in clinical improvement within one month of treatment (ELAbdin et al., 1976). Two separate treatments, which involved the administration of multiple doses

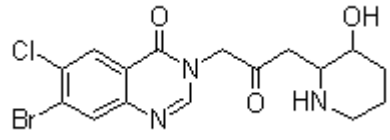


Figure 4: Chemical structure of Halofuginone

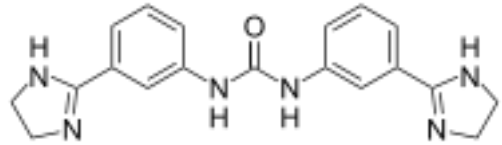


Figure 5: Chemical structure of Imidocarb

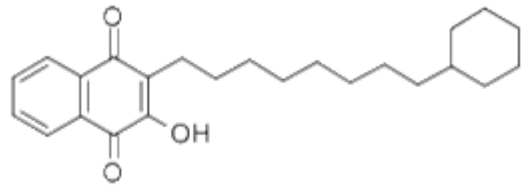


Figure 6: Chemical structure of menoctone

of Amodiaquinine hydrochloride at 10 mg/kg bodyweight orally and Imidocarb diproprionate at 3 mg/kg bodyweight subcutaneously during clinical disease retarded mortality and reduced parasitaemia (Sharma et al., 1977). Imidocarb diproprionate at a dose of 1.2mg/kg bodweight was effective in reducing the parasitaemia in T. sergenti infections by about 80% (Purnell and Rae, 1981), but Brocklesby (1961) reported that Amicarbalideisethionate (3,3 diamidino-carbanalide di-isethionate) was not effective against T. parva. Intramuscular injections of 2 mg/kg bodyweight Imidocarb alone or Imidocarb with sulphapyridazine (10%) 5mg/100 ml and Demaphocycline 7.5 mg/kg bodyweight were effective against theileriosis (Divanov, 1982).

Menoctone

Menoctone's chemical structure is shown in Fig. 6.

The antimalarial activity of menoctone [2-hydroxy-3-(8-cyclohexyloctyl)-1,4 naphthoquinone may be due to blocking of the synthesis of Co-enzyme Q and thereby affecting the electron transport system (Peters, 1974). McHardy et al. (1976) found that menoctone had a high level of antitheilerial activity in vitro and in vivo at a dose of 1.0 mg/ml and 5mg/kg bodyweight followed by five daily doses of 1mg/kg bodyweight. Menoctone was also effective when administered intravenously at

a dose of 5 mg/kg bodyweight and 2.5 mg/kg bodyweight on the day of infection and then daily for 9 days at 0.5 mg/kg bodyweight or 0.1 mg/kg bodyweight (Dolan and McHardy, 1978). McHardy (1978) screened antimalarial, anticoccidial, antibacterial, other antiprotozoals, antimetabolites, anti-inflammatories, antiviral agents and other compounds for activity against T. parva and T. annulata in vitro. Only two compounds, menoctone and methotrexate showed significant activity against T. parva at a concentration of 10-0.001mg/L, and only slightly less effect against T. annulata. Activity was maximal at 0.1 mg/L. Methotrexate, which interferes with folate biosynthesis in the parasite, showed an activity about 1% that of menoctone at 10 mg/L in vitro. EC50 of menoctone was 0.06 mg/L (Boehm et al., 1981). Menoctone was effective against experimentally infected calves at a dose of 10 mg/kg bodyweight (Mishra and Sharma, 1989).

Aminoquinolines

Neitz (1950) reported that the 8-aminoquinolines pamaquine, pentaquine and primaquine affected the piroplasmal stages of the parasite, and the 4-aminoquinoline chloroquine inhibited the schizont stage, although Barnett and Bailey (1957) were unable to confirm the effect of chloroquine. Anjaria et al. (1976) reported that the administration of chloroquine phosphate at doses of 1200-2800 mg intramuscularly, together with intravenous or intramuscular doses of 3mg of quinine dihydrochloride once daily for 4 days provided some beneficial effect in the treatment of T. annulata infections in exotic and cross bred cattle. Resochin diphosphate had no therapeutic or prophylactic value in the treatment of T. annulata infection (Laiblin and Müller, 1977).

Parvaquone

Figure 7 represents the chemical structure of Parvaquone

Parvaquone (2-cyclohexyl-3-hydroxy-1, 4 naphthoquinone; 993C, Clexon:Welcome) and related cyclohexyl analogues are known to be metabolized in mammals via hydroxylation at the 4-position of the cyclohexyl ring (McHardy et al., 1985). McHardy (1979), Dolan (1981) reported the efficacy of parvaquone in the treatment of T. parva infection of cattle at a single dose of 20 mg/kg bodyweight, or 10 mg/kg bodyweight twice at 48 hours intervals, given intramuscularly. McHardy et al. (1983) confirmed these results using cattle artificially infected with T. parva. Dolan et al. (1984) reported that 71% of cattle recovered when treated with parvaquone at 20 mg/kg bodyweight. In a second experiment the same authors reported that 10 mg/kg body weight given 48 hours apart were successful in treating 40% to 90% of animals infected with various T. parva isolates. McHardy and Morgan (1985) concluded that total parasitological cure was not achieved, and symptoms of toxicity were not observed, in any of the calves infected experimentally with T. parva even when inoculated with a single dose of 20 mg/kg bodyweight or 10 mg/kg bodyweight twice. Mbwambo et al. (2002) reported that 80% and 93.3% of cattle naturally infected with T. parva were cured with parvaquone (Parvexon®) at 10 mg/kg bodyweight or with paravaquone-plus-frusemide (Fruvexon®) 5mg plus 1.8mg/kg bodyweight, respectively.

Gill et al. (1981, 1984); Sultan et al. (1981); and Singh et al. (1993) showed that parvaquone was effective against T. annulata infection at a single dose of 20 mg/kg bodyweight. Parvaquone injected once at a dose of 20 mg/kg bodyweight or twice at a dose of 10 mg/

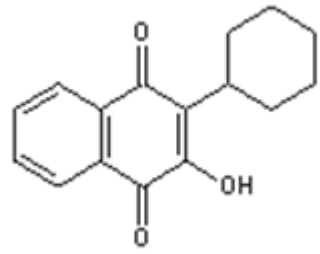


Figure 7: Chemical structure of Parvaquone

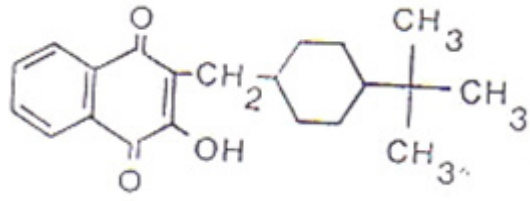


Figure 8: Chemical structure of Buparvaquone

kg bodyweight at 48 hours intervals cured the majority of calves and cattle naturally infected with T. annulata (Hawa et al. 1988; Unsuren et al., 1988). Only bulls treated with parvaquone (1.0 mg/15ml oil suspension) at a dose of 1ml /kg bodyweight at 48 hours intervals after 14 days of experimental infection with T. annulata survived (Stepanova et al., 1988).

Buparvaquone

Chemical structure of Buparvaquonne is shown (Fig. 8)

Buparvaquone (BW 720C: Butalex); 2-trans-(4-t-butylcyclohexyl) methyl-3-hydroxy-1,4-naphthoquinone was eight times more effective than parvaquone or menoctone in vivo against T. parva infection of cattle (Hudson et al., 1985; McHardy et al.,1983). Buparvaquone was twenty times more effective than parvaquone when tested in vitro, and its EC50 against T. parva and T. annulata was 1x10-9 and 16 x 10-9M, respectively; in vivo test confirmed the result as buparvaquone cured all cattle infected with T. parva and T. annulata at a dose rate of 2.5 mg/kg body weight while parvaquone at a of dose 20 mg/kg bodyweight cured 90% of cattle (McHardy et al., 1985), but Muraguri et al. (1999) concluded that parvaquone and buparvaquone were similarly effective as they cured 88% and 90% of cattle naturally infected with T. parva at a dose of 10 mg/kg bodyweight twice, and 2.5 mg/kg bodyweight once, respectively. Dhar et al. (1988); Sharma and Mishra (1990) found that 71% and 100% of cattle treated with a single dose of 2.5 mg/kg bodyweight intramuscularly were cured, respectively. But infections of T. buffeli were eliminated from 68% of calves treated with a combination of buparvaquone at 2.5 mg/kg bodyweight twice, and primaquine phosphate at 2 mg/kg bodyweight three or six times (Stewart et al., 1990b). Dolan et al. (1992); Mishra et al. (1993) concluded that treatment of clinical theileriosis with buparvaquone at 2.5mg/kg bodyweight was necessary. Alternatively, a single dose of 5 mg/kg bodyweight was found more effective (Singh et al., 1993, 1993a).

Antitheilerial chemical drugs (Sudan)

Quinuranium sulphate (Acaprin; *Pirevan) and Quinine dihydrochloride*

Shomein and Obeid (1973) made an experimental treatment trial for T. annulata using Pirevan (quinuronium sulphate, 5%) at a dose of 3 ml/day subcutaneously in all experiments, Pirevan and Terramycin (Oxytetracycline hydrochloride, 50 mg/ml) at a dose of 4 ml intramuscularly; combination of Pirevan and Resactin (2%) at a dose of 10 ml intramuscularly; Quinine dihydrochloride injectable solution (3.5 mg/ml) was given at a dose of 4 ml intramuscularly; combination of Quinine dihydrochloride and Resactin was inoculated at a dose of 4 ml and 10 ml, respectively. These drugs failed completely to suppress the parasite and although the parasites disappeared for some time from the peripheral blood circulation, they reappeared when animals were subjected to stress.

Tetracycline

Combination of pirevan and terrramycin (Oxytetracyline hydrochloride, 50 mg/ml) at a dose of 3 ml/day subcutaneously and 4 ml intramuscularly, respectively was not effective against T. annulata infection (Shomein and Obeid, 1973).

ElGhali et al. (1994) reported that oxytetracycline at 5 mg/kg bodyweight or 10 mg/kg bodyweight showed an efficacy of 70% and 61.1% against moderate cases of naturally acquired malignant ovine theileriosis, and overall efficacy of 52.9% and 55%, respectively. On the other hand, the efficacy and overall efficacy of Diminazene aceturate at 3.5 mg/kg bodyweight was 47.1% and 40%, respectively.

Taha et al. (1997) treated one of two groups of sheep naturally infected with T. lestoquardi with a combination of oxytetracycline at 10 mg/kg bodyweight and Diminazine aceturate at 3.5 mg/kg bodyweight, respectively. The other group was treated with oxytetracycline at 10 mg/kg bodyweight and chloroquine phosphate at 3.5 mg/kg bodyweight. The overall recovery rates were: 60% for the combination of Oxytetracycline and Diminazine aceturate; and 44% for the Oxytetracycline and Chloroquine phosphate.

Buparvaquone

ElHussein et al. (1993) found that 64% of sheep suffering from naturally acquired

malignant ovine theileriosis recovered when treated with buparvaquone at a dose of 2.5 mg/ Kg bodyweight.

Conclusion

It is concluded that treatment of theileriosis could be considered reliable only when the potent Parvaquone and Buparvaquone drugs are used.

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ANTIBODIES TO TOXOPLASMA GONDII IN BACKYARD AND WANDERING PIGS IN IBADAN, NIGERIA: IMPLICATIONS FOR PORK CONSUMPTION.

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Abstract

Toxoplasma gondii the etiologic agent of Toxoplasmosis can be transmitted to the pigs through the ingestion of oocyst, and to humans through the consumption of pork containing viable cysts causing neonatal deaths and abortion in animals, and opportunistic infection in immunocompromised humans. The objective of this study was to investigate the occurrence of antibodies to Toxoplasma gondii in backyard and wandering pigs slaughtered for human consumption in Ibadan, Southwestern Nigeria. Serum samples were collected from 100 pigs and tested for the presence of IgG Toxoplasma gondii-specific antibodies using a commercially available ELISA kit.

The overall frequency of IgG antibodies to T. gondii was 25%, More antibodies were detected in exotic breeds (31.5%) than the local breed (20%), and in pigs raised under the free-range (35.7%) than backyard (20.8%) management systems.

The result of this survey showed that animal raised from both backyard and free-range source are exposed to T. gondii infection, and suggests that the consumption of pork from both sources may be a risk factor for human infection with T. gondii.

Keywords: Toxoplasmosis; Toxoplasma gondii; Wandering Pigs; Backyard Pigs; Pork.

ANTICORPS DE TOXOPLASMA GONDII CHEZ LES PORCS DE BASSE-COUR ET LES PORCS EN LIBERTE : IMPLICATIONS POUR LA CONSOMMATION DE LA VIANDE DE PORC

Resume

L'agent étiologique de la toxoplasmose, Toxoplasma gondii, peut être transmis aux porcs par l'ingestion d'oocystes et à l'homme par la consommation de viande de porc contenant des kystes viables, et causer des mortalités néonatales et des avortements chez les animaux et des infections opportunistes chez les personnes immunodéprimées. L'objectif de cette étude était de rechercher la présence d'anticorps de T. gondii chez les porcs de basse-cour et ceux en liberté, abattus pour la consommation humaine à Ibadan, dans le sud-ouest du Nigeria. Des échantillons de sérum ont été prélevés sur 100 porcs et examinés pour rechercher la présence des anticorps IgG spécifiques à T. gondii à l'aide d'un kit ELISA disponible dans le commerce.

La fréquence globale des anticorps IgG contre T. gondii était de 25%. Un nombre plus élevé d'anticorps a été détecté chez les races exotiques (31,5%) par rapport à la race locale (20%), et chez les porcs élevés en liberté (35,7%) par rapport à ceux élevés en système de basse-cour (20,8%).

Le résultat de cette étude a montré que les porcs, qu'ils soient élevés en basse-cour ou en liberté, sont exposés à l'infection par T. gondii, et laisse entendre que la consommation de viande de porc provenant des deux sources peut être un facteur de risque d'infection de l'homme par T. gondii.

Mots-cles : Toxoplasmose ; Toxoplasma gondii ; Porcs en liberté ; Porcs de basse-cour ; Viande de porc

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Introduction

Toxoplasma gondii, an obligate intracellular apicomplexan protozoan parasite is the etiology of Toxoplasmosis a disease that is widely prevalent in animals and humans worldwide (Dubey, 2010). Toxoplasmosis is an important cause of neonatal deaths and abortion in animals (Aspinall et al. 2002) and it is now increasingly implicated as an opportunistic infection in immunocompromised humans e.g. HIV, pregnant women and cancer patients (Tenter et al., 2000).

T. gondii is mainly transmitted to animals and humans through the consumption of food or drink contaminated with oocyst excreted in the faeces of cats, the definitive host (Dubey, 2009); But there is increasing evidence showing that meat containing tissue cysts (especially undercooked pork) is considered an important source of T. gondii infection in humans in several countries. (Torda, 2001; Dubey, 2009; Tao et al., 2011). Pigs and other warm-blooded animals, act as intermediate hosts and are infected by the ingestion of oocysts present in the environment or by the consumption of cysts present in tissues of infected mammals, commonly small rodents (Kijlistra et al., 2008).

The practice of rearing pigs outdoors in many countries around the world is likely to increase seroprevalence of T. gondii in pigs. Wandering pigs, because of their unique feeding habits are used as indicator to determine the extent of soil contamination with the T. gondii oocyst (Kijlistra et al., 2008).

The inability of beef from cattle to meet the demand of the population for animal protein supply has made the consumption of pork to be on the increase. For example, report from china showed that pork takes about 65% of total meat consumption, an estimate of about 39kg pork consumed per person per year (Wang et. al, 2012). It is then not a surprise that recent reports revealed that the seroprevalence of antibodies to T. gondii in pigs in Northeast China is 12% (Liu et al. 2012); 18.03% in East China (Wang et al., 2012); and 30.6% in Chongquin, China (Wu et al., 2011). Pigs are important in the epidemiology of Toxoplasmosis, especially the consumption of pork is on the increase.

In Nigeria, there is paucity of information on the seroprevalence of T. gondii in Pigs in spite of the increase in pork consumption, especially in the Southwestern and Eastern part of the country where there is no religious restriction on it. Furthermore, there are also very few modern intensive pigs farming facilities in Nigeria. Pigs slaughtered for human consumption are mostly from outdoors rearing systems where pigs are allow wandering around for food, thereby increasing the risk of T. gondii infection to the pigs and consequently pork eaters.

This study is therefore aimed at investigating the occurrence of antibodies to Toxoplasma gondii in backyard and wandering pigs slaughtered for human consumption in Ibadan, Southwestern Nigeria.

Materials and Methods

Animals

A total of one hundred samples were collected by a simple random sampling method (five samples were collected per day; I from every 10 pigs for twenty days) from pigs brought for slaughter at a municipal abattoir in Ibadan Nigeria. Five samples were collected per day for a period of twenty days Information on the breeds, age, sex and where the pigs were raised were obtained before they the animals were taken to the slaughter slab.

Sample collection, and storage

Blood samples were collected into sterile tubes after decapitation and were left at room temperature after which the sera was harvested and stored at -20 °C until analyzed.

Serological assay

Serum samples were tested for the presence of IgG Toxoplasma gondii-specific antibodies using a commercially available ELISA kit (ID Screen® Indirect Multi-species ID VET innovative diagnostic Montpellier, France). The test was carried out according to the manufacturer's recommended protocol and the microplate were read at absorbance of 450nm. The test was validated and the result was interpreted using the specified equations in the instruction manual.

Statistical analysis

Variables were analyzed by Fisher exact test using the GRAPHPAD PRISM 5.01 (GraphPad Software, La Jalla, CA, USA). Association among variables and occurrence of seropositives were estimated from values obtained by the odds ratio (OR), a confidence interval at 95%. $P < 0.05$ was considered to be significant.

Results

The overall frequency of IgG antibodies to T. gondii was 25%. The distribution among breeds was Large White, 31.4%; Derrick, 25.9%; Local breeds, 20%, while male and females had 25% and 27.5% respectively. Antibodies to T. gondii found in less than 1 year old was 26.1%, 1 year old was 28.6%, ages between1.5 to 2 years old was 20% and those greater than 2 years old was 50%. The distribution among the management system was 20.8% and 35.7% for backyard and free-range system respectively (Table 1). The odd ratio analysis among variables showed that the female and the young (≤1 year old) had the odds of getting infected than the male and adult (>1 year old) respectively. While the exotic breeds of pigs and those raised under free-range management system had about twice the odds of getting infected than the local breeds and those raised in the backyard management system respectively

Table 1: Demographic characteristics of the seroprevalence of Toxoplasma gondii antibody among sampled pigs in Ibadan, Nigeria

Variables	Group	No. Tested	No. Positive	Percentage (%)
Breed	Large White (LW)	51	16	31.4
	Durock (D)	27	7	25.9
	LW x D	12	-	0
	Local	10	2	20
Sex	Male	49	11	25
	Female	51	14	27.5
Age (Years)	<1	23	6	26.1
	1	35	10	28.6
	1.5 – 2	40	8	20
	>2	2	1	50
Source	Backyard	72	15	20.8
	Free Range	28	10	35.7
Total	**Sample**	**100**	**25**	**25**

Table 2: Crude odds ratios (OR) with 95% confidence intervals (CI) for various variables associated with seropositivity of Toxoplasma gondii antibodies among sampled pigs in Ibadan, Nigeria

Variables	Group	No. Tested	Percentage (%)
Breed	Exotic (LW & D)	31.5	1.673 (0.330-8.491)*
	Local	20	0.598 (0.118-3.035)
Sex	Male	25	0.765 (0.308-1.901)*
	Female	27.5	1.307 (0.526-3.249)
Age (Years)	Young (≤ 1)	27.6	1.397 (0.548-3.560)*
	Adult (> 1)	21.4	0.765 (0.308-1.901)
Source	Backyard	20.8	0.4737 (0.182-1.237)*
	Free Range	35.7	2.111 (0.808-5.513)

*No significance difference between the variables (P>0.05)

(Table 2). Although there were numerical difference between the various variables. The difference were not statistically significant ($p > 0.05$).

Discussions

Toxoplasma gondii is an ubiquitous parasite that has been reported in mammals worldwide (Dubey, 2010). An overall frequency of 25% obtained in this study is comparable to the report from China where there is increase in the demand for pork as source of protein for the growing population (Wu et al., 2012; Liu et al. 2012; Wang et al., 2012). The findings of our study suggest that the environment where the sample pigs were reared was contaminated with T. gondii oocyst. Pig reared in both backyard and free ranged systems can get infected with the disease through the ingestion of feed contaminated with T. gondii oocyst from (resident or roaming) cats or the consumption of infected rodent.

The findings of this study showed that the free range pigs were more positive and had the odds of getting infected with T. gondii infection than the backyard raised pigs. The difference in type of food and feeding system might account for this difference. Backyard pigs are raised in restricted environment where they are mainly fed with formulated feed and watered with troughs. While the free ranged pigs roam around feeding on any food found in their surroundings are more exposed to T. gondii oocyst from faeces of infected straying cats. There are reports to show that the prevalence of T. gondii in pigs is known to be influenced by management systems (Dubey, 2009).

In this study, while the detection of higher antibodies to T. gondii infection in the young female pigs than the adult male pigs supports a previous report that higher seroprevalence in sows has been associated with access to cat (Dubey, 2009). The reason for detecting higher antibodies in the exotic breeds than in the local breed of pigs is not known. Although, while innate properties could account for increase susceptibility of the exotic breeds, the relatively small number of local pigs found during sampling could also contribute to the difference. The rearing of local breeds is no more a common practice in the studied area. There is therefore the possibility of increase in seroprevalence of toxoplasmosis in pigs in developing countries if the exotic breeds are less susceptible to T. gondii infection, since they are preferred because of their greater feed conversion ability and profitability after slaughter. There is a need for studies that will investigate the level of transmission of T. gondii infection in humans that have consumes pork from different breeds of pigs.

Conclusion

The findings of this survey suggests that the consumption of pork from both backyard and wandering pigs may be a risk factor for human infection with T. gondii in the study area. It is therefore recommended that measures to prevent Toxoplasmosis should be put in place where pigs are raised for human consumption under the backyard and free range management systems. Pork should also be properly cooked or processed before eaten.

Acknowledgement;

This project was partly funded by IFS grant B/4887-1.

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VACCINATION STRATEGIES IN BREEDER AND COMMERCIAL FARMS AND INFECTIOUS BURSAL DISEASE MATERNALLY DERIVED ANTIBODIES IN DAY OLD CHICKS IN NIGERIA

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Summary

In Nigeria infectious bursal disease (IBD) outbreaks have persisted despite routine vaccination. In a quest to determine some of the causes of the vaccination failures, the type of vaccines, vaccination schedules and seromonitoring for antibodies in breeder and commercial farms were investigated using structured questionnaires. The presences and level of maternally derived antibodies in broiler and layer day old chicks from four and six breeders respectively was determined using enzyme linked immunosorbent assay (ELISA) test. Based on the level of antibodies in day old chicks the central vaccination times and vaccination days were calculated using the Deventer formula. Not all breeder farms monitor for antibodies in breeders or chicks and only one of the 17 commercial farms sampled monitor for antibodies in chicks. The type of vaccines used and schedules vary with breeder farm. All broiler and layer commercial farms vaccinate with intermediate vaccine at 5 or 12 and 17 or 22 days of age. The mean IBD maternally derived antibodies (MDA) ELISA titre of day old broilers ranged from 1,564+873 to 2,472+962 while the titre was 2,015+1133 to 3,415+1958 for layers depending on breeder farm. Only the coefficient of variation of the MDA titre of day old chicks from two layer breeders was uniform (less than 30%). The calculated vaccination days using intermediate IBD vaccine for broilers was 14 and 20 days and 26 and 32 days for layers. About 2% to 24% of day old broilers and 2% to 16% layers had IBD MDA ELISA titre below 1,000. Based on the IBD MDA ELISA titre and %CV there is a need to harmonized and improve the vaccination strategies in breeder and commercial farms but breeders and day old chicks should meanwhile each should be seromonitored to determine the best time to vaccinate. In the absence of seromonotoring it is recommended that commercial farmers vaccinate broilers with intermediate at 14 and 20 days of age and layers with intermediate-plus IBD vaccine at 14 and 20 days of age in addition to instituting effective biosecurity to reduce the chances of exposure to IBDV early in life with resultant immunosuppression.

Keywords: Farms, infectious bursal disease, maternal antibodies, vaccination

STRATEGIES DE VACCINATION DANS LES FERMES DE REPRODUCTION ET LES FERMES COMMERCIALES ET ANTICORPS D'ORIGINE MATERNELLE CONTRE LA BURSITE INFECTIEUSE CHEZ LES POUSSINS D'UN JOUR AU NIGERIA

Résumé

Au Nigeria, les foyers de bursite infectieuse (BI) persistent malgré la vaccination de routine. Dans une recherche visant à déterminer quelques-unes des causes des échecs de vaccination, le type de vaccins, les calendriers de vaccination et le suivi sérologique pour les anticorps dans les fermes de reproduction et les fermes commerciales ont été étudiés à l'aide de questionnaires structurés. La présence et le niveau des anticorps d'origine maternelle chez les poussins de chair et de ponte âgés d'un jour issus respectivement de quatre et six fermes de reproduction ont été déterminés en utilisant le test immuno-enzymatique (ELISA). Sur base du niveau des anticorps chez les poussins d'un jour, les temps de vaccination centrale et les jours de vaccination ont été calculés en utilisant la formule de Deventer. Les fermes de reproduction ne surveillent pas toutes les anticorps chez les poulets de reproduction ou les poussins, et seule une des 17 fermes commerciales échantillonnées font un suivi sérologique pour détecter la présence d'anticorps chez les poussins. Le type de vaccins utilisés et les calendriers de vaccination varient suivant la ferme de reproduction. Toutes les fermes commerciales de poulets de chair et de pondeuses effectuent des vaccinations avec un vaccin intermédiaire à 5 ou 12 et à 17 ou 22 jours d'âge. Le titre moyen des anticorps d'origine

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maternelle contre la BI (MDA) déterminé par ELISA des poussins de chair âgés d'un jour variait entre 1,564+873 et 2,472+962 alors que le titre était de 2,015+1133 à 3,415+1958 pour les pondeuses suivant la ferme de reproduction. Seul le coefficient de variation du titre MDA des poussins d'un jour provenant de deux fermes de reproduction était uniforme (moins de 30%). Les jours de vaccination calculés en utilisant le vaccin intermédiaire contre la BI chez les poulets de chair étaient de 14 et 20 jours et de 26 et 32 jours pour les pondeuses. Environ 2% à 24% des poulets de chair d'un jour et 2% à 16% des pondeuses avaient un titre ELISA / MDA / BI inférieur à 1.000. Sur la base du titre ELISA/ MDA/ BI et CV%, il est nécessaire d'harmoniser et d'améliorer les stratégies de vaccination dans les fermes de reproduction et les fermes commerciales, mais les reproducteurs et les poussins d'un jour doivent entretemps être soumis à un suivi sérologique pour déterminer le meilleur moment pour la vaccination. En l'absence de suivi sérologique, il est recommandé aux fermes commerciales de vacciner les poussins de chair avec un vaccin intermédiaire à 14 et 20 jours d'âge, et les poussins de ponte avec le vaccin intermédiaire et le vaccin contre la BI à 14 et 20 jours d'âge et instaurer des mesures de biosécurité efficaces pour réduire les risques d'exposition au virus de la BI tôt dans la vie avec possibilité d'ne immunosuppression conséquente.

Mots-clés : Fermes ; Bursite infectieuse ; Anticorps d'origine maternelle ; Vaccination

Introduction

Infectious bursal disease (IBD) also known as Gumboro is a highly infectious and contagious disease caused by an RNA virus belonging to the genus Avibirnavirus family Birnaviridae1, 2. The disease affects young chickens and is characterized by high morbidity, mortality, atrophy of the bursa of Fabricius and immunosuppression and is of socioeconomic importance causing high mortality, poor feed conversion, growth, subsequent egg production and response to treatment and vaccination against other diseases3, 4, 5, 6, 7, 8, 9.

Because the IBD virus (IBDV) is resistance to physical and chemical agents it persists for long in contaminated poultry houses and equipment8, 10. The virus thus survives harsh conditions and wreck continuous havoc on the poultry industry. Gumboro is therefore controlled by a combination of biosecurity and vaccination of chicks and breeders11, 12, 13, 14, 15. Breeder vaccination is conducted in order to transfer antibodies known as maternally derived antibodies (MDA) to their offspring for protection against immunosuppression and clinical IBD within the first few weeks of age. To protect chicks for the rest of their life they are vaccinated with live vaccines. Live vaccines are however susceptible to neutralization by MDA and vaccinated chicks become susceptible to infection earlier than they would be and respond poorly to vaccination. It is therefore important to determine the most appropriate age to vaccinate chicks to avoid interference by MDA.

Vaccination failures have been reported in Nigeria and neutralization of vaccine virus by MDA has been attributed as one of the causes of the failures16, 17, 18, 19. The success of any IBD vaccination programme depends on the knowledge of breeder vaccination and the level of antibodies in breeders and MDA in chicks20. If chicks are vaccinated when MDA levels are still high, the vaccine may be neutralized by MDA, resulting in a reduced immune response. Conversely, if vaccinations are delayed and MDA levels drops, a severe vaccine reaction may result and the chick will be left vulnerable to the disease prior to vaccination15. This study was therefore conducted to determine the vaccination strategies in breeder and commercial farms, the presence and level of MDA in day old chicks and recommend vaccination schedules for commercial farms in Nigeria.

Materials and Methods

Questionnaires

Two sets of structured questionnaires were distributed each to the management of five major breeders and seventeen commercial farms in southwestern Nigeria respectively. The breeder farms that received the questionnaires were coded as F, P, C, Z and Av. The commercial farms that received the questionnaires were coded 1 to 17.

The questionnaire for the breeder farms were meant to answer the following questions. The type of breeder operation and vaccination schedule meant for the transfer

of adequate IBD maternal derived antibody and the type of vaccine that is administered; whether live, killed monovalent or polyvalent. Determine if there is periodic seromonitoring for IBD antibody titre in breeders and chicks, if information on the antibody titre in breeders was communicated to commercial farmers, laboratory tests were conducted at all, by an in house laboratory or outside laboratory or both.

The questionnaire for commercial farms was meant to answer the following questions. The type of operation, source of day old chicks, if the levels of IBD MDA level in chicks were provided by the breeder operators, whether on arrival of day old chicks they test for IBD MDA, whether IBD breeder vaccination schedule was communicated to them, whether breeder farms advised them on when to vaccinate with IBD vaccines, the strain of IBD vaccine they used in their commercial operation and where they derived their respective IBD vaccination schedules from.

Determination of maternal derived antibody in day old chicks

Fifty 50 sera from 50 day old chicks in a batch of broilers obtained from the hatcheries of four breeder farms coded as Am, O, C and Av were analyzed for IBD MDA using ELISA test. Fifty sera from 50 day old chicks in a batch of pullets obtained from the hatcheries of six breeder farms coded as Av, C, Am, F, O and Z were analyzed for IBD MDA using ELISA test.

Determination of central vaccination time and vaccination days

The central vaccination times and vaccination days were determined based on the Deventer formula using the MDA half life of 3.5 and 5.5 in broilers and layers respectively20. If the uniformity of the MDA was poor (coefficient of variation is > 30%) two vaccinations were considered necessary. A break through MDA of < 100, < 125 and < 500 was used for mild, intermediate and intermediate-plus vaccines respectively21, 22, 23, 24

Results

The type of breeder operation

All the breeder farms stated that they have both layer and broiler breeder operations.

Vaccination schedule and type of IBD vaccine used in breeder farms

All breeder operators vaccinate at point of lay. Only farm P does not administer boosters at intervals. Only breeder farm F vaccinates breeders with live vaccines. Only breeder farm C provided the average IBD MDA titre as >4000 and was the only farm that informed commercial farmers on MDA level and test for MDA in its laboratory (Table 1).

Table 1: Vaccination schedule and type of IBD vaccine used in breeder farms.

Farm	Vaccination	Booster	Type of vaccine	Monitor breeder for IBD antibody titre	Monitor for MDA titre	Test for MDA in an outside laboratory
F	16th wk	Every 8 wk	Live	No	No	Yes
P	16th wk	Does not	Polyvalent inactivated	No	No	Yes
C	Depending on antibody titre	3 times	Monovalent inactivated	Yes	Yes	Yes
Z	Not provided	Not provided	Polyvalent inactivated	Yes	Yes	Not provided
Av	18th wk	20th and 45th wk	Monovalent inactivated	Yes	No	Not provided

Table 2: Type of operation, source of birds and IBD vaccination strategy in commercial farms.

Farm	Bird type		Bird source	Source of vaccination schedule	Age at vaccination in days		
	Layer	Broiler			First	Second	Third
1	+	-	F	Hatchery	9	21	
2	+	-	F	Hatchery	9	21	
3	+	-	Z	Hatchery	9	21	
4	+	-	C	Hatchery	7	21	
5	+	-	C	Hatchery	7	21	32
6	+	-	Am	Hatchery	8 or 9	20 or 22	
7	+	+	Z	Hatchery	9	21	
8	+	-	C	Hatchery	7	21	
9	+	-	F	Hatchery	9	21	
10	+	-	Av	Hatchery	8 or 9	20 or 22	
11	+	-	F	Hatchery	9	21	
12	+	-	C	Veterinary hospital	14	28	
13	+	-	F	Hatchery	10 or 12	20 or 21	
14	+	-	Various	Animal laboratory	8	18	28
15	+	-	C and Am	Animal laboratory	5 or 9	17 or 21	
16	+	+	C	Not stated	7	21	
17	+	+	C	Farm veterinarian	Not stated		

Table 3: Mean IBD MDA titre in day old broilers and layers and calculated vaccination time and days.

Farm	Type of bird	Target mean titre	%Coefficient of variation	Central vaccination time in days			Vaccination days		
				Type of vaccine			Type of vaccine		
				Mild	Intermediate	Strong	Mild	Intermediate	Strong
Am	Broiler	1564+873	56.5	17	16	10	14/20	13/19	7/13
Av	Broiler	2414+909	37.3	18	17	11	17/21	14/20	8/14
C	Broiler	1747+678	38.4	17	16	10	14/20	13/19	7/13
O	Broiler	2472+962	43.1	19	18	12	16/20	17/21	9/15
Am	Layer	2614+937	35.1	31	29	18	28/34	26/32	15/21
Av	Layer	3390+1204	37.6	34	32	21	31/37	29/35	18/24
C	Layer	2513+758	29.9	30	29	18	30	29	18
O	Layer	3415+1958	56.8	33	31	20	30/36	28/34	17/23
F	Layer	2015+1133	56.9	30	28	17	27/33	25/31	14/20
Z	Layer	2647+723	27.0	31	29	18	31	29	18
Overall		**1564-3415**	**27-56.9**	**17-34**	**16-31**	**10-21**	**14/37**	**13/35**	**7/24**

Type of operation and sources of day old chicks for commercial farms

Out of the 17 commercial farms sampled all rear layers while only three rear both layers and broilers. Breeder farm C supplied seven commercial farms, Z supplied two, F supplied five, Am two and Av supplied one farm. Most (71%) commercial farmers obtain their day old chicks from farm C and F (Table 2).

Vaccination schedule and type of IBD vaccine used in commercial farms

All commercial farms do not have information on IBD titre of breeders and IBD MDA titre of day old chicks. None of the commercial farms determine the IBD MDA in day old chicks. All commercial farms sampled used live intermediate strain of IBD vaccine and reported a successful vaccination. About 65% of the farms obtain their vaccination schedules from information provided by the various hatcheries supplying them with chicks. About 12.5% farms derive their vaccination schedule from a veterinary laboratory, 6.25% from the farm veterinarian and 6.25% from a veterinary hospital. All commercial farms vaccinated twice for IBD at 5 or 12 and 17 or 22 days of age. Only two commercial farms vaccinated thrice against IBD (Table 2).

The IBD-MDA of broilers and pullets

The IBD MDA ELISA titre of day old broilers range from 1,564+873 to 2,472+962 while the titre was 2,015+1133 to 3,415+1958 for layers depending on breeder farm. Only the coefficient of variation (%CV) of the MDA titre of the day old chicks from two layer breeders was less than 30%. The calculated vaccination days using live intermediate IBD vaccine for broilers was 14 and 20 days and 26 and 32 days for layers (Table 3). Broilers from farm Am (12/50; 24%), O (3/50; 6%) C (9/50; 18%) and Av (1/50; 2%), and layers from farm Av (3/50; 6%), C (0/50; 0%), Am (1/50; 2%), F (8/50; 16%), O (3/50; 6%) and Z (1/50; 2%) had IBD MDA ELISA titre below 1,000.

Discussion

The existence of %CV of over 30% in layer and broiler day old chicks from 80% of the hatcheries sampled is of concern. The implication of this is that chicks from the different breeder farms sampled should have different vaccination schedules due to the variation in uniformity of MDA titre. High %CVs for a flock suggests poor uniformity of response to vaccination and implies that the vaccination programs need improvement25, 26. The causes of variation of MDA in day old chicks include difference in titre between individual breeder birds as not every bird responds equally to the same vaccination. Others causes are difference in average titres between different breeder flocks due to age difference, types of vaccines, vaccination programmes, mixing of eggs from different breeder flocks and difference in resorption speed of the yolk sac27. Because broiler flocks are often derived from several breeding flocks of different ages, chickens will have varying levels of MDA when hatched5. In fact in this study the mean IBD MDA titre was lower and %CV higher in the broilers than in pullets sampled. The high %CV observed in this study might be a result of hatching birds from different batches of hens or inappropriate vaccination of hens. All the breeder farms have different vaccination schedules. The highest %CV of 56.9 and 56.8 recorded in pullets from farm F and O respectively might be as a result of the use of live IBD vaccines in breeders. It is worthy of note that of the six layer breeder farms sampled, farm F had the lowest MDA titre which also reflects the use of live vaccines. Although farm Am used inactivated oil emulsion IBD vaccines, the mean MDA titre was low and the %CV high (56.5%).

As MDA levels decrease, even in chickens from well-vaccinated hens, the chickens become susceptible to infection28. In case of big variation in MDA titre in the chicks, different groups of chicks within the flock will reach the time of susceptibility and stage of vaccination at different times. Consequently there could be more than one best time for vaccination and repeated vaccination may be needed to reach all categories in time27. Based on the value of the %CV of > 30% broilers from all but two of the breeder farms require two vaccinations against IBD. However, it has been shown that broiler farmers in Nigeria vaccinate only once and the risk of IBD outbreak was

higher in broiler than in layer farms29. The high %CV is an indication of lack of uniformity of MDA in day old chicks and by extension their hens. To achieve uniformity in MDA level in chicks, it has been recommended that breeder titres should be maintained at high (8,000) and uniform levels by priming with live vaccine during growing stage and boosted during the production period with killed oil-adjuvant vaccines to extend MDA to 4 or 5 weeks21, 26. Hens from older breeding flocks (>50 weeks) may transfer lower levels of MDA, leaving progeny relatively more susceptible to infection with IBD virus28. It is pertinent to note that the type of vaccine and vaccination schedule used by breeder farmers varied. Periodic checks of IBD MDA of day old chicks by the breeder operators are therefore necessary in order to maintain uniform IBD MDA and for formulating uniform IBD vaccination programmes for the progeny.

Precipitin IBD antibodies were prevalent in breeders and their level depends on the type of vaccine administered and the age of the birds30, 31, 32. Young breeders tend to have high antibody levels especially those that received inactivated oil emulsion vaccine following priming with a live vaccine earlier in life33. As antibodies in breeders are transferred to their chicks and such antibodies are widely available in chicks, their concentrations in birds vary with age and vaccination practice. Thus, it is expected that the antibodies will be prevalent with varied level depending on the source of chicks. Since MA interferes with vaccination, the presence of MA in chicks will make the formulation of a uniform vaccination schedule in Nigeria very difficult. The need to introduce a uniform vaccination regime for breeders and chicks in Nigeria is therefore imperative.

Only breeder farm C claimed it transferred information on IBD MDA titres to commercial farms. This claim was not supported by information obtained from the commercial farms receiving birds from this source. Farm C also provided the MDA in chicks titre as 4,000, but the mean IBD MDA titres in broilers sampled from the same farm was 1,747+678 for broilers and 2,513+758 for layers. In order words there was no information flow from breeder farms to commercial farms on IBD antibody titres in hens and MDA in chicks.

All breeder farms sampled were involved in both broiler and layer breeder operation and and most commercial farmers obtained their vaccination schedules and day old chicks from the breeder farms C and F. This explains why most of the commercial farms sampled have similar vaccination schedules for broiler and layer farms. This schedule is however inappropriate because the half life of MDA in broilers is shorter (3.5 days) than that of layers (5.5 days) and would therefore loss their MDA earlier than layers and requiring early vaccination than layers20, 24.

Based on the vaccination days it is more appropriate to vaccinate broilers with intermediate at 14 and 20 days of age and layers with intermediate-plus IBD vaccine at 14 and 20 days of age5. This is because the day on which to vaccinate with an intermediate and intermediate-plus vaccine is when ELISA titre is approximately 125 and 500 respectively in the face of virulent IBDV. However, all the commercial farmers sampled used intermediate strain of IBD vaccines for broilers and layers. Mild IBD vaccine is not suitable for use in Nigeria as vaccination has to be delayed to between three to five weeks an age when chickens are most susceptible29.

Based on the protective ELISA titre of 1,000 day old chicks from 90% of breeder farms in Nigeria would be infected and be immunosuppressed if exposed naturally to IBDV26, 34. Also all flocks with %CV of more than 50% would have a significant number of birds susceptible to IBDV infection before 10 days of age and would develop bursal atrophy and permanent immunosuppression5. This emphasized the need for combining biosecurity and vaccination for the effective control of IBD. Chicks obtained from all breeder farms should be prevented from exposure to a virulent IBDV within the first seven to thirty days of life before vaccination because from the seventh day of age they have none protective MDA titre and would be vaccinated only after 14 to 31 days of age depending on the source and type of bird with intermediate vaccine to avoid neutralization of vaccine virus by MDA. The efficacy of IBD vaccination program was

related to the level of MDA in the chickens. The MDA of chickens can impede the virus in vaccine to infect the target cells and also reduce the ability of virus in vaccine to stimulate the chicken's immune system35, 36. Anyhow, the MDA is of benefit to IBDV infection in chickens at the age of 1-4 weeks37.

Results from various studies on precipitin MDA in chicks revealed that MDA are present and widespread31, 38, 39, 40, 41. The level of MDA varied with the source and age of birds. The higher the level of MDA at day-old the longer they lasted in circulation. The levels of MDA and the percentage of birds with MA are low or absent in chicks 3 weeks of age. By 4 weeks of age all chicks were devoid of MDA31, 41. The MDA does interfere with vaccination preventing active antibody production42. The `safe' age to vaccinate for IBD appears to be between 3 and 4 weeks. However, any bird that has not been vaccinated at 3 weeks or older stands the risk of suffering from IBD.

Conclusion

In conclusion the following recommendations are made. Breeder farms should avoid using live vaccines as boosters for breeder vaccination. Breeder farms should adopt a uniform and effective vaccination strategy. Uniformity of IBD MDA will translate into a uniform and effective vaccination strategy in all commercial farms. Otherwise may be better that breeder farms uniformly desist from vaccinating at all so that commercial farmers start vaccinating chicks at day old due to low IBD MDA. Intermediate and intermediate-plus vaccines are recommended for commercial operations of broilers and layer respectively. There should be flow of information within the up and down stream levels of the poultry industry in Nigeria. Such information should include IBD MDA titres from the breeders to the commercial farms. This implies that every breeder should screen various batches of day old chicks for IBD MDA titre for transmission of information to commercial farms.

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REPRODUCTIVE PERFORMANCE IN RESPONSE TO DIFFERENT ENERGY AND PROTEIN FEEDING REGIMENS OF NUBIAN GOATS IN THE SUDAN

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Abstract

This study was conducted to investigate the effects of feeding different levels of energy and protein on the reproductive performance of Sudanese Nubian goats. Forty- four female Nubian goats (3-5 years old) were divided into four equal groups and fed on different levels of energy (2.22 -3.12 Mcal /kg DM) and protein (7.3% - 13.1%dry matter). The animals were synchronized for estrous with progesterone - impregnated intravaginal sponges + PMSG (400 - 500 IU). About 80-90% of treated goats showed estrus response within 48h. The conception rate (72%) and kidding rate(63% and 54%) are higher in goats fed high level of protein (group HEHP and LEHP) these goats showed significant (P<0.05) shorter time for resumption of ovarian activity after kidding. kid survival is 100% in group HEHP whereas, high mortality rates occurred in groups fed low levels of energy (LELP and LEHP).

Key words: Nutrient Supplementation, Energy, Protein, Reproductive Performance, Nubian goats

PERFORMANCE DE LA FONCTION REPRODUCTIVE DES CHEVRES NUBIENNES EN REPONSE AUX DIFFERENTS REGIMES ALIMENTAIRES ENERGETIQUES ET PROTEIQUES AU SOUDAN

Résumé

Cette étude a été réalisée dans le but d'évaluer les effets de l'alimentation avec différents niveaux d'énergie et de protéines sur la performance de la fonction reproductive des chèvres nubiennes soudanaises. Quarante- quatre chèvres nubiennes femelles (3-5 ans) ont été réparties en quatre groupes égaux et reçu des régimes contenant différents niveaux d'énergie (2,22 -3,12 Mcal / kg MS) et de protéines (7,3% - 13,1% de matière sèche). Les animaux ont été soumis à une synchronisation de l'œstrus au moyen d'éponges intravaginales imprégnées de progestérone + PMSG (400 - 500 UI). Environ 80-90% des chèvres traitées ont montré une réponse œstrale dans les 48h. Le taux de conception (72%) et de mise bas (63 % et 54%) étaient plus élevés chez les chèvres recevant un niveau élevé de protéines (groupe HEHP et LEHP) ; ces chèvres ont montré un temps significativement (P <0,05) plus court pour la reprise de l'activité ovarienne après la mise bas. La survie des chevreaux était de 100 % dans le groupe HEHP alors que des taux de mortalité élevés ont été enregistrés chez les groupes recevant de faibles niveaux d'énergie (LELP et LEHP).

Mots-clés : Supplémentation en nutriments ; Energie ; Protéine ; Performance de reproduction ; Chèvres nubiennes

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Introduction

The level of reproductive performance is dependent on the interaction of genetic and environmental factors but it is particularly susceptible to the latter; seasonal availability of nutrients can considerably affect reproduction (Riera, 1982). Pre- and post- mating nutrition affects ovulation rate directly by acting on the gonadotrophic axis or on the ovary (Landau and Molle, 1979). Jahid et al., (2010) demonstrated that short-term intermittent nutritional stimulus from the luteal phase increased the total number of ovulatory follicles and the ovulation rate in association with increasing plasma concentrations of glucose and insulin in goats. Reproduction efficiency of female goat can be measured as length of breeding season, cyclic activity, ovulation rate, fertilization rate, post-partum anoestrus period and growth and viability of the offspring also it can be expressed by the kidding rate, weaning rate, kidding intervals, live weight of kids and the length of the reproductive cycle (Greyling, 2000). Failure of female to return to oestrus is the first sign of pregnancy. It could be defined as the period from successful fertilization of the ova to parturition. About 20-30% of fertilized ova in normal does have genetic or other defects and die as young embryos failed to implant (Walkden- Brown, 2001). These early embryonic losses results in normal returns to service and are usually wrongly considered as fertilization failure. The effect of nutrition on embryo survival is limited as reviewed by Abecia and Rhind (1994) and Hanrahan (1994), and only 10% of the probability of embryo survival can be explained by changes in feeding levels within the range of 0.5- 1.5 of maintenance requirements. An unscientific approach to animal feeding during pregnancy may lead to reproductive wastage that result in either abortion or neonatal death due to low birth weight resulting from malnutrition of pregnant does (Osuagwuh and Akpokdje 1981) or dystocia due to absolute foetal oversize as a result of high level of feeding throughout the gestation (Osuagwuh et al., 1980).

The objective of this study is to investigate the effects of feeding different energy and proteins levels on the reproductive performance of Nubian goats.

Materials and Methods

Experimental animals:

A total of 44 healthy adult mature female Nubian goats and 3 adult mature bucks were selected from the above mentioned flock they were 3-5 years old and approximately weighing 18.5- 32.0 kg body weight. Before the commencement of this experiment the animals were kept for 4 weeks in cages to adapt and to be clinically examined for freedom from diseases specially brucellosis, any reproductive disorders or abnormalities.

Feeding rations:

Four experimental diets were used as follows: High energy, high protein (HEHP); Low energy, low protein (LELP); High energy, low protein (HELP); and Low energy, high protein (LEHP). The rations were basically calculated using the production ration for lactating does as given in Nutrient Requirements of Goats (Anon, 2000). The high energy diets comprised 3.12 Mcal /kg DM and the high protein diet had a total protein of at least 13.1%. The low energy diets had 2.22 Mcal/kgDM and the low protein had for at least 7.3% of dry matter (maintenance ration).

The rations were formulated using the computer Programme Feedwin (win95) for feed formulation (1997-1998, ICP – Livestock, Barneveld). The ingredients for all diets were mainly grounded sorghum stalks, sorghum, molasses, ground nut cakes and wheat bran. The chemical compositions of ingredients were calculated according to the values obtained from the laboratory analyses.

Oestrous Synchronization:

After Ten weeks from the start of adoption of feeding regime, animals of each group were synchronized in the same day with an interval of 4 days between one group and the other. Synchronization was done by insertion of Intra-vaginal Drug Release (CIDR) device that contains 0.3 gm slow release progesterone, (Inter Ag, Hamilton, Netherlands). The CIDR remained in situ for 14 days (Ritar et al., 1984). At time of CIDR withdrawal, the goats, according

to their live weight, received an intramuscular injection of 400 - 500 IU of PMSG (Intervet, UK). Heat was detected at 48 and 60 hours following the removal of CIDR and injection of PMSG. Animals that showed heat signs were hand mated by the buck, and ensuring that breeding occurred at least twice per mating. The bucks were selected on the basis of their sexual desire (libido) and they were also tested for their semen quality.

Serum , Plasma and Milk Samples:

Weekly blood samples of 5.0 ml were collected into plain and heparinized vacutainer tubes from each goat throughout the study. Plasma obtained by an immediate centrifugation of heperinized blood at 3000 rpm for 5 minutes. Whereas, plain blood samples were first allowed to clot. The collected plasma and sera samples were stored at -200C until analyzed. Milk samples for progesterone measurement were collected as 10 ml into Mac-Carteny bottles which contained one tablet of sodium azide (100mg) each, at weekly intervals throughout. Milk samples were centrifuged at 3000 rpm for 15 minutes to remove fat and the skimmed milk was then stored in sealed plastic containers at -20^{0}C until analyzed.

Measurement of Progesterone:

Progesterone levels in serum samples were determined by Radio-immuno assay technique. The RIA kits were supplied by the IAEA under the project No. RAF 5046 (SUD). Serum progesterone concentrations were measured using RK-460 M progesterone 125I RIA kits (supplied by the institute of Isotopes Co. Ltd Budapest). The inter assay coefficient of variation for the QC value was 7.09 %.

In all assays the inter-assay coefficient of variation (CV %) for two sets of internal laboratory quality control values were 12.9% and 12.6%.

Evaluation of Progesterone Profiles:

Progesterone concentrations greater than 1.0 ng/ml were considered an indication of cyclic ovaries. From the progesterone profile and the mating schedule, the conception date of each doe was determined and confirmed later by the date of delivery in pregnant does. True pregnancy was detected by three successive increases in progesterone profile level following mating (> 3.0 ng/ml).

Results

All resulting data are presenting in Table (1) with the following details:

Oestrus response to synchronization and conception rates:

Reproductive data were recorded after two months of the commencement of the ration (intervention) all goats showed response to heat after synchronized with CIDR and PMSG protocol. Ten goats from group HEHP showed signs of heat at 48 hours post injection of PMSG and only one goat showed signs of heat at 72 hours. Whereas, in group LELP, ten does showed heat signs at 48 hours while one doe did not showed heat at all but left with the buck overnight. In group HELP, ten does showed heat signs at 48 hours and the remaining one was doubtful till 72 hours post injection despite that it was introduced to the buck. Nine does from group LEHP, showed heat signs at 48 hours, one doe at 72 hours whereas the last doe showed heat signs 10 days post injection of PMSG.

The conception rate to the first service is higher in group HEHP and group LEHP, which is 72.2%, followed by group HELP then group LELP which are 63.6% and 54.5%, respectively.

Kidding rate and embryonic losses:

Group HEHP showed the highest kidding rate (87.5%) followed by group LEHP (75%) whereas, higher embryonic losses occurred in group LELP and to a less extent in group HELP.

The gestation length and post- partum activity:

Gestation length of goats in group LELP was found to be shorter (139–144 days) compared to the other three groups. Group LELP showed the longest period from delivery to the first serum P4 rise with (83 to 159 days) which is significantly ($P<0.05$) longer than in group HEHP and LEHP. After parturition, the progesterone profile of all kidded does in group HEHP showed regular cycles and they

Table 1: Reproductive parameters in response to different levels of energy and protein

Trait	Groups			
	HEHP	LELP	HELP	LEHP
Mean time from end of treatment to the onset of oestrus (h)	50.18±7.2[a]	49.09±3.6[a]	50.18±7.2[a]	67.63±57.6[b]
Rate of oestrous response at 48 h	90.9%	90.9%	90.9%	81.8%
Rate of oestrous response at 72 h	9.1%	9.1%	0.0%	9.1%
Conception rate N (%)	8 (72.2)	7 (63.6)	6 (54.5)	8 (72.2)
Kidded N (%)	7 (87.5)	4 (57.1)	4 (66.7)	6 (75.0)
Gestation length (days)	157.00 ± 5.24[a]	141.25[b] ± 2.22	144.33[ab] ± 4.04	153.25[ab] ±14.66
P4 rise post partum (days)	44.60±18.08[a]	121.0± 53.74[b]	83.50±81.30[ab]	47.33±42.19[a]
Resumed normal cycle (%)	100	50	50	66.6
Non cyclic post- partum (%)	0.0	50	50	33.3
Kids/doe (Prolificacy)	1.80±0.84[ab]	2.25±0.96[b]	1.50±0.71[a]	1.00±0.0[a]
Kid weight (kg)	1.55±0.26[a]	1.40±0.13[a]	1.30±0.18[a]	1.40±0.00[a]
Kid survival rate (%)	100%	0.0%	33.33%	0.0%

HEHP: High energy high protein LELP : Low energy low protein
HELP : High energy low protein LEHP : Low energy high protein
Values with different superscripts within the same row were significantly different at P<0.05.

all resumed their ovarian activity in short time (44.6 ±18 days). Whereas, 50% of does in group LELP; 50% of does in group HELP; and 33.3% of does in group LEHP did not resume ovarian activity as detected by the low level of P4 (<1.0 ng/ml) and the remaining does showed delayed cycles and resulting in long mean period to first P4 rise.

Body weight at delivery and Kidding conditions:

Higher body weight of kidded goats was in observed group HEHP and LELP followed by group LEHP whereas lower body weight was observed in HELP. Similarly, the kid's body weight followed the same sequence i.e. higher in group HEHP, followed by LELP and LEHP then group HELP. Twins are more in group LELP resulting in prolificacy as 2.25 while it is 1.8 and 1.5 in group HEHP and HELP, respectively whereas, in group LEHP each kidded doe gave a single kid resulting in lower prolificacy (1.0). Kids of group HEHP showed the highest birth weight (1.55 ±0.26 kg) and best survival rate (100%) compared to the other three groups. On the other hand, all kids in group LELP and LEHP died immediately after birth or within few hours later.

Discussion

The level of reproductive performance is dependent on the interaction of genetic and environmental factors as well as seasonal availability of nutrients. Severe under- nutrition may lead to cessation of all reproductive activities regardless of other factors (Walkden-Brown, 2000).

In the present study, the proportion of goats that showed heat signs at 48-72 hours post injection of PMSG was higher (100%) in group HEHP than those in the other three groups where it ranged from 81- 90%. Thus following synchronization, there may be an effect of pre-mating supplementation of different levels of crude protein and energy on the onset of oestrus; this finding agrees with that of Kusina et al. (2001) in Mashona goats where dietary energy restriction during pre-mating was found to decrease the proportion of does showing onset of oestrus. although their study has demonstrated that feeding energy in excess of maintenance requirement immediately before mating did not improve the reproductive performance. This is also true as reported elsewhere in sheep (Gunn and Doney, 1979; Gunn et al., 1983) that feeding in excess of maintenance requirement was not desirable

and would account for reduced prolificacy compared to those fed on a moderate energy level. The delay in appearance of oestrus signs in animals received low level of energy was also shown by (Mansour et al., 2000 ; Mani et al. ,1992) who found that low level of feeding results in delay and suppression of oestrus in goats following synchronization with $PGF_{2\alpha}$; it also lowers ovulation rate, incidence of multiple ovulations and further reduces pregnancy rate in goats. Does of group LELP and group HELP were not affected by energy level and oestrus tended to be a slightly longer in those groups compared to the others. Mani et al (1992) found that the proportion of Saanen and Toggenburg does coming to oestrus were not affected by energy deprivation; they did observe that onset of oestrus after $PGF_{2\alpha}$ tended to be longer in feed restricted group.

Therefore, it can be concluded from the present study, which is comparable to the above mentioned findings, that energy restriction does not affect oestrus synchronization in does whereas feeding balanced energy and protein diets improves synchronization almost up to 100% as shown in group HEHP.

Conception rate :

In the present study the conception rate (54–72.2%) Chi square test did not reveal any significant difference between the different groups. This finding is similar to that shown by Damascus goats (Shalaby et al., 2000) and in agreement with that of Kochapakdee et al. (1994) who found that different regimens of concentrates supplementation did not increase the conception rate. In this study, it is clearly demonstrated that high protein fed groups (HEHP and LEHP had higher conception rate (72.2%) than low protein fed groups (LELP and HELP); similarly high energy fed groups had high conception rate but to a lesser extent than those fed high protein. Howevere, Kusina et al (2001) Hossain et al. (2003b) Conroy et al. (2002) found high conception rate in high energy supplemented groups compared to the low and medium energy supplements.

Does that received low energy diet (LELP) were in very poor condition at the time of mating. It is probable that this breed of does has adapted itself to bouts of under nutrition by, to mobilizing body reserves and to successfully ovulate and conceive under such conditions. Kusina et al. (2001) have found that, despite low energy diets and loss of body weights by magnitude of 12%, about 73% of does bred and got pregnant to full term suggesting that their loss in body weight was testimony to their energy deficit and they were, therefore, mobilizing body reserves for maintenance.

Embryo survival:

The effect of nutrition on embryo survival has been claimed to be linked with peripheral progesterone concentrations. Overfeeding during early pregnancy results in reduced progesterone levels and increases embryo loss in sheep (Williams and Cumming, 1982; Parr et al., 1987). In beef heifers, an increase in feed intake before AI decreases embryonic survival (Dunne et al., 1999). A statistical study summarizing 55 trials with swine found that high levels of feed intake after breeding led to an increase in embryonic mortality (Den Hartog and Kempen, 1980). On the other hand, severe energy deprivation of goats through the third month of pregnancy was associated with increase in embryo loss (Mani et al., 1992). Whereas, high post mating nutrition was beneficial to survival of triple embryos only and otherwise deleterious to conception rate in sheep (West et al., 1991). The high protein intake elevates urea nitrogen in plasma (Carroll et al. 1988; Howard et al. 1987; Jordan et al., 1983) and uterine and vaginal concentration of ammonia (Duby et al., 1984). This was assumed to occur however, as more energy is required for liver conversion of excess ammonia to urea (Chalupa, 1984; Visck, 1984). On the other hand, Landau and Molle, (1979) reported that both an acute deficit of energy and an over-fattening of ewes reduce progesterone concentration in the blood and embryo survival during the first stage of pregnancy. Moreover, Hossain et al. (2003a) speculated that increased level of dietary energy supplementation together with other nutrients from the concentrate mixture might increase the availability and proper balance of nutrients to the host animal, which might, in turn result in high supply of nutrients to the foetus.

Kidding rate :

The present finding shows that the kidding rate was high in group HEHP and group LEHP (high protein diet groups) . Sahlu et al. (1992) found that provision of a poor CP (9%) diet to Alpine does from week 12 of pregnancy through parturition was associated with decreased kidding rate compared to a diet containing 11% or 14% CP. whereas no effect on kidding rate was observed in another study with similar treatments (Sahlu et al., 1995). Conroy et al. (2002) in their study on poor goat- keepers in India concluded that the combination of high conception rates and high twining resulted in high kidding rates, i.e. mean number of kids per doe in treatment groups. One possible explanation for this is that these goats were protein- deficient but not energy- deficient, in the late dry season, as a large proportion of their feed was composed of chopped cactus at that time of the year, which is a good source of energy, but not protein (Wood et al., 2001). On the contrary, severe energy deprivation such as an energy supply at 70% of the maintenance requirements from day 91, increased the rate of abortion between 91 and 120 days of pregnancy and reduced significantly the crop of live kids (Hussain , 1993). Clearly, poor energy and protein feeding lead to high embryonic losses which are clearly demonstrated in the present study in group LELP goats. Research has shown that nearly 80% of lamb mortality was related to ewes nutrition during the last weeks before lambing and the first week after lambing (Seymour, 1998). On the other hand, Acute deficit of energy and over-fattening of ewes reduce progesterone concentration in the blood and hence embryo survival during the first stage of pregnancy (Landau and Molle, 1979).

The mean gestation length in group LELP is significantly shorter ($P<0.05$) than that of group HEHP with a least range of 139-144 days whereas, no significant difference is observed in the other two groups. Salim et al.(2002) reported similar gestation lengths in control and supplemented goats; 143 and 144 days, respectively, which are close to that reported by Hussain (1993) and agree with that of Sabra et al.(1997) where the level of supplementation had insignificant effects on gestation length of Barki ewes or dairy goats.

However, Sahlu et al. (1992) found that crude protein intake did not significantly affect the mean gestation length although it tended to be shorter in does fed high protein diets. Because of the fact that metabolizable energy concentrations were similar among the treatments it may be speculated that low protein intake has resulted in a low foetal growth rate and consequently prolonged the gestation length. It is possible that the effect of protein intake on foetal growth is mediated by the effect of dry matter (DM) and metabolizable energy (ME) intakes. In another study, increased final body weight, along with reduced gestation length were observed with increasing ME intake (Sahlu et al., 1995). Those authors related the insignificant difference in the gestation length to the good body condition of the does at the beginning.

It may be worth mentioning, however, that group LELP goats carried multiple foetuses compared to the other three groups; this might have led to shortening of gestation length. This finding is in close agreement with that of Sousa et al. (1999) in Caninde goats with multiple pregnancies. The controversial literature on the gestation length was clarified by Riera (1982) that the influence of nutrition on foetal development during certain months of pregnancy tend to shorten or lengthen the gestation period by only 1.5 days.

The kid birth weight:

The kid birth weight was heavier in group HEHP, 1.55± 0.26 kg, compared to those of the other three groups. The survival rate was the highest (100%) in group HEHP compared to kids of the other groups (0.0 - 33.33%). Salim et al. (2002) reported 1.45kg birth weight of kids in supplemented group compared to 0.85 kg in the controls. It is obvious that supplementation did improve the kid birth weight. Doney et al., (1982) observed that provision of proper nutrition before mating is associated with complex interrelation between body weight gain and body condition of lambs. On the other hand, Hossain et al. (2003a) reported that birth weight of lambs was high in high energy diet. It is speculated that increased levels of dietary energy supplementation along with other

nutrients from the concentrate mixture might increase the availability and proper balance of nutrients to the host animal. This in turn resulted in high supply of nutrients to the foetus that may be reflected in high birth weight. Sahlu et al. (1992) found that adequate protein supply during late pregnancy in dairy goats improved body weight of kids while in another study Sahlu et al. (1995) concluded that neither crude protein nor energy had an effect on kid birth weight. However, size, weight, and health status of does may be the other factors that may affect kid's birth weight. Previous studies have shown that the numbers of kids per doe (prolificacy) increased by high energy diets (Zezza et al., 1991; Kusina et al., 2001) and decreased by restriction of dietary energy (Kusina et al., 2001). In the contrary, no significant increase in both kidding rate or multiple births was produced by supplementation of goats 15 days before mating or 15-45 days during mating (Kochapakdee et al., 1994).

In the present study, prolificacy was highest in group LELP followed by group HEHP, then group HELP and group LEHP, respectively which is a contradiction to the pervious studies but the number of animals per group was too small to draw a definite conclusion on this parameter.

This might also be proved by our present finding that kids of group LELP which were totally lost due to the poor nutrition and multiple pregnancies compared to those of the other groups. It can be concluded from our results, that a single and twin kids have high birth weights than triplets and, hence, a better chance for survival. (Song et al., 2001).

Acknowledgments

The authors thank the director of the Central Veterinary Research Laboratories, Khartoum for the financial support of this study. Special thanks were due to the staff of the Centre of Animal Production and Nutrition, Khartoum North-Kuko where these experiments were conducted.

Impact

Feeding high energy and high protein levels during the breeding time of goats is required for better reproductive and productive performance.

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Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

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Examples of References

- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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