ISSN 0378 – 9721

Volume 63 No 4

December / Decembre 2015

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 30786, NAIROBI, KENYA

BULLETIN

December
2015 Volume 63 No. 4
Decembre

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

Special edition December 2015 to commemorate the importance of animal genetic resources in Africa

Annual subcription: US$ 100.00

ISSN 0378-9721

Abonnement pour un an : 100$

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

VOL. 63 NO. 4 **CONTENTS** **DECEMBER, 2015**

1. **ABSORPTION AND BIOACCUMULATION OF HEAVY METALS IN GIANT AFRICAN LAND SNAILS (Archachatina marginata).** *Alabi J O, Fafiolu A O, Ekemezie A A O and Alimba C G.* 389

2. **EFFECT OF STAGE OF MATURITY ON DRY MATTER YIELD, MORPHOLOGICAL CHARACTERISTICS AND NUTRITIVE VALUE OF BURGUNDY BEAN (MACROPTILIUM BRACTEATUM).** *M Baba, A B Nasiru and N Ashahabu.* 397

3. **PERFORMANCE OF BROILER CHICKENS FED NEEM (AZADIRACHTA INDICA) LEAF MEAL AS AN ALTERNATIVE TO ANTIBIOTICS.** *Egbeyale, L. T., Ayo-Ajasa O Y Ayoola, A A, Ogunkunle N F, Okuboyejo O A.* 403

4. **METHODS OF ISOLATION AND IDENTIFICATION OF MYCOPLASMA SPECIES OF RUMINANTS IN AFRICA - A REVIEW.** *Francis M I, Ejeh E F, Raji M A and Egwu G O.* 411

5. **ACUTE TOXICITY STUDY AND EFFECT OF ETHANOLIC LEAF EXTRACT OF GUIERA SENEGALENSIS J. F. GMEL (COMBRETACEAE) ON *TRYPANOSOMA BRUCEI* BRUCEI INDUCED PATHOLOGY IN ALBINO RATS.** *Badau S J, Luka J, Igwenagu E and Suleiman I E.* 433

6. **RESPONSE OF FINISHING BROILER CHICKENS TO SUPPLEMENTAL NEEM (AZADIRACHTA INDICA) AND GARLIC (ALLIUM SATIVUM) ON OOCYST COUNT, BACTERIA COUNT AND GUT MORPHOLOGY.** *Sobayo R A, Muhammad S B, Sogunle O M, Adegbenjo A A, Adejola A Y, Oso A O and Adeyemi O A.* 443

7. **ARTERIAL BLOOD PRESSURE CHANGES IN ACUTE *T. BRUCEI* INFECTION OF DOGS.** *Ajibola Eyitayo Solomon, Oyewale Johnson Oluwayemisi and Oke Bankole Olusiji.* 455

8. **INTAKE AND DIGESTIBILITY OF NUTRIENTS BY WAD GOATS FED DIETS CONTAINING VARYING LEVELS OF CASHEW NUT SHELL LIQUID INCLUSION.** *Olagoke K O, Aderinboye R Y, Onwuka C F I and Ojo V O A.* 461

9. **KOBS (KOBUS KOB ERXLEBER, 1777) ACTIVITIES AT SALT-LICK SPOTS IN KAINJI LAKE NATIONAL PARK, NIGERIA.** *Ogunjobi J A and Ayodele I A.* 467

10. **ANTIBIOTICS PROFILING OF PROTEUS MIRABILIS AND PSEUDOMONAS AERUGINOSA ISOLATED FROM SOME COMMERCIAL POULTRY FARMS IN NSUKKA, ENUGU STATE, NIGERIA.** *Ogunleye A O, Oyeleye Oluwatosin A. and Ajuwape A T P.* 475

11. **MÉTHODOLOGIE POUR L'IMPLÉMENTATION DU RECENSEMENT NATIONAL DU CHEPTEL ANIMAL EN RÉPUBLIQUE DE DJIBOUTI.** *Matete G O, Idriss Ali Soultan, Omar Abdoulkader Mohamed, Farah Abdi Djama Abdoulkader Ismail and Fallou Gu èye.* 485

12. **PREVALENCE OF FISH DISEASES IN FISH FARMS IN KATSINA STATE, NIGERIA.** *Dauda A B and Ibrahim H I.* 495

13. **HEALTH PROPHYLAXIS IN GOAT BREEDING IN SOUTHERN AND CENTRAL CÔTE D'IVOIRE PROPHYLAXIE SANITAIRE EN ÉLEVAGE CAPRIN AU SUD ET AU CENTRE DE LA CÔTE D'IVOIRE.** Emanfo A S A, Esmel M E, Komoin-Oka A M A C, Koné M W..... 505

14. **BIOECOLOGIE DES TABANIDÉS DANS LA ZONE PÉRIPHÉRIQUE DU DISTRICT DE BAMAKO AU MALI.** *Traore D, Bass B and Kone F.*..... 519

ABSORPTION AND BIOACCUMULATION OF HEAVY METALS IN GIANT AFRICAN LAND SNAILS (Archachatina marginata)

Alabi J O*[1], Fafiolu A O[1], Ekemezie A A O[2] and Alimba C G[3]
[1]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Federal College of Animal Health and Production Technology, Ibadan, Oyo State, Nigeria,
[3]Department of Zoology, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract

Interest in the production of micro-livestock to supply the required protein has resulted in the intensive production of Snails. Wild and domesticated snails fed on contaminated feed may accumulate heavy metals which may be injurious to the consumers. Although toxicity and the resulting threat to human health of any contaminant are, of course, a function of concentration, it is well-known that chronic exposure to heavy metals and metalloids at relatively low levels can cause adverse effects. This study aimed at investigating the ability and effects (if any) of heavy metal accumulation in Giant African Land Snails (Archachatina marginata). 120 *A. marginata* growers were randomly allotted to 2 feed treatments (T1: Heavy metal contaminated pawpaw fruits and leaves; and T2: Heavy metal free pawpaw fruits and leaves) in a Completely Randomized Design. Each treatment has 4 replicates containing 15 snails per replicate. The experimental diets were fed ad libitum for the period of 12 weeks during which recommended management practices were strictly adhered to. Data were collected on shell length, shell width, and live weight. Results obtained revealed that giant land snails possess the ability to bio-accumulate heavy metals in their tissues and this accumulation could have a reducing effect on all studied parameters, caused pathological lesions of the edible foot and mortality. It was thus recommended that feeds given to caged snails, and even other animals, should be ascertained heavy metals free before offered.

Keywords: Heavy metals, Bioaccumulation, Giant Land Snails, Contamination, Food safety.

ABSORPTION ET BIOACCUMULATION DE METAUX LOURDS CHEZ LES ESCARGOTS TERRETSRES GEANTS D'AFRIQUE (Archachatina marginata)

Résumé

La pratique croissante du micro-élevage dans le souci d'obtenir les protéines nécessaires au besoin nutritif human, a conduit à une production intensive d'escargots. Les escargots sauvages et domestiques nourris avec des aliments contaminés peuvent accumuler des métaux lourds susceptibles d'être préjudiciables à la santé du consommateur. Bien que la toxicité de tout contaminant et la menace qu'elle constitue pour la santé humaine soit, bien entendu, fonction de la concentration du contaminant, on sait que l'exposition chronique aux métaux lourds et aux métalloïdes à des taux relativement faibles peut avoir des effets néfastes. Cette étude avait pour objet d'étudier la possibilité et les effets (le cas échéant) de l'accumulation de métaux lourds dans les escargots terrestres géants d'Afrique (Archachatina marginata). Cent vingt (120) éleveurs d'escargots *A. marginata* ont été répartis de manière aléatoire à 2 régimes alimentaires (T1: fruits et feuilles de papayer contaminés par des métaux lourds ; et T2 : fruits et feuilles de papayer non contaminés par des métaux lourds) dans un dispositif complètement aléatoire. Chaque traitement comportait 4 répétitions, avec 15 escargots par répétition. Les escargots ont été nourris ad libitum avec des régimes expérimentaux pendant une période de 12 semaines durant laquelle les pratiques de gestion recommandées ont été strictement suivies. Les données ont été recueillies sur la longueur et la largeur de la coquille des escargots, ainsi que sur leurs poids vifs. Les résultats obtenus ont révélé que les escargots terrestres géants peuvent accumuler des métaux lourds dans leurs tissus, et cette accumulation est susceptible d'avoir un effet réducteur sur tous les paramètres étudiés, causer des lésions pathologiques

*Corresponding author: joelalabi@gmail.com, +2347066486949

du pied comestible des escargots et engendrer leur mortalité. L'étude a donc recommandé que les aliments destinés aux escargots en cage - et même à d'autres animaux - soient soumis à une analyse de leur concentration en métaux avant leur utilisation.

Mots-clés : métaux lourds, bioaccumulation, escargots terrestres géants, contamination, sécurité sanitaire des aliments.

Introduction

Protein is required for normal growth and repair of body tissue, and it is principal constituents of the organ and soft structures of animal body. This makes the importance of protein not to be overemphasized in the nutrition of man (Ani *et al.,* 2013). In Nigeria, animal protein sources are mainly from cattle, pig, sheep, goats, poultry, fish and game animals in the rural areas (Ani *et al.,* 2013). The cost per unit (kg) of these meat and the purchasing power of average Nigerians could limits their meat consumption pattern thus resulting to low animal protein intake (Agbogidi *et al.,* 2008; Omole *et al.,* 2010). However, snail meat holds the potential to bridge the gap of this would-be nutritional deficiency owing to its nutritional profile, availability and affordability.

Snail meat is high in protein content, rich in amino acids and essential fatty acids, contains less fat and cholesterol, and supply sufficient amount of Iron and other essential minerals (Ademolu *et al.,* 2004; Agbogidi *et al.,* 2008; Anthony *et al.,* 2010). Its medicinal uses for prophylactic and therapeutic purposes against several ailments and diseases such as anemia, tuberculosis, diabetes, ulcer, hypertension, asthma, circulatory diseases, kidney diseases and stomach disorders have been established (Omole and Kehinde, 2005; Abere and Lameed, 2008). These potentials are being harnessed due to the fact that snails are easily accessible either by production, purchase or picking (hunting) in the wild (Anthony *et al.,* 2010). Though snails are omnivores (Omole and Kehinde, 2005), they have preference for fruits, leaves and vegetables (Akinnusi, 1998; Eruvbetine *et al.,* 1999). However, wild snails, which are commonly found in the bush, have free access to soil, vegetables, fruits, leaves of plants which might have grown in heavy metals contaminated areas. More so, domestic snails fed with plant food materials that have been contaminated can accumulate such heavy metals. This could adversely affect the growth and reproductive capacity.

Plants that grows near the roadside, domestic and industrial waste dump site have tendency to absorb and accumulate heavy metals (Singh *et al.,* 2012). It is of interest to note that some of these elements are important for the normal functions of the body but could cause acute and chronic poisoning when their concentrations exceed tolerable limit. Continuous consumption of vegetables or fruits grown in heavy metal contaminated areas and eating of animals such as snails that feeds on these plants grown in such area are the main avenue through which man gets infected (Florijin and Van Beusichem, 1993). Heavy metals such as Lead (Pb), Cadmium (Cd), Copper (Cu), Chromium (Cr), and Arsenic (Ar) have been indicted as primary causes of cancer, neo-natal mortality, kidney, liver and nerve damage, osteoporosis and other serious human and livestock health problems (EFSA. 2010; Taghipour and Mosaferi, 2013; Ogendi *et al.,* 2014). Although the need for increased production of snails is important particularly for supply of animal protein, consumer's right to consumption of safe snail meat devoid of contamination beyond safe limits must be protected. Therefore, this study focused on assessing the ability of snails to bio-accumulate heavy metals and to study the effect (if any) of heavy metal accumulation in snails.

Materials and Methods

Experimental Location and Housing

The study was carried out at the Snailery Unit, Southern Farm of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. The Snails (*A. marginata*),

purchased from the Snailery Unit of the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, were reared under intensive system of production using rectangular wooden cages of 2.44 x 0.90 x 0.33m in dimension. The cages were raised 23cm above the ground, partitioned into 6 divisions and placed in a shed made of concrete and wire netting surrounded by trees which provide shade, adequate ventilation and cool environment for the snails.

Experimental Design and Feeding

120 growers African giant land snails (*A. marginata*), whose weight ranged from 25 - 30g, were randomly allotted to 2 dietary treatments in a completely randomized design. Each treatment was further subdivided into 4 replicates of 15 snails per replicate. Snails in treatment 1 were fed heavy metal contaminated pawpaw fruits and leaves (HMCD) obtained from Aba-Eku waste dump, Akanran Road, Ibadan; and those in treatment 2 were fed heavy metal free pawpaw fruits and leaves (HMFD) obtained from IAR&T, Ibadan. The fruits were cut into small pieces while the leaves were chopped and offered to snails ad libitum in flat trays. Clean water were also provided in flat drinkers. The experimental period lasted 14 weeks out of which 2 weeks was used for acclimatization while the feeding trial lasted for 12 weeks. Management practices recommended for optimum growth and prevention of insects and predator attack were strictly adhered to. Data were collected at the commencement of the study and at fortnightly thereafter on shell length and shell width (using vernier caliper) and body weight (using electric weighing balance).

Chemical and Statistical Analyses

At the end of the feeding trial, three snails taken from each replicate were washed and killed by carefully cracking the shell with iron rod. Following careful separation of the snails' parts, the edible portions were oven-dried at 65oC to constant weight. Chemical analysis for the proximate composition (body protein, body fat, body ash, & protein to ash ratio) of the dry sample was carried out using the procedures of AOAC (2000). Presence and concentration of heavy metals (Pb, Fe, Cu, Cd, Ni and Zn) were analyzed by atomic absorption spectrophotometer (AAS). Statistical analysis was done by subjecting the results obtained to one-way analysis of variance (ANOVA) using SAS (1999) while variation among the means were separated using Tukey Studentized Range (HSD) test at 5% level of significance.

Results

There was an appreciable gain in the shell length, shell width and live weight of snails fed experimental diets. Significant difference ($P<0.05$) in shell length began at the 4th week of the study with higher values obtained in snails fed HMFD. Similarly, higher ($P<0.05$) shell width commenced from 6th week with snails fed HMFD having a total gain of 1.30cm compared to those fed HMCD having a total gain of 0.83cm. Significant variation ($P<0.05$) in weight gain commenced from 2th week, except at 4th week, with snails fed HMCD and HMFD having a total weight gain of 17.58 and 21.63g, respectively.

Table 2 shows the effect of dietary treatment imposed on the growth and shell performance of *A. marginata*. The result revealed that shell length gain, shell width gain, weight gain and mortality varied significantly ($P<0.05$). Snails fed HMFD had higher values for final shell length (7.12cm), shell length gain (1.63cm), final shell width (4.68cm), and shell width gain (1.30cm) compared to their counterparts fed HMCD which had the values of 6.43cm, 0.95cm, 4.20cm and 0.83cm for final shell length, shell length gain, final shell width and shell width gain, respectively. Similarly, final weight (50.52g) and weight gain (21.63g) were significantly higher ($P<0.05$) in snails fed HMFD while those fed HMCD had lower values of 46.48g and 17.56g for final weight and weight gain, respectively. Onset of pathologic lesion (swelling) on the foot was observed in snails fed HMCD at the 6th week which led to incidence of mortality at day 52 of the study. Mortality (2.75%) was recorded in snails fed HMCD whereas no

mortality was observed in snails fed HMFD.

The results showing the proximate content and heavy metal concentration in the edible portion (foot) of *A. marginata* as presented on Table 3 revealed significant variation ($P<0.05$) for all parameters except body fat. Body protein (87.52%) and body ash (2.40%) were higher in snails fed HMFD while those fed HMCD had reduced values (85.54% and 2.02%, respectively). Snails fed HMCD had higher protein: ash ratio (42.39) compared to those fed HMFD which had lower value (36.48). Higher ($P<0.05$) heavy metals concentration were found in snails fed HMCD. Pb, Fe, Cu, Cd, Ni, and Zn had the values of 64.00, 129.00. 79.01. 16.00, 64.00, and 175.02 mg/kg, respectively for snails fed HMCD and 5.01, 47.01, 8.00, 4.95, 2.85, and 28.00 mg/kg, in the same order for snails fed HMFD.

Discussion

Results from the study (Table 1) showed significant increase in shell length, width and average live weight in snails fed heavy metal free diets (HMFD) which could be attributed to the quality of the diets. Growth, being the major indicator of performance, is usually influenced by the quality and quantity of dietary nutrient intake. This better explains the reason for retarded growth, swellings of the foot, and mortality observed in snails fed HMCD. Odo and Orji (2010) had reported that not all feed exert appreciable change in snail growth, and that some food materials might even cause growth retardation, elicit some pathological conditions and negative body gain. Positive correlation observed between shell length, shell width and body weight gain in this present study agrees with those earlier reported for growing snails (Odunaiya and Akinnusi, 2008; Ani *et al.,* 2013).

The gain in shell length (0.95 & 1.63cm) and shell width (0.83 & 1.30cm) values obtained in this present study (Table 2) fall below the values earlier reported (Ademolu *et al.,* 2004; Ejidike, 2004; Ani *et al.,* 2013). The variation with these reports could arise from differences in size, age, species of snails used, feeds offered and management practices. Weight gain values (17.58 and 21.63g) in this study were higher than the values (5.97-14.02g) reported by Ademolu *et al.,* (2004) in snails fed diets containing varying protein levels but lower than that (21.40-35.30g) reported by Ejidike, (2004) when snails were fed diets with different nitrogen sources. Furthermore, significant variation in weight gain and shell length observed in this study is in tandem with that of Odo and Orji, (2010) when snails were fed different forages but, however, contradicts the observation of Okonta, (2012) who reported no significant difference in shell length and weight gain of growing snails fed palm fruits and potato leaves. This discrepancy, therefore, cannot be unconnected with the variation in the types, quality and nutritive value of diets used in various experiments, among other factors. Survivability was influenced by the experimental diets. Snails fed HMCD recorded 2.75% mortality following pathological lesions observed in the edible foot. This implies that heavy metals ingested has serious implications on health status of *A. marginata*.

Crude proteins of snails flesh obtained in this present experiment (Table 3) is higher than those reported by Ademolu *et al.,* (2004), Ejidike, (2004), Kalio and Etela, (2011). This suggests the appropriateness of the pawpaw fruits and leaves for effective tissue protein accretion. This finding is in congruence with the previous findings (Kalio and Etela, 2011) that snails have preference for pawpaw leaves over other experimental diets. Higher ash values in snails fed HMFD suggests the appropriateness of snail meat in supplying the essential minerals required in human nutrition.

Heavy metals concentration in the edible portion of snails were influenced by the treatment imposed with significantly higher concentration observed in snails fed HMCD. This implies that pawpaw fruit and leaves harvested from the contaminated area absorbed high amount of heavy metals that could translate into animal tissue when consumed. This corroborates the findings of Singh *et al.,* (2012) who found that plants have potential to accumulate heavy metals from

Table 1: Shell length, shell width and live weight of *A. marginata* fed experimental diets

	Weeks							
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**Total**
Shell length (cm)								
Heavy metal contaminated diets	5.48	5.88	6.26[a]	6.32[b]	6.34[b]	6.40[b]	6.43[b]	0.95[b]
Heavy metal free diets	5.49	5.87	6.24[b]	6.39[a]	6.54[a]	6.62[a]	7.12[a]	1.63[a]
SEM	0.013	0.003	0.004	0.008	0.004	0.006	0.008	0.020
Shell width (cm)								
Heavy metal contaminated diets	3.37	3.63	4.14	4.14[b]	4.16[b]	4.19[b]	4.20[b]	0.83[b]
Heavy metal free diets	3.38	3.61	4.14	4.21[a]	4.30[a]	4.43[a]	4.68[a]	1.30[a]
SEM	0.009	0.006	0.016	0.008	0.012	0.006	0.014	0.021
Average live weight (g)								
Heavy metal contaminated diets	28.90	34.57b	37.92	42.46[b]	43.01[b]	44.16[b]	46.48[b]	17.58[b]
Heavy metal free diets	28.89	34.80a	38.16	42.72[a]	45.90[a]	49.23[a]	50.52[a]	21.63[a]
SEM	0.030	0.020	0.208	0.007	0.006	0.012	0.004	0.030

[ab]*Comparative means between treatment in a column that contain different superscripts are significant (P<0.05). SEM – Standard error of Means*

Table 2: Growth and shell performance of *A. marginata* fed experimental diets

Parameters	**Heavy metal contaminated diets**	**Heavy metal free diets**	**SEM**
Initial shell length (cm)	5.48	5.49	0.013
Final shell length (cm)	6.43[b]	7.12[a]	0.008
Length gain (cm)	0.95[b]	1.63[a]	0.020
Initial shell width (cm)	3.37	3.38	0.009
Final shell width (cm)	4.20[b]	4.68[a]	0.014
Width gain (cm)	0.83[b]	1.30[a]	0.021
Initial weight (g)	28.90	28.89	0.030
Final weight (g)	46.48[b]	50.52[a]	0.004
Weight gain (g)	17.58[b]	21.63[a]	0.030
Daily weight gain (g)	0.21[b]	0.26[a]	0.000
Mortality (%)	2.75[a]	0.00[b]	0.604

[ab] *Comparative means in a row that contain different superscripts are significant (P<0.05)*
Daily weight gain = Weight gain/Duration of experiment (days), SEM – Standard error of Means

the environment or contaminated land on which they are grown. The profile followed the sequence: Zn>Fe>Cu>Pb>Ni>Cd. This could be attributed to their various chemical properties which influences their mobility and bioaccumulation. The finding is similar to those of previous studies by Singh *et al.,* (2012) and Ogendi *et al.,* (2014) who reported similar trend in the heavy metal concentration in fish and sediments, and in different crops and plant parts, respectively. Fe, Cu and Zn has been classified as essential metal nutrients while Pb, Cd and Ni are referred to as non-essential heavy metal Singh *et al.,* (2012). Fe is an integral component of haemoglobin which helps to prevent haemolytic anaemia, and ensures normal serum bilirubin (Babalola and Akinsoyinu, 2009; Jummai and Okoli, 2013).

Table 3: Proximate content and Heavy metals concentration in the flesh of *A. marginata* fed experimental diets

Parameters	Heavy metal contaminated diets	Heavy metal free diets	SEM
Proximate content (%)			
Body protein	85.54[b]	87.52[a]	0.209
Body fat	3.59	3.84	0.078
Body ash	2.02[b]	2.40[a]	0.22
Protein: Ash	42.39[a]	36.48[b]	0.508
Heavy metals (mg/kg)			
Pb	64.00[a]	5.01[b]	0.035
Fe	129.00[a]	47.01[b]	0.074
Cu	79.01[a]	8.00[b]	0.027
Cd	16.00[a]	4.95[b]	0.028
Ni	64.00[a]	2.85[b]	0.311
Zn	175.02[a]	28.00[b]	0.219

[ab] *Comparative means in a row that contain different superscripts are significant (P<0.05)*
Daily weight gain = Weight gain/Duration of experiment (days), SEM – Standard error of Means

More so, its involvement in in oxidation of carbohydrates, protein and fats could be attributed to its necessity in psychomotor development, maintenance of physical activity and work capacity as well as resistance to infection (Babalola and Akinsoyinu, 2009; Walker *et al.,* 2005). Cu is a vital element necessary for normal organism growth, immune function and metabolism (Ogendi *et al.,* 2014). It forms an essential part of several enzymes systems including cytochrome oxidase and tyrosinase. Zn, being a component of more than 50 enzymes, is required for proper growth, maintenance of immune function, and prevention of and quick recovery from infections. In addition, Fe and Zn are both important components of neural function and are essential for childhood growth and development (Walker *et al.,* 2005). This confirms the reason for recommending snail meat for pregnant women, nursing mothers and children. However, eventhough Fe, Cu and Zn were considered to be essential metals, the chemistry of interaction among these metals, and with other non-essential ones in body tissues as well as their permissible tolerable limits in snails is not known.

Intake of Pb causes depression, behavioural disorders, and neo-natal mortality. Acute Pb poisoning in humans could result to severe dysfunction of kidney, reproductive system, liver, brain and CNS, which leads to sickness and death (Taghipour and Mosaferi, 2013). Furthermore, several studies reported by European Food Safety Authority showed higher positive correlation between dietary lead intake and blood lead levels with significant effects on neurodevelopmental effects in children, neurotoxicity, cardiovascular and kidney defects in adults (EFSA, 2010). Cd bio-accumulation has been related to hypertension and cardio-vascular diseases, destruction of RBC and testicular tissue, organs and CNS dysfunction, osteoporosis and pulmonary emphysema (Ogendi *et al.,* 2014).

It is worthy of note that dietary intake of contaminated food remains the major route of ingesting heavy metals, and that absorbed nutrients accumulates with age or duration of exposure. The potential health risk, however, depends on the chemical composition, concentration and consumption rate of the ingesta. In man, maternal transfer could also occur through placenta and subsequently during breast feeding, especially for lead (EFSA,

2010). Therefore, picking of wild snails around waste dumpsite should be discouraged in order to reduce the risk of bioaccumulation and biomagnification of heavy metals.

Conclusion

It is evident from the present study that snails could accumulate heavy metals from the feed consumed with resultant adverse effects on their growth and health status. To this end, feeds given to caged snails, and even other animals, should be ascertained heavy metals free before offered. Wild snails with swollen foot (edible portion) should not be consumed, particularly by the children and women of child-bearing age. This is necessary to protect the consumer against collected snails that may have ingested toxic plants or potentially harmful chemicals.

List of Abbreviations: HMCD: Heavy metal contaminated diet, HMFD: Heavy metal free diet.

Competing interests: The authors hereby declared that there is no competing interest.

Authors' contributions: JO participated in the design of the study and data collection, performed the statistical analysis and drafted the manuscript. AO participated in the design of the study, coordinate the chemical analysis and drafted the manuscript. AAO conceived of the study, participated in its design and coordination and helped to draft the manuscript. CG participated in the design of the study, source for the test ingredients throughout the feeding trial and assisted in the chemical analysis. All authors read and approved the final manuscript.

References

Abere SA, Lameed GA. 2008. The medicinal utilization of snails in some selected states in Nigeria. In the Proceeding of the 1st National conference of the Forests and Forest Products Society, Akure, Ondo State Nigeria,pp: 233–237.

Ademolu KO, Idowu AB, Mafiana CF, Osinowo OA. 2004. Performance, proximate and mineral analyses of African giant land snail (Achachatina marginata) fed different nitrogen sources. African Journal of Biotechnology, 3(8): 412-417.

Agbogidi OM, Okonta BC, Ezeani EL. 2008. Effects of two edible fruits on the growth performance of African giant land snail (Archachatina marginata). Journal of Agricultural and Biological Sciences, 3(3):26-29.

Akinnusi O. 1998. A practical approach to backyard snail farming. Nigerian Journal of Animal Production, 25: 193-197.

Ani AO, Ogbu CC, Elufidipe CO, Ugwuowo LC. 2013. Growth performance of African giant land snail (Achachatina marginata) fed varying dietary protein and energy levels. ARPN Journal of Agricultural and Biological Science, 8(2):184-190.

Anthony AA, Adebayo-Tayo CB, Inyang UC, Aiyegoro AO, Komolafe OA. 2010. Snails as meat source: Epidemiological and nutritional perspectives. Journal of Microbiology and Antimicrobials, 2(1):001-005.

AOAC. 2000. Official methods of Analysis. Association of Official Analytical Chemists International. 17th ed. 2 volume set.

Babalola OO, Akinsoyinu AO. 2009. Proximate composition and mineral profile of snail meat from different breeds of land snail in Nigeria. Pakistan Journal of Nutrition. 8(12): 1842-1844

EFSA. 2010. Scientific Opinion on Lead in Food. EFSA Journal, 8(4):1570.

Ejidike BN. 2004. Growth performance and nutrient utilization of African giant land snail (Archachatina marginata) hatchlings fed different protein diets. Food Agriculture and Environment, 2(1):160-162.

Eruvbetine D, Oguntona EB, Adoye UA. 1999. Preliminary studies comparing different diets for intensive rearing of snails. In the Proceedings of the 1997 Biennial Conference of the Ecological Society of Nigeria (ECOSON) held at the University of Agriculture, Abeokuta, pp: 93-97.

Florijin PJ, Van Beusichem ML. 1993. Uptake and distribution of Cd in Maize inbreed line. Plant Soil,

150:125-193

Jummai AT, Okoli BJ. 2013. Compositional Evaluation of Giant East African Snail. Research Journal in Engineering and Applied Sciences, 2(5):397-401

Kalio GA, Etela I. 2011. Nutritional and sensory profiling of the African Giant Land Snail fed commercial-type and leaf-based diets in a rain-forest ecology. African Journal of Food, Agriculture, Nutrition and Development (AFJAND Online), 11(5): 1-12

Odo BI, Orji AC. 2010. Evaluation of the growth performance of snails fed different forages under intensive management. Pakistan Journal of Nutrition, 9(6): 543-546.

Odunaiya O, Akinnusi FAO. 2008. Effect of some cheap locally available feeding material on the growth performance of edible land snail. In the Proceedings of the 33rd Annual Conference of Nigerian Society for Animal Production (NSAP). College of Agricultural Sciences, Olabisi Onabanjo University, Yewa Campus, Ayetoro, Ogun State Nigeria. Pp: 126-128.

Ogendi GM, Maina GM, Mbuthia JW, Koech HK, Ratemo CM, Koskey JC. 2014. Heavy metal concentrations in water, sediments and Common Carp (Cyprinus carpio) Fish species from lake Naivasha, Kenya. Research Journal of Environmental and Earth Sciences, 6(8):416-423.

Okonta BO. 2012. Performance of African land snail Archachatina marginata (Swainson) fed with selected diets. Global Journal of Bio-Science & Biotechnology, 1(2): 182-185

Omole AJ, Kehinde AS. 2005. Backyard snail farming at a glance. Back to Agricultural Series (1). Ibadan, Technovisor Agricultural Publications.

Omole AJ, Obi OO, Soetan KO, Olaseinde TO. 2000. Influence of stocking rate on growth and reproduction performance of breeding snails (Archachatina marginata). Journal of Cell and Animal Biology, 4(4):064-7.

SAS. 1999. Statistical Analysis System User's guide: Statistical. (Version 6). 12th ed. SAS Institute Inc. USA, Cary, North Carolina.

Singh S, Zacharias M, Kalpana S, Mishra S. 2012. Heavy metals accumulation and distribution pattern in different vegetable crops. Journal of Environmental Chemistry and Ecotoxicology, 4(10):170-177.

Taghipour H, Mosaferi M. 2013. Heavy Metals in the vegetables collected from production sites. Health Promotion Perspectives, 3(2):185-193.

Walker CF, Kordas K, Stoltzfus RJ, Black RE. 2005. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. The American Journal of Clinical Nutrition, 82:5-12.

EFFECT OF STAGE OF MATURITY ON DRY MATTER YIELD, MORPHOLOGICAL CHARACTERISTICS AND NUTRITIVE VALUE OF BURGUNDY BEAN (MACROPTILIUM BRACTEATUM)

M Baba, A B Nasiru and N Ashahabu
Deparment of Animal Science, Bayero University Kano, Nigeria.

Abstract

The experiment evaluated effect of stage of maturity on dry matter yield, morphological characteristics and nutritive value of burgundy bean (Macroptilium bracteatum) at the screen house of Department of Agronomy, Bayero University Kano, Nigeria. The treatments were 3 stages of growth repeated 3 times in a completely randomized design. Burgundy seeds were scarified and later sown in plastic buckets containing soil-sand mixture (3:1). Plants were harvested at 47, 61 and 82 days post sowing representing vegetative pre flowering and flowering stages of growth. Parameters such as dry matter yield, morphological characteristics and nutritive value indices were evaluated.

There was no significant effect of stage of maturity on dry matter yield and morphological characteristic of burgundy bean, however, numerically, dry matter yield of burgundy bean tended to increase linearly with age from 2.09 to 6.7 g pot-1 at vegetative and flowering stages respectively. Significant differences were observed among stages of growth in terms of nutritive value parameters. Crude protein was significantly greater ($p<0.05$) at vegetative (20.8%) compared to pre flowering (17.6) and flowering (17.9) stages. ADF and NDF were significantly higher ($p<0.05$) at flowering compared to other stages while parameters such as DMI, DDM and RFV were superior ($p<0.05$) at vegetative and pre flowering than flowering stages. Pre flowering stage was recommended as the best stage to harvest burgundy bean for improved animal performance.

Key words: Dry matter, Morphological, Chemical, flowering, pre flowering vegetative

EFFET DU STADE DE MATURATION SUR LE RENDEMENT EN MATIERE SECHE, LES CARACTERISTIQUES MORPHOLOGIQUES ET LA VALEUR NUTRITIVE DU HARICOT MACROPTILIUM BRACTEATUM

Résumé

La présente étude a évalué l'effet du stade de maturation sur le rendement en matière sèche, les caractéristiques morphologiques et la valeur nutritive du haricot Macroptilium bracteatum, à la serre du Département d'agronomie de l'Université de Bayero à Kano au Nigeria. Les traitements consistaient en 3 stades de croissance répétés 3 fois selon un schéma complètement aléatoire. Les graines du Macroptilium bracteatum ont été scarifiées puis semées dans des seaux en plastique contenant un mélange sol-sable dans la proportion (3:1). Les plantes ont été récoltées 47, 61 et 82 jours après le semis, représentant les stades de croissance végétatifs, pré-floraux et floraux. Des paramètres tels que le rendement en matière sèche, les caractéristiques morphologiques et les indices de valeur nutritive ont été évalués.

L'étude n'a relevé aucun effet significatif du stade de maturation sur le rendement en matière sèche et les caractéristiques morphologiques du haricot Macroptilium bracteatum. Cependant, numériquement, le rendement en matière sèche du haricot Macroptilium bracteatum avait tendance à augmenter de façon linéaire avec l'âge de 2,09 à 6,7 g pot-1, respectivement aux stades végétatif et floral. Des différences significatives ont été observées entre les stades de croissance en termes de paramètres de valeur nutritive. La protéine brute était significativement plus élevée ($p <0,05$) au stade végétatif (20,8%) par rapport aux stades pré-floral (17,6) et floral (17,9). Les teneurs en ADF et NDF étaient significativement ($p <0,05$) plus élevées au stade floral par rapport aux autres stades, tandis que les paramètres DMI, DDM et RFV étaient

*Corresponding author email: babamuhammad01@ yahoo.com

supérieurs (p <0,05) aux stades végétatif et pré-floral par rapport au stade floral. Le stade pré-floral a été recommandé comme étant le meilleur stade de récolte des haricots Macroptilium bracteatum pour améliorer la performance des animaux.

Mots-clés : matière sèche, morphologique, chimique, floral, pré-floral, végétatif

Introduction

Animal production in Nigeria is beset with a variety of problems prominent among which is the issue of feed adequacy during a particular period of the year (dry season). This period is characterized by decline in quantity and quality of the available forages that animals graze on with attendant negative consequences on overall animal productivity.

Most tropical livestock are grazed on poor quality forages and crop residues during critical period (dry season) that do not add any reasonable value to the animals in question, hence animals suffer from loss in body weight, reduction in meat and milk production as well as reduced reproductive capacities among other problems.

The need for supplementation during critical period is therefore very imperative. Utilization of forage legume as supplement have proven to be successful in whittling down the effect of poor quality feed on animal production as reported by Markovic et al, (2007). However, productivity and quality of legume forages may be influenced by factors such as species and stage of growth (Van Soest, 1987).

One of the forage legumes that could be used as supplement to improve poor quality feed is burgundy bean albeit not cultivated in Nigeria. Burgundy bean is a twining and trailing perennial which grows up to 80cm in height (Whitebread *et al.,* 2005). It establishes easily and can quickly provide a canopy suitable for grazing; in addition, it is a persistent perennial with robust root system (Conway, 2000). Under a good growing condition, burgundy bean can produce 5-8 tons/hectare/year of dry matter (Cox *et al.,* 2007). The experiment therefore aimed at evaluating the dry matter yield and quality parameters at different stages of growth under Nigeria's condition.

Objectives

The objectives of the study were

- To determine the dry matter yield of burgundy bean under three growth stages (Vegetative,pre flowering and flowering stages)
- To evaluate the nutritive value of burgundy bean under the 3 growth stages.

Materials and Methods

Experimental location:

The experiment was conducted at the screen house of the Department of Agronomy, Bayero University Kano, Nigeria, in the year 2014. Kano lies between longitude 9° 31′ and 12° 30′ North, and latitude 9° 30′ and 8° 42′ East in the Sudan savannah ecological zone of Nigeria. The zone is characterized by two distinct seasons, wet season (May-September) and dry season (October-April). Mean annual rainfall ranges from 500-1000 mm while mean annual temperature from 21°C to 39°C (KNARDA, 2001). The soil used for the experiment is a sandy loam soil with the following characteristics (pH=6.47, N=0.08%, P=22.53ppm and K=0.03cmol/kg)

Treatments and experimental design:

The experimental treatments were the three stages of growth repeated four times in a completely randomized design

Seed treatment soil preparation and sowing operation:

Burgundy seeds were procured from the Department of Animal Science Bayero University Kano, Nigeria. The seeds were scarified by immersing in hot water boiled at 80°C for 3 minutes to break dormancy. Soil mixture in the ratio (3 soils to 1 sand) was prepared and 5 kg each was put in 12 plastic buckets with the dimensions (21.5 cm width,

15.5 cm base width and 20 cm, height). Water was sprinkled onto the soil mixture contained in the buckets. The seeds were later sown (10 per bucket) on 13/08/2014. The seedlings were later trimmed to 5 seedlings per bucket 3 weeks post sowing. The plants were irrigated initially and subsequently fed by rainwater till harvest. Harvesting was done at 47, 61 and 82 days post sowing representing vegetative, pre flowering and flowering stages.

Harvesting:

The parameters measured at each harvest were, dry matter yield, leaf to stem ratio, root length, number of leaves and number of nodules. Dry matter yield was measured by harvesting the forage at the desired stage of growth to a stubble height of 5cm to the soil level, fresh weights were taken using digital balance, the materials were then oven dried at 65°C for 72 hours to determine the weight on dry matter basis. Leaf to stem ratio was determined by separating the harvested materials into leaf and stem, the respective weights were taken on dry weight basis after oven drying at the same temperature and duration.

Number of leaves was determined by counting the number of leaves on each plant stand while root length and number of nodules were determined by washing the root with flowing tap water free of soil, thereafter, number of nodules was counted and the root length measured using meter rule.

Chemical Analysis:

Chemical composition of forage was determined by grinding the dried samples with hammer mill to pass through 2 mm sieve. Crude protein content was determined by Kjeldahl's procedure (AOAC, 1984). ADF and NDF were determined according to procedure described by Goering and Van Soest (1970). Other quality parameters were calculated as follows

Dry matter intake (DMI) = 120/%NDF

Digestible Dry Matter (DDM) = 88.9-(%ADF*0.779)

Relative Feed Value (RFV) = (%DDM*%DMI)/1.29

Total Digestible Nutrient (TDN) = (-1.291*ADF) (Horrock and Vallentine 1999)

Data Analysis:

The data generated were analyzed by analysis of variance using SAS (9.2 2009)

Results

Dry matter yield and morphological characteristics:

The dry matter yields and morphological characteristics of burgundy bean harvested at three stages of growth are shown in Table 1. There were no significant effects of stages of growth on dry matter yield and morphological characteristics of burgundy bean, however, in numerical terms, dry matter yield was found to increase with increased stage of growth from 2.09 g pot-1 at vegetative stage to 6.7 at flowering stage. Dry matter yield value was highest at flowering stage compared to pre flowering (3.03 g pot-1) and vegetative stages (2.09 g pot-1). Number of leaves was numerically greater at flowering stage (90.5) compared to pre flowering (71) and vegetative (60.5) stages. Leaf to stem ratio root length and number of nodules were observed to have increased from vegetative to pre flowering (1.34-1.91, 30.45-45.75 and 26-40.5) respectively, followed by a decline at flowering stage (1.18, 43.28 and 32.75).

Chemical composition and other nutritive value indices: The chemical composition and nutritive value indices of burgundy bean are shown in Table 2. Significant differences were observed among stages of growth in the following parameters CP, ADF, NDF, DMI DDM and RFV. Crude protein was significantly greater ($p<0.05$) at vegetative stage (20.8%) compared to pre flowering (17.6%) and flowering (17.9%). Conversely, ADF was significantly higher ($p<0.05$) at flowering (41.1%) compared to pre flowering (31.7%) and vegetative (27.6%) stages. The ADF

at pre flowering stage was in turn superior ($p<0.05$) to that of vegetative stage. NDF was significantly higher ($p<0.05$) at flowering stage (52.3%) compared to pre flowering (41.1%) and vegetative (41.5%) stages. However, no significant difference was observed between vegetative and pre flowering stages.

Total digestible nutrient was not significantly different among stages of growth, however, in absolute terms, vegetative (65.7%) and pre flowering (60.4%0 tended to have higher TDN than flowering stage. DMI DDM and RFV were observed to be significantly greater ($p<0.05$) at vegetative and pre flowering stages than flowering stage

Discussion

Forage dry matter yield, crude protein, fiber content and digestibility are good indicators of availability and nutritive value. Although, there were no significant differences among stages of growth in terms of dry matter yield, flowering stage showed numerically higher dry matter yield value compared to other stages, this may be attributable to the seemingly higher number of leaves at this stage (Table 1) which could have led to higher rate of photosynthesis and by extension higher dry matter yield.

The significantly higher crude protein content observed at vegetative stage compared with other stages agrees with the reports of (Razec *et al.,* 2002 and Turgut et al 2008) on fresh forage and hay respectively. The significantly higher ADF and NDF content at flowering compared to other stages is consistent with the results of Ammar et al, (2010) and Turgut et al, (2008) and might be attributed to increased lignification with age. Ammar *et al.,* (2004) reported that as plants matured photosynthetic products are more rapidly converted to structural carbohydrate thus decreasing protein and soluble carbohydrate and increasing cell wall component. The significantly higher DDM, DMI and RFV values recorded at vegetative and pre flowering stages compared to flowering may viewed in the context of higher higher ADF and NDF values recorded at flowering stage. Furthermore, ADF gives a measure of digestibility, the higher the ADF the less the digestibility. Similarly, NDF value is negatively related to intake, the higher the NDF the lower the intake. The digestibility of forages decreased with advancing maturity in approximately lower fashion (Ammar *et al.,* 2010).

Table 1: Dry matter yield and morphological characteristics of burgundy bean as affected by three stages of growth.

Stages of growth	DMY (g pot-^{1})	L/S	Root length (cm)	Number of leaves	Number of nodules
Vegetative	2.09	1.34	30.45	60.5	26
Pre flowering	3.03	1.91	45.75	71	40.5
Flowering	6.7	1.18	43.28	90.5	32.75

DMY = Dry matter yield, L/S = leaf to stem ratio

Table 2: Chemical composition and nutritive value parameters of burgundy bean harvest at three stages of growth.

Stage of growth	Cp (%)	ADF (%)	NDF (%)	TDN (%)	DMI (%)	DDM (%)	RFV (%)
Vegetative	20^{a}	27.6^{c}	41.5^{b}	65.7	2.9^{a}	65.7^{a}	151^{a}
Pre flowering	17.6^{b}	31.7^{b}	41.1^{b}	60.4	2.92^{a}	64.2^{a}	145^{a}
flowering	17.9^{b}	41.1^{a}	52.3^{a}	48.3	2.29^{b}	56.89^{b}	101^{b}

Means within the same column with different superscripts were significantly different.

Conclusion

Based on the dry matter yield and nutritive value results, it can be concluded that pre flowering stage is the best stage to harvest burgundy bean for improved animal production. This is predicated on the fact that fiber fractions (NDF and ADF) were comparatively lower at this stage compared to flowering while other nutritive value parameters (DDM. DMI and RFV) were significantly greater. The higher dry matter yield recorded by burgundy bean at flowering stage notwithstanding given that dry matter yield is not the only determinant of animal performance, other quality parameters are important as well.

References

Ammar H, Lopez S and Andres, S 2010 Influence of maturity stage of forage grasses and legumes on the chemical composition and in vitro dry matter digestibility. In Porqueddu, C. (ed) Rios, S (ed). The contribution of grasslands to the conservation of Mediterranean biodiversity Zaragoza: CIHEAM/ CIBIO/FAO/ SEEP no 92 p199-203.

Ammar H, Lopez S, Gonzalez J S and Ranilla M J 2004 Chemical composition and in vitro digestibility of some Spannish browse plants species. Journal of Science and Food Agriculture, 84:197-204.

AOAC 1984 Association of Official Analytical Chemist. Official Methods of Analysis. Agricultural Handbook no 379, US Department of Agriculture.

Conway M 2000 Animal productivity from Milgarra butterfly pea (Clitoria ternatea) and burgundy bean (Macroptilium bracteatum). Central Queensland Sustainable Farming Systems Project (CQSFSP)

Cox C, Whitebread A M and Pengelly B C 2007 Establishing grass-legume pastures on rundown cropping soils of the Western Downs in southern Queensland. Procceedings of the 11th Australian Agronomy Conference, Geelong.

Goering H K and Van Soest P J 1970 Forage fiber analysis. Agricultural Handbook no 379, US Department of Agriculture.

Harrocks R D and Vallentine J F 1999 Harvested forages, Academic Press, San Diego, California USA. 415 pp.

KNARDA 2001 Kano Agricultural and Rural Development Authority. Meteorological Station Record Book and Management. Vol 11 p 1-3.

Markovic J, Radovic J, Lugic Z and Sokolovic D 2007 The effect of development stage on chemical composition of alfalfa leaf meal, Biotechnology in Animal Husbandry 23(5-6):283-338.

Razec I, Rajec M, and Opera G 2002 Dynamics of DM accumulation and feeding value of the first cycle of growth of some association of grasses with white clover in lowland grasslands of Europe . Utilization and development FAO/CIHEAM. Interegional and Cooperative Research and Development Network for Pastures and Fodder Crop Production. FAO Technical Series no 64 p 133-140

SAS 1989 Statistical Analysis System. SAS Users Guide Version 9.2 SAS Institute Inc CARY, NC, USA.

Turgut L, Yanar M, Tuzemen N, Tan M, and Comakli B 2008 Effect of maturity stage on chemical composition and insitu ruminal degradation kinetics of meadow hay in Awassi sheep. Jouranal of Veterenary Advances 7(9) 1061-1065.

Van Soest P J 1987 Nutritional ecology of the ruminants. Comstock Publishing Associates. A division of Cornell University Press, Cornell, New York.

Whitebread A M, Pengelly B C and Smith B R 2005 An evaluation of three tropical legume hays for use in mixed farming systems on clay soils in Southern Queensland Australia, Tropical grasslands, 39:9-21

PERFORMANCE OF BROILER CHICKENS FED NEEM (AZADIRACHTA INDICA) LEAF MEAL AS AN ALTERNATIVE TO ANTIBIOTICS

*Egbeyale, L. T., Ayo-Ajasa, O. Y. Ayoola, A. A., Ogunkunle, N. F., Okuboyejo, O. A.
Department of Animal Production and Health, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria.

Abstract

One hundred and ninety-two day-old marshal broilers were used in an eight weeks feeding trial to evaluate the effects of neem (Azadirachta indica) leaf meal on growth performance and haematological parameters of broiler chickens. The birds were randomly assigned into four (4) groups of forty eight (48) birds each in a Completely Randomized Design. Each group was sub-divided into three (3) replicates of sixteen (16) birds each. The neem leaf meal (NLM) was included at 0, 0.5, 1.0 and 1.5% as a replacement of wheat offal.. Feed and water were provided ad libitum. The inclusion levels of A. indica reduced ($p<0.05$) the growth performance of the birds during starter phase but 0.5% NLM produced highest ($p<0.05$) final weight at finisher phase. The best values of feed conversion ratio (FCR) and protein efficiency were recorded in the control while all the other groups had similar values at starter phase. At finisher phase, the highest ($p<0.05$) weight gain was recorded from birds fed 0.5%NLM while the control and 1.0%NLM had similar ($p>0.05$) values. The FCR and protein efficiency were similar. There was no mortality throughout the finisher phase while the values at starter phase were similar ($p>0.05$) across the groups. Among all the haematological parameters that were measured, the PCV, Hb, heterophils and lymphocytes were significantly ($p<0.05$) influenced at starter phase while PCV, WBC, platelets and heterophils were influenced at finisher phase. It could be recommended that NLM should not be included in broiler chicken diets at starter phase. However 0.5% NLM inclusion level could be adopted during finisher phase for better growth performance without any adverse effect on the haematological parameters of the broiler chickens.

Keywords: Neem, growth performance, haematology, broiler chickens

PERFORMANCE DES POULETS DE CHAIR NOURRIS AVEC DE LA FARINE DE FEUILLES DE MARGOUSIER (AZADIRACHTA INDICA) UTILISEE COMME ALTERNATIVE AUX ANTIBIOTIQUES

Résumé

Cent quatre-vingt-douze poulets Marshal âgés d'un jour ont été utilisés dans un essai alimentaire de huit semaines qui avait pour objectif d'évaluer les effets de la farine de feuilles de margousier (Azadirachta indica) sur la croissance et les paramètres hématologiques des poulets de chair. Les oiseaux ont été répartis de manière aléatoire en quatre (4) groupes de quarante-huit (48) oiseaux chacun, dans un dispositif complètement aléatoire. Chaque groupe a été divisé en trois (3) répétitions de seize (16) oiseaux chacune. L'étude a inclus la farine de feuilles de margousier (NLM) aux niveaux de 0 ; 0,5 ; 1,0 ; et 1,5% en remplacement des abats de blé. La nourriture et l'eau ont été fournies ad libitum. L'on a constaté que les niveaux d'inclusion de l'A. indica ont réduit ($p <0,05$) la performance de croissance des oiseaux pendant la phase de démarrage, mais le taux de 0,5% de NLM a produit le poids final le plus élevé ($p <0,05$) à la phase de finition. Les meilleures valeurs du taux de conversion alimentaire (FCR) et de l'efficacité protéique ont été enregistrées dans le groupe témoin, alors que tous les autres groupes avaient des valeurs similaires à celles de la phase de démarrage. Durant la phase de finition, le gain pondéral le plus élevé ($p<0,05$) a été enregistré chez les oiseaux nourris avec la NLM à 0,5% alors que le groupe témoin et le groupe à NLM à 1,0% avait des valeurs similaires ($p> 0,05$). Le FCR et l'efficacité protéique étaient similaires. L'on n'a noté aucune mortalité pendant toute la phase de finition, et les valeurs de la phase de démarrage étaient semblables ($p> 0,05$) entre les groupes. Parmi tous les paramètres hématologiques mesurés, l'hématocrite,

*Corresponding author email: *egbeyalelt@funaab.edu.ng*

l'hémoglobine, les hétérophiles et les lymphocytes ont été significativement (p <0,05) influencés lors de la phase de démarrage tandis que l'hématocrite, la numération leucocytaire, les plaquettes et les hétérophiles ont été influencés à la phase de finition. L'on pourrait recommander d'éviter d'inclure la NLM dans les régimes des poulets de chair lors de la phase de démarrage. Cependant, un taux d'inclusion de NLM de 0,5% pourrait être adopté lors de la phase de finition pour une meilleure performance de croissance, et il n'aurait aucun effet négatif sur les paramètres hématologiques des poulets de chair.

Mots-clés : margousier, performance de croissance, hématologie, poulets de chair

Introduction

Many synthetic drugs and growth promoters are supplemented to broiler chickens to effect rapid growth, prevention, treatment and control of diseases. However, their use have shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties etc. For example resistance by Enterococcus faecium to the antimicrobials tetracycline and erythromycin were 20% and 76%, respectively and the resistance to growth promoters was 80-100% (Danmap, 2005). These products (antibiotics) are also considered as human health risk factors due to their residual effects on the animal products consumed by man. Study has shown that frequent use of antibiotic in controlling colibaillosis in poultry can result into prevalence of fluoroquinolone-resistant E. coli, consequently playing a role on the treatment of E. coli in human (Kolář *et al.,* 2005).

The restrictions to the use of antibiotic growth promoters have stimulated the search for alternative additives (Nasir and Grashorn, 2006). Hence, an intensive search for alternatives such as probiotics, prebiotics, symbiotics, enzymes, toxin binders, organic acids, organic minerals, oligosaccharides and other feed additives has started in the last decade (Fulton *et al.,* 2002). Herbs and plant extracts represent common responses to these demands. At present, scientists are working to improve feed efficiency and growth rate of livestock using useful herbs (Bunyapraphatsara, 2007).

One of such plants is neem. Neem (Azadirachta indica) is an indigenous plant of Asian subcontinent known for its useful medicinal properties since ancient times. Neem has attracted world-wide prominence due to its vast range of medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepato-protective and various other properties without showing any adverse effects (Kale *et al.,* 2003). This study is therefore targeted at observing the effect of neem (Azadirachta indica) as an alternative to antibiotics on the growth and haematological indices of broiler chickens.

Materials and Methods

Experimental Site: The experiment was carried out at the Poultry Unit of Teaching and Research Farm Directorate, (TREFAD), Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.

Sources and Processing of Experimental Diet: Neem leaves (Azadirachta indica) were collected from neem trees within the Federal University of Agriculture, Abeokuta campus. The leaves were air dried for twelve days and milled into neem leaf meal. The Neem leaf meal was used to replace wheat offal in broiler chicken diet with varying inclusion levels 0%, 0.5%, 1.0% and 1.5%. The ingredient composition of the experimental diet is shown in Table 1

Experimental Birds and Management: A total of 192 day-old Marshal broiler chicks were sourced from a reputable hatchery in Abeokuta, Ogun State, Nigeria. They were randomly assigned to four treatments with 48 birds per treatment in a Completely Randomized Design. Each treatment was further replicated thrice with sixteen birds per replicate. The chicks were reared on a deep litter system under standard management practices throughout the experimental period. Broiler starter diet was given to the chicks up to four weeks and

Table 1: Composition (%) of experimental diets

Ingredients	Inclusion Level of NLM (%)							
	Starter diet				Finisher diet			
	0	0.5	1.0	1.5	0	0.5	1.0	1.5
Maize	47.00	47.00	47.00	47.00	53.50	53.50	53.50	53.50
Soya bean meal	18.50	18.50	18.50	18.50	16.50	16.50	16.50	16.50
Fish meal	2.00	2.00	2.00	2.00	0.40	0.40	0.40	0.40
Groundnut cake	17.50	17.50	17.50	17.50	13.80	13.80	13.80	13.80
Wheat offal	10.00	9.50	9.0	8.5	10.80	10.30	9.80	9.30
Azadirachta indica	0.00	0.5	1.0	1.5	0.00	0.50	1.00	1.50
Bone meal	3.00	3.00	3.0	3.00	3.00	3.00	3.00	3.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	`1.00	1.00	1.00
Vit/min. Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	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
Total	100	100	100	100	100	100	100	100
Calculated analyses								
Crude protein (%)	23.02	23.03	23.03	23.04	20.17	20.25	20.25	20.19
ME (MJ/KG)	2930.28	2903.82	2895.0	2886.18	2834.22	2816.58	2370.16	2591.02

finisher diet was fed ad libitum from fifth to eighth week. Antibiotics were not administered to the birds on the diets containing A. indica throughout the experimental period.

Data Collection: The chicks were weighed at the commencement of the experiment and subsequent weeks to determine the weight gain which was calculated by subtracting initial weight from final weight. The feed intake was determined by deducting the left over feed from feed supplied. Other growth parameters calculated were feed efficiency, protein intake and protein efficiency ratio.

Haematological Analyses: At the fourth and eighth week of the study, 2.5ml of blood was collected from three birds randomly selected from each replicate via the wing vein into specimen bottles containing Ethylene Diamine Tetra Acetic (EDTA) to prevent coagulation. The blood samples were labeled according to the replicates and taken to laboratory for analysis. The following haematological parameters were taken on the collected blood samples; haemoglobin concentration (Hb), red blood cells (RBC), white blood cells (WBC), Neutrophils (Neut.), basophils (Baso), lymphocytes (Lymph), eosinophils (Eos), haematocrit (PCV), Mean corpuscular volume (MCV), complete blood count (CBC) and mean corpuscular hemoglobin concentration (MCHC).

Statistical Analysis: All data collected were subjected to a one-way analysis of variance (ANOVA) in a Completely Randomized Design. The probability level of 95% was used (SAS, 1999), while significant ($p<0.05$) means were separated using Duncan's Multiple Range Test in the software package.

Results

The proximate analysis of neem leaf meal (NLM) showed that the test ingredient contained moisture content of 12.80%, 16.59% crude protein, 21.97% crude fibre, 13.99% ash and 32.30% nitrogen free extract.

The growth performance of broilers fed neem leaf meal at the starter phase is shown in Table 2. Final weights were significantly ($p<0.05$) reduced across the treatments as the inclusion level of NLM increased in the experimental diets. Birds on control diet had higher weight compared to their counterparts fed neem leaf meal, however, there was no significant ($p>0.05$) difference between the treatment groups fed neem leaf meal. The values of feed intakes among the treatments were not influenced ($p>0.05$) by the NLM levels. There was significant ($p<0.05$) difference in the feed conversion ratio with birds on control diet having the best conversion ratio. The neem leaf meal inclusion does not affect the protein intake of the birds. However protein efficiency ratio was influenced ($p<0.05$) by the inclusion levels of neem leaf meal. Birds in the control group recorded the best value of 1.96 while the birds subjected to varying levels of neem leaf meal showed similar results.

Table 3 showed the growth performance of broiler chickens fed varying levels of neem leaf meal at finisher phase. The final weight of birds fed 0.5%NLM had significant ($p<0.05$) highest value among the treatment groups. The value decreased as the inclusion level increased. Total and daily weight gain were significantly ($p<0.05$) reduced as the level of NLM increased. Birds fed with 0.5% NLM had the highest value, birds in the control group and their counterpart fed 1.0% NLM were similar while birds fed 1.5% NLM recorded the least value (972.74 and 34.74g, respectively). The total feed intake, feed intake per day, feed conversion ratio, protein efficiency ratio, protein intake were not affected ($p>0.05$) by the inclusion levels of neem leaf meal.

The results for the overall growth performance are shown in Table 4. The final weights were significantly ($p<0.05$) influenced by the inclusion of neem leaf meal in the diet. Weight gain and weight gain per day were also influenced ($p<0.05$) by the neem leaf meal inclusion in the diet. The values of total and daily weight gain ranged from 1523.69 to 1733.58g and 26.27 to 29.89g/day, respectively. Birds fed 0.5% NLM had the highest ($p<0.05$) weight gain while the least value was recorded from the birds fed 1.5% NLM. Feed intake, feed intake per day, feed conversion ratio, protein intake and protein efficiency ratio were not influenced ($p>0.05$) by the neem leaf meal inclusion.

The haematological indices of the starter phase of broiler chickens fed graded levels of Neem leaf meal are presented in Table 5. There were significant ($p<0.05$) differences in the PCV, Hb and differential leucocyte count HET and LYM. The PCV value ranged from 26.30% which was recorded in birds fed 1.0% NLM to 34.70% recorded in birds fed 1.5% NLM. Control group recorded lowest

Table 2: Effects of neem leaf meal on growth performance of broiler chickens at starter phase

	Inclusion level (%) of NLM			
Parameters	**0**	**0.5**	**1.0**	**1.5**
Initial Weight (g)	39.74±0.265	39.75±0.267	39.72±0.035	39.64±0.064
Final Weight (g)	638.56±7.426^{a}	600.50±16.428^{b}	591.39±7.668^{b}	590.74±15.793^{b}
Weight Gain (g)	598.82±7.220^{a}	560.75±16.176^{b}	551.67±7.644^{b}	551.10±15.777^{b}
Weight Gain/day (g)	19.96±0.242^{a}	18.69±0.541^{b}	18.39±0.255^{b}	18.37±0.527^{b}
Total Feed Intake (g)	1330.51±36.946	1329.71±42.751	1351.04±20.188	1322.23±42.037
Feed Intake(g/d/bird)	44.35±1.232	44.32±1.425	45.03±0.673	44.07±1.401
Feed Conversion Ratio	2.22±0.043^{b}	2.37±0.048^{a}	2.45±0.030^{a}	2.40±0.090^{a}
Protein Intake	10.21±0.284	10.21±0.328	10.37±0.155	10.15±0.323
Protein Efficiency Ratio	1.96±0.038^{a}	1.83±0.037^{b}	1.77±0.021^{b}	1.81±0.067^{b}
Mortality	1.00±0.00	0.67±0.00	1.00±0.00	0.67±0.00

^{ab}; Means in the same row with different superscripts in a row differ significantly ($p<0.05$)

Table 3: Effects of Neem leaf meal on growth performance of broiler chickens at finisher phase.

	Inclusion level (%) of NLM			
Parameters	**0**	**0.5**	**1.0**	**1.5**
Initial Weight (g)	638.56±7.426^{a}	600.50±16.428^{b}	591.39±7.668^{b}	590.74±15.793^{b}
Final Weight (g)	1676.67±25.166^{b}	1773.33±15.275^{a}	1626.67±15.275^{c}	1563.33±15.275^{d}
Weight Gain (g)	1038.11±19.015^{b}	1172.83±19.345^{a}	1035.47±17.167^{b}	972.74±30.516^{c}
Weight Gain/day (g)	37.075±0.679^{b}	41.887±0.691^{a}	36.98±0.613^{b}	34.74±1.090^{c}
Total Feed Intake (g)	2924.51±118.253	2786.97±161.396	2493.16±483.762	2726.30±13.332
Feed Intake (g/d/bird)	104.45±4.223	99.53±5.764	89.04±17.277	97.37±0.476
Feed Conversion Ratio	2.81±0.064	2.38±0.168	2.41±0.499	2.80±0.075
Protein Intake	21.07±0.852	20.16±1.167	18.03±3.498	19.66±0.096
Protein Efficiency Ratio	1.76±0.040	2.08±0.153	2.11±0.492	1.77±0.047
Mortality (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

^{abcd}; Means in the same row with different superscripts in a row differ significantly ($p<0.05$)

Table 4: Effects of Neem leaf meal on overall growth performance of broilers (0-8 weeks)

	Inclusion level (%) of NLM			
Parameters	**0**	**0.5**	**1.0**	**1.5**
Initial Weight (g)	39.74±0.265	39.75±0.267	39.72±0.035	39.64±0.064
Final Weight (g)	1676.67±25.166^{b}	1773.33±15.275^{a}	1626.67±15.275^{c}	1563.33±15.275^{d}
Weight Gain (g)	1636.93±25.065^{b}	1733.58±15.127^{a}	1586.95±15.301^{c}	1523.69±15.263^{d}
Weight Gain/day (g)	28.22±0.432^{b}	29.89±0.261^{a}	27.36±0.264^{c}	26.27±0.263^{d}
Total Feed Intake (g)	4254.99±154.862	4116.68±199.129	3844.20±485.790	4048.53±39.419
Feed Intake (g/d/bird)	73.36±2.670	70.98±3.433	66.28±8.376	69.80±0.680
Feed Conversion Ratio	2.60±0.055	2.37±0.117	2.42±0.319	2.66±0.027
Protein Intake	31.69±1.153	30.72±1.486	28.69±3.625	30.18±0.294
Protein Efficiency Ratio	0.89±0.019	0.97±0.049	0.96±0.137	0.87±0.009
Mortality (%)	1.00±1.00	0.67±0.577	1.00±0.00	0.67±0.577

^{abcd}; Means in the same row with different superscripts in a row differ significantly ($p<0.05$)

value (7.59g/d) of Hb while the highest value (10.09g/d) was recorded in 0.5% NLM.

At the finisher phase, there were significant ($p<0.05$) differences in the PCV and WBC values of the birds. The highest value of PCV was recorded from the birds fed the control diet while the least value was from those fed 1.0%NLM. The white blood cell (WBC) was significantly ($p<0.05$) reduced as the level of NLM increased. The birds fed 0.5%NLM had highest value of WBC whereas their counterparts in the other groups had similar values. The values of Hb and RBC in all the groups were not significantly ($p>0.05$) different. It was only the heterophils that was influenced ($p<0.05$) among all the leucocytes differential counts. The values increased ($p<0.05$) across the groups and ranged from 23.33 to 45.00%.

Discussion

The crude protein value of neem leaf meal obtained in this study was lower to the crude protein value of 24.06% reported by Onyimonyi et al. (2009) and 21.87% by Odoh and Bratte (2015) but similar with the value reported by Bonsu et al. (2012). The crude

Table 5: Effects of graded levels of neem leaf meals on the haematological parameters of broiler chickens at starter phase

	Inclusion level (%) of NLM			
Parameters	**0**	**0.5**	**1.0**	**1.5**
4th Week				
Pack Cell Volume (%)	33.13±2.82^{ab}	33.56±2.76^{ab}	26.30±1.47^{b}	34.70±0.75^{a}
Haemoglobin (g/dl)	7.59±0.42^{b}	10.09±0.08^{a}	8.69±0.61^{a}	9.02±0.24^{a}
Red Blood Cell (x 109/L)	2.16±0.23	2.37±0.12	2.18±0.12	2.29±0.15
White Blood Cell (x 109/mm3)	13.84±0.13	14.55±0.54	13.01±0.70	14.33±0.34
Platelets (%)	24.66±6.66	25.33±3.48	36.66±6.93	24.66±3.71
Heterophil (%)	25.89±1.04^{b}	28.81±1.95^{ab}	37.69±1.69^{a}	22.19±5.56^{b}
Lymphocyte (%)	72.41±1.68^{a}	69.39±2.13^{ab}	61.64±1.62^{b}	76.64±5.29^{a}
Eosinophils (%)	0.66±0.17	0.60±0.11	0.43±0.18	0.70±0.36
Basophils (%)	1.03±0.48	1.20±0.52	0.23±0.06	0.46±0.14
Monocytes (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8th Week				
Pack Cell Volume (%)	38.66±1.45^{a}	33.33±0.88^{b}	28.00±1.52^{c}	36.33±0.88^{ab}
Haemoglobin (g/dl)	12.33±1.32	11.33±0.14	11.00±1.76	12.7±0.27
Red Blood Cell (x 109/L)	2.45±0.31	2.26±0.35	2.35±0.30	2.61±0.02
White Blood Cell (x 109/mm3)	15.73±0.95^{a}	10.06±0.37^{b}	10.13±0.23^{bc}	8.63±0.32^{c}
Platelets (%)	25.33±3.48^{b}	39.00±1.15^{a}	25.00±5.56^{b}	16.66±1.45^{b}
Heterophil (%)	23.33±1.20^{b}	26.33±1.76^{b}	27.33±0.66^{b}	45.00±1.73^{a}
Lymphocyte (%)	74.33±2.02	72.33±2.40	70.33±1.45	52.66±1.66
Eosinophils (%)	0.33±0.33	1.33±0.66	2.00±0.57	0.33±0.33
Basophils (%)	2.00±0.57	1.33±0.66	2.00±0.57	2.00±0.57
Monocytes (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

^{abcd}; Means in the same row with different superscripts in a row differ significantly ($p<0.05$)

fibre value was higher than the findings of Onyimonyi et al. (2009) and Bonsu et al. (2012) who reported 12.00 and 14.6%, respectively but lower than 33.77% reported by Sokunbi et al. (2003). The nitrogen free extract value was similar to that of Odoh and Bratte (2015). These differences in the chemical composition of NLM may be related to the ages of the leaf. As leaf ages, there are variations in the nutrient composition of leaf (Onyimonyi *et al.,* 2009).

The feed intake obtained in this study was similar with the report of Bonsu et al. (2012) who also reported non-significant values in the feed intake at both starter and finisher phases. However, this negates the findings of Onyimonyi et al. (2009) who reported significant decrease in the feed intake as the level of neem leaf meal increased. Wankar et al. (2009) also reported that supplementation of neem leaf meal caused non- significant increase in mean feed consumption in all the treatment groups at 0%, 1%, 2% and 3% inclusion level.

At the starter phase, the slow growth rate of broiler chicks as the level of neem leaf meal inclusion increased above 0.5% despite the fact that their feed intake was similar with their counterparts in other groups is in agreement with observations of Bonsu et al. (2012) who also experienced decrease in the live weight of broiler chicks as the level of inclusion increased. This could be attributed to the poor feed utilization as seen in the values

of feed conversion ratio of birds fed diets containing neem leaf meal. The best protein efficiency ratio observed in the control diet when compared to the other treatments shows that the protein in neem diets was not adequately utilized by the birds (Esonu *et al.,* 2006). The best growth performance in term of live weight at finisher phase recorded at 0.5%NLM corroborates research findings by Onyimonyi et al. (2009) and Kharde and Soujanya (2014) who supplemented neem leaf meal and neem leaf powder, respectively in the diets of broiler chickens and reported that the inclusion of neem leaf influenced the weight gain. The decreased in live weights of birds on 1.0% and 1.5% NLM could be attributed to high crude fibre and the probable effects of bioactive compounds in the leaf meal (Obun *et al.,* 2013). The reduction in live body weight of birds with increasing NLM in the diets is in agreement with findings of Esonu et al. (2006) and Obikaonu et al. (2012). The mortalities recorded were during the brooding period which was very low. Lower mortality in treated groups could be due to medicinal value of neem leaves in preventing losses and eliminating stress (Tipu *et al.,* 2002).

The values of Hb recorded by all groups were however within the normal range (7.0g-18.6g/dl) reported by Mitruka and Rawnsley (1977) in chicken. The results showed no traces of anaemia. Anaemia is defined as a reduction in the haemoglobin concentration of blood (Moss, 1999). The significant variations in the values of PCV and Hb falls in line with the report of Obikaonu et al. (2012) where broiler starter were fed diets containing neem leaf meal at 2.5, 5.0, 7.5 and 10% inclusion levels. However, the values in this present study were higher than the values reported by the researchers. The variations could be attributed to the low levels of neem leaf meal inclusion in this study when compared with the levels used by Obikaonu et al. (2012). Differential leucocytes counts were within the normal ranges of 76.64-61.64, 37.69-22.19 and 0-5 x 103/L respectively for clinically healthy birds (Aiello and Mays, 1998).

Conclusion and Recommendation

Neem leaf meal causes decreased growth rate at the starter phase with birds in the control group having the best growth performance while at the finisher phase birds fed 0.5% NLM had the best growth rate. It is therefore recommended that NLM inclusion in the broiler diet should not exceed 0.5% for better growth performance.

Conflicts of interest: The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

Aiello SE, Mays A, 1998. The Merck Veterinary Manual, 8th Edition, Merck and Company Inc., White House Station, UJ, USA. pp18 – 1477.

Bonsu FRK, Kagya-Agyemang JK, Kwenin WKJ, Zanu HK, 2012. Effects of direct fed microbial and antibiotics supplementation on the health status and growth performance of broiler chickens under hot humid environmental conditions. World appl Sci J. 19(6): 800 - 805.

Bunyapraphatsara N, 2007 Utilization of medicinal plants in animal production. 11th International Congress, Leiden, The Netherlands, Phytopharmcology.

DANMAP 2005. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. 2005 ISSN 1600-2032. (http://www.danmap.org).

Esonu BO, Opara MN, Okoli IC, Obikaonu HO, Udedibie C, Iheshiulor OOM, 2006. Physiological Response of Laying Birds to Neem (Azadirachta indica) Leaf Meal- Based Diets: Body Weight, Organ Characteristics and Haematology. Online Journal of Health and Allied Sci, 2:4.

Fulton RM, Nersessian BN, Reed WM, 2002. Prevention of Salmonella enteritidis infection in commercial ducklings by oral chicken egg-derived antibody alone or in combination with probiotics. Poult Sci 81: 34 - 40.

Iheukwumere FC, Ndubuisi EC, Mazi EA, Onyekwere MU, 2008 Performance, Nutrient utilization and organ characteristics of broilers and cassava leaf meal (Manihot esculenta Crantz). Pak. J. Nutri. 7: 13-16.

Kale BP, Kothekar MA, Tayade HP, Jaju JB, Mateeddin M, 2003. Effects of neem (A. indica) supplementation on performance and carcass characteristics of broilers. Indian J. Pharmacool 35: 177.

Kharde KR, Soujanya S, 2014. Effect of garlic and neem leaf powder supplementation on growth performance and carcass traits in broilers.Veterinary World 7: 799-802.

Kolář M, Bardoň J, Sauer P, Kesselova M, Čekanová L, Vagnerova I, Koukalova D, Hejnar P, 2005. Fluoroquinolone-resistant Escherichia coli and Proteus mirabilis in poultry of middle Moravia. Czech Republic Acta Vet Brno 74: 249-253

Mitruka BM, Rawnsley HM, 1977. Clinical Biochemical and Haematological Reference Value in Normal Experimental Animals. Masson Publication, New York, USA. pp. 21-64.

Moss PP, 1999. Blood banking: Concepts andApplications. W.B. Saunders Co. Philadelphia, 27: 12-34

Nasir Z, Grashorn MA, 2006. Use of Black cumin (Nigella sativa) as alternative to antibiotics in poultry diets. In: Proceedings of the 9th Tagung schweine- und geflügelernährung, Halle, Germany. pp. 210-213.

Obikaonu HO, Okoli IC, Opara MN, Okoro VMO, Ogbuewu IP, Etuk EB, Udedibie ABI, 2012 Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (Azadirachta indica). J of Agric Tech 8(1): 71-79.

Obun CO, Ukim CI, Olatunji EA, Kehinde AS, 2013. Health and carcass implication of dietary inclusion of graded level of sun-cured neem (Azadirachta indica, A. Juss) leaf meal for broilers. Greener J of Agric Sci 3: 048 - 054

Odoh LI, Bratte L, 2015. Effects of Varying Levels of Neem (Azadirachta indica) Leaf Meal in Layer Diets on the haematological and Serological Indices, and Faecal Bacterial Counts of Layers . J. Natural Sci Res; 5(4): 37-44.

Onyimonyi AE, Adeyemi O, Okeke GC, 2009. Performance and Economic Characteristics of Broilers Fed Varying Dietary Levels of Neem Leaf Meal (Azadirachtaindica). International Journal Poultry Science 8 (3): 256-259.

SAS Institute, 1999. SAS User's Guide: Statistics. Inc., Cary, N.C. pp. 923.

Sokunbi OA, Egbunike GN, Salako AO, Bobadoye AO, 2003. The performance of pre-pubertal female swine fed diet containing sun-cured neem (Azadirachta indica) leaf meal. Proceedings of the 8 Annual conference of the Animal Science Association of Nigeria (ASAN), 28-31.

Tipu MA, Pasha TN, Alli Z, 2002. Comparative effect of Salinomycine sodium and Neem fruit (A. indica) as feed additive anticoccidials in broilers. Int. J. Poult. Sci. 1(4): 91-93.

Wankar AK, Shirbhate RN, Bahiram KB, Dhenge SA, Jasutkar RA, 2009. Effect of Neem (Azadirachta indica) leaf powder supplementation on growth in broilers: Vet. World 2 (10): 396-397.

METHODS OF ISOLATION AND IDENTIFICATION OF MYCOPLASMA SPECIES OF RUMINANTS IN AFRICA - A REVIEW

Francis M I[1]*, Ejeh E F[1], Raji M A[1] and Egwu G O[2]
[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, Nigeria

Abstract

Mycoplasmas are responsible for causing many important diseases of ruminants in Africa. Of particular importance are Mycoplasma mycoides subspecies mycoides and Mycoplasma capricolum subspecies capripneumoniae, the causative agents of contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) respectively. These are the two most important diseases of ruminants causing significant losses in livestock production and have been reported to be widely distributed in Africa. Diagnosis of CBPP and CCPP can be performed by taking a good quality samples either in live animals or at necropsy. Culturing of these agents is difficult and time consuming; therefore, the method of choice is molecular identification by polymerase chain reaction, which has been recommended by various studies for disease surveillance, control and eradication.

Key words: Contagious Bovine Pleuropneumonia , Contagious Caprine Pleuropneumonia , Mycoplasma, Africa

METHODES D'ISOLEMENT ET IDENTIFICATION DES ESPECES DE MYCOPLASME DES RUMINANTS EN AFRIQUE

Résumé

Les mycoplasmes sont a l'origine de nombreuses maladies importantes des ruminants en Afrique. Les mycoplasma mycoides sous-espèce mycoides et les mycoplasma capricolum sous-espèce capripneumoniae sont d'une importance particulière, respectivement comme agents responsables de la péripneumonie contagieuse bovine (PPCB) et de la péripneumonie contagieuse caprine (PPCC). La PPCB et la PPCC sont les deux maladies les plus importantes des ruminants, à l'origine de pertes importantes en production animale, et elles ont été déclarées comme étant largement répandues en Afrique. Le diagnostic de la PPCB et de la PPCC peut être effectué en prélevant des échantillons de bonne qualité, soit sur des animaux vivants, soit à l'autopsie. La culture de ces agents est difficile et prend beaucoup de temps ; par conséquent, la méthode de choix est l'identification moléculaire par réaction en chaîne de la polymérase recommandée par diverses études pour la surveillance, le contrôle et l'éradication des maladies.

Mots-clés : pleuropneumonie contagieuse bovine, pleuropneumonie contagieuse bovine caprine, mycoplasme, Afrique

*Corresponding author email: markfrancis4u@gmail.com

Introduction

The name "mycoplasma" is conventionally used to designate Mollicutes, a class of smallest prokaryotes that lack a cell wall and have often been portrayed as "minimal self-replicating organisms" because of their small genome size and the paucity of their metabolic pathways (Chazel *et al.*, 2010; Amin *et al.,* 2013). The class Mollicutes (mollis; soft, cutis; skin, in Latin) are separated from all other bacteria by the total lack of a rigid cell wall envelope, reduced cellular (0.1 - 0.3 µm diameter) and genome (0.58 - 2.20 Mbp) sizes, low G + C content (24 - 33 mol %) and sterol incorporation into their own plasma membrane (Razin *et al.,* 1998; Chazel *et al.,* 2010). The class Mollicutes consists of nine genera (Mycoplasma, Ureaplasma, Entomoplasma, Mesoplasma, Spiroplasma, Acholeplasma, Anaeroplasma, Asteroleplasma and Phytoplasma) and over 200 species and subspecies have been identified to date, amongst which are severe pathogens of humans, animals and plants (Rottem, 2003), 119 of which are under genus Mycoplasma (Bizarro and Schuck, 2007). The trivial terms mycoplasmas and mollicutes have been used interchangeably to designate any species within the Mollicutes class of bacteria (Walker, 1999).

Many mycoplasmas have been reported to cause disease in animals as shown in Table 1. In ruminants, Mycoplasmas are responsible for causing many important diseases and some are listed by the World organisation for Animal Health (Chazel *et al.,* 2010). Of particular importance are Mycoplasma mycoides subsp. mycoides (Mmm) and Mycoplasma capricolum subsp. capripneumoniae (Mccp), two members of the 'Mycoplasma mycoides cluster' (Cottew *et al.,* 1987; Fischer *et al.,* 2012), which are responsible for contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP), respectively. Both diseases cause significant losses in livestock production particularly in Africa and Asia, and are a threat to disease-free countries (Cottew *et al.,* 1987).

The causative agent of CBPP was first cultivated and characterized by Nocard and Roux in 1898. It was introduced into Africa from Europe during the colonial period in the 18th and 19th centuries via livestock movement (Fisher, 2006). Today, CBPP is endemic in sub-Saharan Africa (Fischer *et al.,* 2012).

Contagious caprine pleuropneumonia (CCPP) was first described in Algeria in 1873 by Thomas and known under the local name of "bou frida" because, in the majority of diseased goats, only one lung was affected (McMartin *et al.,* 1980). Its highly contagious nature was acknowledged after an outbreak in South Africa in 1881. It is a significant disease of goats in Africa causing mortalities of up to 80%.

Besides the causative agents of CBPP and CCPP, the Mycoplasma mycoides clusters encompass additional pathogens including Mycoplasma leachi, Mycoplasma mycoides subsp. capri and Mycoplasma capricolum subsp. capricolum (Manso-Silvan *et al.,* 2009). This write up focuses mainly on the isolation and identification of different Mycoplasma species in Africa more especially Mmm and Mcc which are on the list of diseases notifiable to the World Organisation of Animal Health (OIE).

Overview of CBPP and CCPP

The causative agent of contagious bovine pleuropneumonia (CBPP) is Mycoplasma mycoides subspecies mycoides; was described by Nocard and Roux in 1898 (Nocard and Roux, 1898).

CBPP is a respiratory illness characterized by the presence of sero-fibrinous, interstitial pneumonia, interlobular oedema and hepatization giving a marbled appearance of the lung and capsulated lesions termed sequestra in the lungs of affected cattle. The occurrence of subacute, symptomless infections and chronic carriers after the clinical phase of the disease is generally believed to create major problems in the control of this disease. CBPP is present in the Middle East, Asia, and is now considered the most significant disease of cattle in Africa (AU-IBAR, 2013).

Table 1: Major pathogenic mollicutes of animals (Joachim, 2002).

Animal Hosts/Mollicutes species	Diseases
Bovines	
Mycoplasma mycoides subsp. mycoides SC	Contagious bovine pleuropneumonia (CBPP)
Mycoplasma sp. bovine group 7	Pneumonia and arthritis
Mycoplasma bovis	Mastitis, pneumonia(calf), polyarthritis(calves), metritis, abortion, sterility
Mycoplasma dispar	Pneumonia (calves)
Mycoplasma californicum	Mastitis
Mycoplasma canadense	Mastitis
Mycoplasma bovigenitalium	Mastitis and genital disease
Mycoplasma bovocculi	Conjunctivitis
Mycoplasma wenyonii	Anaemia
Sheep and goats	
M. capricolum subsp.capripneumoniae	Contagious caprine pleuropneumonia (CCPP)
M. capricolum subsp. Capricolum	Mastitis, arthritis
M. mycoides subsp. Capri	Pneumonia, mastitis, arthritis, septicaemia (goats)
M. mycoides subsp. mycoides LC	Pneumonia, mastitis, arthritis, septicaemia (goats)
M. agalactiae	Infectious agalactia
M. ovipneumoniae	Atypical pneumonia (lambs)
M. conjunctivae	Infectious conjunctivitis (sheep)
Wild caprine (ibex, chamois, gazelles)	
M. conjunctivae	Infectious conjunctivitis (ibex, chamois)

The Consultative Group on CBPP was reconvened for the first time in over 25 years in October 1998 by the OIE (1998) to discuss the deteriorating situation of the disease in Africa. Over the previous decade, an alarming spread of the disease was seen in Africa along two fronts: one in the east where it had invaded the whole of Tanzania, the north of Zambia threatening Malawi and Mozambique; and the other on the south-west where CBPP appeared after a long absence in Botswana in 1995 and subsequently in west Zambia. Simultaneously, the incidence of CBPP continues to increase in the endemic areas of West and Central Africa as well as in the Horn of Africa. The meeting concluded that CBPP "can now be considered as the most important threat to the cattle industry in Africa". The group met again in October 2000 to review the situation in Africa and the conclusion remained unchanged. Although deterioration could not be demonstrated it was noted that the inadequacy of veterinary services which led to a lack of epidemiological knowledge, and the failure to control coupled with the lack of regional coordination, and civil unrest contributed to the spread of CBPP in the African continent. Further meetings in 2003 and 2006 recommended research into improving vaccines and assessing the possible use of antibiotic treatments as an option to control CBPP. They also recommended carrying out serological prevalence studies and using mathematical modelling in CBPP research. The meeting in 2006 went as far as recommending the use of antibiotics in defined scenarios, but abattoir surveillance, culling, zoning and controlling cattle movement along with vaccination are the main holistic strategies for controlling CBPP (AU-IBAR, 2013).

Tambi et al. (2006) estimated the cost of CBPP in twelve sub-Saharan African countries to 3.7 million Euros per country and suggested an investment of 14.7 million Euros to control CBPP would prevent the loss of 30 million Euros.

The causative agent of contagious caprine pleuropneumonia (CCPP) is Mycoplasma capricolum subsp. capripneumoniae (Mccp), which was previously known by the strain name of its type species, F38. It is also a member of the Mycoplasma mycoides cluster. CCPP is a significant disease of goats in Africa, the Middle East and Western Asia, and is characterized primarily by its contagious nature. The disease causes interstitial, fibrinous pleuropneumonia, interlobular oedema and hepatization of the lung causing high mortality rates of up to 80% (AU-IBAR, 2013). There is no thickening of the interlobular tissue in the diseased lung of the goat, which forms such a striking feature in bovine pleuropneumonia; the section of the diseased lung in the goat has the appearance of a somewhat granular-looking liver (Hutcheon, 1889). These features also clearly differentiate disease caused by M. mycoides subsp. capri. The pathological lesions are localised exclusively in the lung and pleura and consist of a pleuropneumonia, unilateral hepatisation, adhesions, pleuritis and an accumulation of pleural fluid. The pleural exudate can solidify to form a gelatinous covering on the lung (Nicholas, 2002).

Distribution of CBPP and CCPP in Africa

There are a number of different theories to explain the spread of CBPP into eastern Africa. It may have been present in pre-colonial times and some workers cite Thomson's description of a cattle disease resembling CBPP in Maasai cattle in the1880s in eastern Africa (Thomson, 1885). Today, CBPP is endemic in much of sub-Saharan Africa and its incidence is increasing. An examination of the number of outbreaks reported by African countries in the late 1990s showed that large increases in incidence occurred in Burkina Faso and Ghana in West Africa, Ethiopia, Kenya and Tanzania in East Africa, and Namibia and Zambia as well as Botswana between 1995 and 1996 in Southern Africa. Outbreaks of CBPP in previously uninfected areas occurred in Kenya, Rwanda, Tanzania, Botswana, Zambia, Burundi and West Guinea. The reasons for the increase in CBPP incidence relate to the decrease in efficiency and financing of national veterinary services and specifically to reduced funding for vaccination, possibly linked to the success of the rinderpest campaign, changes in vaccines and vaccine usage, cost recovery for CBPP vaccination and reduced disease surveillance (Amanfu *et al.,* 1998; AU-IBAR, 2013).

In 2011, CBPP was reported to the AU-IBAR in 18 African countries; spreading across the west, central, east and southern Africa regions. During the reporting period, 304 epidemiological units were affected by CBPP across Africa involving 16,836 cases and 3007 deaths, with an estimated case fatality rate of 17.9% . The highest number of CBPP outbreaks was reported in Ghana (75), followed by Central African Republic (43) and Ethiopia (29).

Although a precise description of the distribution of CCPP is not available, the clinical disease has been reported in 30 countries mainly in Africa and Asia (Thiaucourt and Bölske, 1996). The African countries where M. capricolum subsp. capripneumoniae has been isolated are Chad (Lefèvre *et al.,* 1987), Eritrea, Ethiopia (Thiaucourt *et al.,* 1992), Kenya (MacOwan and Minette, 1976), Mauritius (Srivastava *et al.,* 2010), Niger, Sudan (Harbi and El-Tahir, 1981), Tanzania (Kusiluka *et al.,* 2000), Tunisia (Perreau *et al.,* 1984), and Uganda (Bölske *et al.,* 1995). In Mali, goats have been suspected of infection based on serological evidence (Rurangirwa *et al.,* 1990).

According to AU-IBAR (2011) seven countries reported the occurrence of CCPP in 2011, with Ethiopia, Somalia and Tanzania having reported the disease since 2008. The disease seems to be confined to the central and eastern Africa regions based on the reports received. It is, however, impossible to rule out the presence of CCPP in other parts of the continent due to possible under-reporting of cases, and lack of adequate laboratory support

to correctly diagnose the disease.

AU-IBAR (2011) reported that the disease affected 280 epidemiological units on the continent causing 5833 cases and 1342 deaths, with a case fatality rate of 23%. The highest fatalities were recorded in Somalia with 567 deaths of goats followed by Ethiopia and Chad with 486 and 213 deaths, respectively.

Diagnosis of CBPP And CCPP

Definite diagnosis is made by culture of the causative agent from lung samples or pleuritic fluid taken at postmortem. Pleural fluid was described as the sample of choice (Nicholas and Baker, 1998), but recent reports from an experimental infection indicate detection as low as 25% in pleural fluid (Schnee *et al.,* 2011). Liquid and solid mycoplasma media are inoculated and filtered subcultures from liquid media may be required if there is evidence of bacterial contamination. Isolates may be identified by biochemical, immunological and molecular tests. Serological tests for the detection of specific antibodies have relied on the complement fixation test and more recently the competition ELISA which are tests prescribed by the Office International des Epizooties (OIE) for international trade. Both these tests are only reliable for herd diagnosis. The immunoblotting test has been developed and is useful as an additional specific serological test to support other serological diagnostic methods. A penside latex agglutination test has also been developed and is commercially available (Churchward *et al.,* 2007).

The preferred method for the control of CBPP is the restriction of animal movement and stamping out by slaughter of entire infected herds. There is only one vaccine, T144, recommended by the OIE and FAO. Antibiotic therapy is discouraged on the basis that it may promote the formation of carriers. In vitro minimum inhibition concentration tests indicate tilmicosin, oxytetracycline and the fluroquinolones are active against M. m. mycoides SC (Ayling *et al.,* 2007). In vivo studies have demonstrated that danofloxacin, a fluoroquinolone was effective at treating a CBPP outbreak in Namibia and no further cases occurred (Nicholas *et al.,* 2007). Treatment of infected animals with oxytetracycline was shown to have a positive impact on the course of the disease and prevent the spread of disease to in-contact animals, however Mycoplasma mycoides mycoides could still be recovered from the treated animals (Niang *et al.,* 2007).

Definite diagnosis is made by culture of the causative agent from lung samples or pleuritic fluid taken at postmortem. Liquid and solid mycoplasma media are inoculated and filtered subcultures from liquid medium may be required if there is evidence of bacterial contamination. Isolates may be identified by biochemical, immunological and molecular tests. Serological tests for the detection of specific antibodies have relied on the complement fixation test which is the test prescribed by the Office International des Epizooties (OIE) for international trade. A latex agglutination test is also available, and is used routinely in some parts of Africa. A specific competitive enzyme-linked immunosorbent assay has been developed (Thiauourt *et al.,* 1996).

Growth and Nutritional Requirements of Mycoplasmas

The difficulty of culturing mycoplasmas in vitro is a major setback to research and laboratory diagnosis of these fastidious organism, and it is highly likely that many more mycoplasmas exist in nature but have not yet been isolated, despite great effort over many years (Razin *et al.,* 1998) including the introduction of PCR; in addition, many isolated mycoplasmas still grow very poorly even on the best mycoplasma media (Razin, 1994).

The inability of mycoplasmas to synthesized necessary nutrients for their growth reflects their evolutionary development which resulted in their small size. Therefore, complex media are used for their invitro cultivation in order to overcome these deficiencies. The medium normally used for mycoplasmas is based on beef heart infusion, peptone, yeast extract and serum with various supplements (Razin, 1991). The medium must contain

sterol such as cholesterol since mycoplasmas completely depends on their host for fatty acids, and require amino acids, nucleic acid precursor, lipid precursor molecules and vitamins. Glucose is the main source of energy in fermentative mycoplasma, pyruvate can replace glucose in non-fermentative mycoplasma such as M. bovis and M. agalactiae (Miles *et al.,* 1988).

Peptones provide the media with different polypeptides, di-peptides and amino acids (Miles *et al.,* 1992).

Different types of animal sera (calf, horse, porcine) are used at 5-20% as a source of essential lipids. Animal sera are usually inactivated by heating in a water bath at 56°C for 30 minutes to decrease the complement component of the serum which can cause cell lysis.

Beef heart infusion and yeast extract provide a variety of nutrients including nucleotides, vitamins and mineral salts. The addition of organic components including DNA and NADH may enhance the growth of different types of mycoplasmas (Miles, 1992). Energy source are provided through the inclusion of glucose, pyruvate or urea.

Mycoplasmas lack a cell wall and therefore they are more susceptible to cell lysis in hypo-osmotic media than any other cell-walled bacteria, so they need sodium chloride to increase medium tonicity. They also require osmotic pressure of 7-14 atmosphere for optimal growth. For most mycoplasmas, growth is best at pH 7-8 and a typical media have a pH of 7.6 (Miles, 1992).

Oxygen plays an important role in mycoplasma growth. The need for oxygen depends on the strain; some mycoplasma prefers to grow in anaerobic conditions while others prefer microaerophilic conditions (Miles and Agbanyim, 1998).

Although DNA amplification techniques are being used frequently for the detection and identification of mycoplasmas, the isolation of a mycoplasma by conventional techniques is still required by most national and international authorities, particularly where diseases of importance such as contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) are concerned. The used of high yielding and selective media is essential since live mycoplasmas are required for vaccine production, molecular typing, antibiotic sensitivity tests and for use as antigens in diagnostic procedures (Nicholas *et al.,* 2008).

Samples

A key to isolation success lies in collecting good quality samples. Mmm can be isolated from samples taken either in live animals or at necropsy. Samples to be collected for mycoplasma isolation are shown on Table 2. Samples taken from live animals are: nasal swabs or nasal discharges, broncho-alveolar lavage or transtracheal washing and pleural fluid collected aseptically by puncture made in the lower part of the thoracic cavity between the seventh and eighth ribs. Samples taken at necropsy are: lungs with lesions, pleural fluid, lymph nodes of the broncho-pulmonary tract, and synovial fluid from those animals with arthritis. The lung samples should be collected from lesions at the interface between diseased and normal tissue (Nicholas *et al.,* 2008).

When collecting nasal swab samples, a transport medium should be used to protect the mycoplasmas and prevent proliferation of cell-walled bacteria (heart-infusion broth without peptone and glucose, 10% yeast extract, 20% serum, 0.3% agar, 500 IU/ml penicillin, thallium acetate 0.2 g/litre).

After collection all samples must be kept refrigerated at 4°C and sent to the laboratory within 24 hours. For longer periods they should be frozen at or below -20°C.

Mycoplasma Culture

Mycoplasma in general needs selective media to grow but some are considered to be fastidious in growth more especially Mccp, M. dispar and M. conjunctivae (Nicholas *et al.,* 2008). There are several media compositions used in different reference laboratories but, essentially, they should contain: a basic medium such as heart-infusion broth or PPLO broth

Table 2: Samples for mycoplasmas isolation in ruminants (Nicholas et al., 2008)

Species	Growth in vitro	Host	Samples from live animals	Samples from dead animals
M.m.mycoides	Good	Cattle	Nasal swabs, nasal discharges, bronchoalveolar washings, pleural fluid.	Lung lesions, pleural fluid, bronchopulmonry lymph nodes.
M.c. capripneumoniae	Fastidious	Goats	Nasal and ear swabs	Lung lesions, pleural fluid, mediastinal lymph nodes.
M. agalatiae	Good	Sheep/ goats	Milk, joint fluids, ocular swabs, nasal and ear swabs.	Udder and associated lymph nodes, joint fluid, lung lesions, brain.
M. bovis	Good	Cattle	Nasal swabs, joint fluids, ocular swabs, milk, bronchoalveolar washings, pleural fluid, semen.	Lung lesions, joints fluid, udder, brain.
M.m capri	Good	Goats	Milk, joint fluid, ocular swabs, nasal swabs.	Udder and associated lymph nodes, joint fluid, lung lesions.
M.c. capricolum	Good	Sheep/ goats	Milk, joint fluid, ocular swabs, nasal swabs.	Udder and associated lymph nodes, joint fluid, lung lesions.
M. dispar	Fastidious	Cattle	Nasal swabs, bronchoalveolar washings, pleural fluid.	Lung lesions
M. bovigenitalium	Good	Cattle/ sheep	Vulvo-vaginal swabs, discharge from reproductive systems, milk, semen.	Endometrium and other reproductive sites.
M. conjunctivae	Fastidious	Sheep, goats, wild small ruminants	Eye swabs.	Eye swabs.
M. putrifaciens	Good	Cattle	Milk	Udder and associated lymph nodes
M. ovipneumoniae	Varies	Sheep/ goats	Nasal swabs, nasal discharges, bronchoalveolar washings, pleural fluid.	Lung lesions, pleural fluid, bronchopulmonry lymph nodes.
M. canis	Good	Cattle	Nasal swabs, nasal discharges, bronchoalveolar washings, pleural fluid.	Lung lesions, pleural fluid, bronchopulmonry lymph nodes.
Ureaplasma spp.	Requires specialize medium containing urea	Cattle, sheep	Semen, vulvovaginal swabs/ washes/discharges, nasal swabs.	Lung lesions, pleural fluid, bronchopulmonry lymph nodes.

(pleuropneumonia-like organisms), 1-2.5% yeast extract, 10-20% inactivated horse serum, 0.1% glucose, 1% triptose, and 0.0024% DNA. To avoid growth of other bacteria, the media can also contain an antibiotic of the penicillin family (for example, 500 IU/ml penicillin G) as mycoplasmas are naturally resistant (OIE, 2014). The media should be used both as broth and solid. All culture media prepared should be subjected to quality control and must support growth of low passage Mycoplasma spp. from small inocula. The reference strain should be cultured in parallel with the suspicious samples to ensure that the tests are performed correctly.

Tenfold dilution (10-1 to 10-6) is made of liquid sample (pleural fluid, nasal exudates, synovial fluid) or lung homogenate in appropriate medium. Tissue samples are best chopped with scissors then shaken vigorously in medium. A few drops of each sample are deposited and spread on the solid medium and 10% inoculums dispensed into liquid medium. In addition, a direct impression should be made on the solid medium with the cut surface of a lung lesion or lymph node without spreading it. Swabs are streaked directly on to solid medium. The broths and plates are incubated at 37°C in humidified atmosphere with 5% CO2 (Nicholas *et al.,* 2008).

The broths are examined daily for signs of growth or pH changes, indicated by a colour change in the medium. Mycoplasma growth will appear between 3 and 5 days and is usually seen as a very fine cloudiness, usually described as 'opalescence': it may be necessary to compare with an uninoculated broth to see the growth, particularly in the case of M.c. capripneumniae. Mmm grows well and usually produces 'whirls' from the bottom of the tube when shaken. If film appears on the surface of the liquid medium which also has an orange colour, M. bovis should be suspected if the isolate is from cattle and M. agalactiae if from small ruminants.

The plates are inspected after 2-3 days under 35X magnification, or 100X if colonies are small, for the typical 'fried egg' appearance. Mycoplasmas grow into the agar, which make the use of loop for subculturing unsatisfactory.

All members of M. mycoides clusters except Mccp grow within 3 days, producing colonies of between 1 -3 mm. M. ovipneumoniae can be suspected when isolated from small ruminat lung when the colonies are centreless and do not stick to the agar surface (Nicholas *et al.,* 2008).

Subculturing

For most serological test, it is necessary to grow the mycoplasma on solid medium. Broths should be subcultured immediately growth is apparent or pH change is seen. Suspected samples are subcultured three times before rejecting the material as negative. Plates are not normally incubated for more than a week (Nicholas *et al.,* 2008).

For plate to plate subculturing, a block with colonies is cut out and placed face down on the new plate, and is then carefully slid about on the surface and incubated. For plate to broth subculturing, a block with colonies is cut out with spatula and dropped into the broth and incubated. For broth to broth subculturing, 10% inoculums should be placed into the new broth using Pasteur pipette and incubated (Nicholas *et al.,* 2008).

Definite diagnosis is made by the isolation of Mccp from clinical samples, usually lung tissue and may be a long and difficult process. The success of isolation depends primarily on the attention that is given to sample collection. The main difficulties in isolating Mycoplasma capricolum subsp. capripneumoniae (Mccp) is that it grows very poorly in vitro and samples are often contaminated by other mycoplasmas (Freundt, 1983; Thiaucourt *et al.,* 1996) which are generally faster growing and overgrow Mccp. In addition to this the frequent use of antibiotic treatment has impaired the growth of these mycoplasmas from clinical material.

A number of studies were conducted in Africa to isolate mycoplasmas. Mccp was isolated by culture during respiratory outbreaks in goats in Tanzania (Noah *et al.,* 2011), whereas

Table 3: Isolation rates of Mycoplasmas of ruminants in Africa from 2005-2015

Media used in isolation	Method of inoculation	Source of sample	Isolation rate	Species	Reference
Heart infusion agar(HIA) and broth(HIB)	Impression smear, & serial dilution then inoculation	Lungs, pulmonary lymph node (LN)	1.6% (6/384)	Mmm (Cattle)	Wade et al., 2015 (Cameroun)
Eaton's medium (culture) GIT & IFAT	Impression smear, & serial dilution then inoculation	Pneumonic & apparently healthy lungs (goats & sheep)	44.5% (89/200) goats & 25% (50/200) sheep	M. ovipneumoniae, M.m capri, Mmm M.c capricolum, M. bovis	Egwu et al., 2012 (Nigeria)
Modified Hayflick's medium, Biochem tests: GF, AH, TR, film & spot	Serial dilution of samples then inocultion	Pneumonic lung tissues	2.0% (2/100)	M. bovis (cattle)	Francis et al., 2015b (Nigeria)
PPLO agar; Biochem test: TR, PT, GF, AH, GIT	Serial dilution of samples then inocultion	Lungs	-	Mmm, M. bovis, M. argini (cattle)	Ajuwape et al., 2003 (Nigeria)
Modified Hayflick's medium, & Commercial freeze dried diagnostic medium	Serial dilution of samples then inocultion	Lungs, LNs, & pleural fluid	18.7% (47/251)	M.c. capripneumoniae (goats)	Noah et al., 2011 (Tanzania)
Modified Hayflick, Beef heart infusion agar	Serial dilution of samples then inocultion	Lung tissues & nasal swabs	0.56% (3/529)	Mmm (cattle)	Elghazali et al., 2011 (Sudan)
Modified Hayflick medium	Serial dilution of samples then inocultion	Lung tissues, nasal swabs, tracheal swabs, LNs & vaginal swabs	12.6% (86/685)	M. bovis, M. bovigenitalium, & other M. species	Marouf et al., 2011 (Egypt)
Modified Hayflick medium, Biochem tests: GF, AH, UH, GIT	Fluid inoculate directly, Tissues homogenized then serial dilution	Pleural fluid, LNs, lung tissues	Not determined	Mycoplasma species	Teshale et al., 2005 (Ethiopia)

Media used in isolation	Method of inoculation	Source of sample	Isolation rate	Species	Reference
PPLO medium, heart infusion medium. Biochem: GIT, GPT, immuno-flurescence	Serial dilution then inocula-tion	Lung tissues & pleural fluid	7.2% (18/249)	M.c. capripneu-moniae, M.m capri, M. ovipneumo-niae,	Abbas et al., 2013 (Sudan)
Modified Hay-flick's + Colis-tin sulphate	Direct plating unto the agar plates	Milk sam-ples, prepucial swabs, vaginal swbs	12.5% (32/255)	M. bovis, M. bovigeni-talium	Yassim et al., 2012 (Egypt)
PPLO medium, Biochem tests: GF, AH, PT, TR, urea hydrolysis, film & spot test	Serial dilution then inocula-tion	Lung tissues, nasal swabs, ear swabs	4.4% (21/480)	Mmm/Mmcapri, M. bovirhinis, M. alkalense, M. bovis	Ankeli, 2015 (Nigeria)

NB:
PPLO: pleuropneumonia-like organism
GF: glucose fermentation
AH: arginine hydrolysis
PT: phosphatise test
Mmm: Mycoplasma mycoides subsp. mycoides
TR: tetrazolium reductase test
GIT: growth inhibition test
IFAT: indirect fluorescent antibody test
GPT: growth precipitation test

M. c. capricolum, Mccp as well as Mmm LC, M. ovippneumoniae and M. argini were isolated from lungs and pleural fluid in Sudan (Abbas *et al.,* 2013). Mycoplasma ovipneumoniae, M. arginini and M. mycoides subspecies capri were isolated from respiratory tract of small ruminants in North-central Nigeria (Akwuobu *et al.,* 2014). Egwu et al. (1999; 2012) isolated Mycoplasma agalactiae, M.c.capricolum as well as M. bovis and Mmm LC from milk samples collected form udder of goats infected by mastitis in Nigeria. A study was conducted in a tertiary care Hospital where genital mycoplasmas such as M. hominis, M. fermentans and M. penetrans were isolated in semen of married men with problems of infertility (Agbakoba *et al.,* 2007); in the same vein, Mycoplasma hominis and Ureaplasma urealyticum were isolated from urine samples collected from females attending University of Abuja Teaching Hospital Gwagwalada, Nigeria (Ugoh, *et al.,* 2014). Mycoplasma bovis was reported to be isolated in Nigeria (Ajuwape *et al.,* 2003; Egwu *et al.,* 2012; Francis, 2014; Francis *et al.,* 2015). Mycoplasma mycoides subsp. mycoides was isolated from lung tissues and pulmonary lymph node in Cameroun (Wade et al., 2015). Table 3 shows various methods and isolation rates of mycoplasmas of ruminants from different parts of Africa from 2005-2015.

Biochemical and Immunological Identification Tests

Once isolated, mycoplasmas can be identified by conventional biochemical and immunological methods, or increasingly by molecular methods such as polymerase chain reaction (PCR) assays. Biochemical tests were used routinely in the past but have now been superseded by other tests, namely the PCR. Biochemical tests alone do not allow identification of a precise Mycoplasma species because of overlapping of the few phenotypic traits that can be evaluated. Therefore, molecular tests such as PCR are recommended for identification of isolates. The biochemical methods are given below for historic reasons. Following subculture, antibiotics should be

omitted from the medium to check if the isolate is a mycoplasma or an L-form of a bacterium that will regain its original form in the medium without inhibitors. Once this test is done, the organism can be identified using biochemical tests (Freundt *et al.,* 1979).

Mmm is sensitive to digitonin (like all members of the order Mycoplasmatales), does not produce 'film and spots', ferments glucose, reduces tetrazolium salts (aerobically or anaerobically), does not hydrolyse arginine, has no phosphatase activity, and has no or weak proteolytic properties.

For these tests, special media have been developed that include the same basic ingredients (heart infusion broth or Bacto PPLO broth, horse serum, 25% yeast extract solution, 0.2% DNA solution), to which is added 1% of a 50% glucose solution for glucose hydrolysis, 4% of a 38% arginine HCl solution for arginine hydrolysis, and 1% of a 2% triphenyl tetrazolium chloride solution for tetrazolium reduction, as well as a pH indicator (e.g. phenol red). (NOTE: a pH indicator should not be added to a medium containing triphenyl tetrazolium chloride). For demonstration of proteolysis, growth is carried out on casein agar and/or coagulated serum agar. Table 4 shows biochemical differentiation of various mycoplasmas of ruminants.

Once the biochemical characteristics have been checked, one of the following immunological tests can be performed to confirm the identification: disk growth inhibition test (DGIT) (Freundt *et al.,* 1979), direct or indirect fluorescent antibody test (FAT), agar gel immunodiffusion test (AGID) (Provost, 1972), or dot immunobinding on a membrane filter (MF-dot) test (Brocchi *et al.,* 1993). DGIT and FAT are the preferred immunological tests for M.c. capripneumoniae, DGIT is particularly useful in identifying M.c. capripneumoniae because they appear to be serologically homogenous with wide zone of inhibition.

At present, few laboratories use immunochemical tests on a routine basis for detection and identification of Mycoplasmas because of the development of PCR-based tests that is more specific, sensitive, rapid and easy to perform and standardise.

Molecular Identification of Mycoplasmas

PCR has become the method of choice for the rapid and specific identification of MmmSC when the organism is isolated from a clinical sample. To avoid cross-contamination and carryover-contamination, strict separation of laboratory rooms, used for PCR preparation and handling of reactions, is needed.

DNA Extraction

Any accepted method for DNA extraction would be appropriate. A simple and effective method is to select a single colony, resuspended it in 100 µl of PCR-grade water, boil for 15 minutes to lyse the cells and release the DNA, and centrifuge at 1500 g for 1 minute. The supernatant with the DNA will be used in the PCR reaction after diluting 1/10 in PCR-grade water. Alternatively 500 µl of a 4-day broth culture, from a single colony, is centrifuged at 1500 g for 5 minutes, the pellet is resuspended in 100 µl of PCR-grade water and boiled and centrifuged as above. After diluting 1/10, 1 µl of the supernatant is used in the PCR. Alternatively, DNA extraction kits are available by many manufacturers and are being employed for extraction of various species of mycoplasmas.

Molecular Diagnosis

Powerful diagnostic systems based on PCR have been developed for the rapid detection, identification and differentiation of members of the M. mycoides cluster and the specific identification of Mmm. An arbitarily-primed PCR (AP-PCR) of Mmm with Mlip1 and Mlip4 primers produced a fingerprint with little genomic polymorphism and thus of limited epidemiological use. Two bands of 900 bp and 100 bp for strains PG1, PO, KH3J and Fatick were produced, although the later had a faint band at 400 bp, in contrast to the five M. capricolum subsp. capricolum strains tested which produced four different patterns (Rawadi *et al.,* 1995). These tests were designed from sequences of unknown functions or from

Table 4: Biochemical properties of the most frequent mycoplasmas of ruminants (Nicholas and Ayling, 2003; Nicholas et al., 2008).

Mycoplasma	Glu-cose	Argi-nine	Ure-ase	Film	Casein	Phos-phatase	Tetrazo-lium/ Aerobic	Tetrazo-lium/ Anaerobic
M. bovis	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve
M. bovirhinis	+ve	-ve	-ve	-ve	+/-ve	+/-ve	+ve	+ve
M.bovigenitalium	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve
M. bovoculi	+ve	-ve	-ve	+ve	-ve	+/-ve	+ve	+ve
M. canis	+ve	-ve	-ve	-ve	+/-ve	-ve	-ve	+ve
M. californicum	-ve	-ve	-ve	-ve	nk	+ve	-ve	+**ve
M. canadense	-ve	+ve	-ve	-ve	nk	+*ve	-ve	+ve
M. dispar	+ve	-ve	-ve	-ve	nk	-ve	+ve	+ve
M. mycoides SC	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
U. diversum	-ve	-ve	+ve	-ve	-ve	-ve	nk	nk
M.c. capripneumoniae	+ve	+ve	nt	-ve	nt	-ve	+ve	+ve
M. agalactiae	-ve	-ve	nt	+ve	nt	+ve	+ve	+ve
M. argini	-ve	+ve	nt	-ve	nt	-ve	+/-ve	+/-ve
M.m. Capri	+ve	-ve	nt	-ve	nt	-*	+ve	+ve
M. ovipneumoniae	+ve	-ve	nt	-ve	nt	-*	+ve	+ve

NB: nk = not known +/- ve= variable; = weak; nt = not tested; **= most strains; +ve = positive; -ve= negative*

known genes (Bashiruddin *et al.,* 1994b; Dedieu *et al.,* 1994; Hotzel *et al.,* 1996; Miserez *et al.,* 1997; Rodriguez *et al.,* 1997; Persson et al., 1999). With many of these tests, confirmation of the presence of Mmm or the production of the expected amplification product was possible by the digestion of the product with specific restriction enzymes. In some cases the standard detection of PCR products by agarose gel electrophoresis was replaced with enhanced methods which improved the sensitivity and allowed some degree of automation (Bashiruddin *et al.,* 1999a; Persson *et al.,* 1999). They have been used for the detection and identification of Mmm from culture and clinical materials including nasal mucous, pleural fluid, tissue from lung, lymph node, kidney, spleen, and semen from bovines (Bashiruddin *et al.,* 1994a; 1994b; Nicholas *et al.,* 1994; Bashiruddin *et al.,* 1999a; 1999b; Stradaioli *et al.,* 1999); and from the milk and respiratory tract of small ruminants (Brandao, 1995; Kusiluka *et al.,* 2000; Srivastava *et al.,* 2000). In some cases they had superior diagnostic sensitivities compared with conventional diagnostic tests. Particularly sensitive nested PCR systems have been used for the detection of Mmm from culture and clinical material where the target organism may be in low numbers such as in nasal swab samples (Hotzel *et al.,* 1996; Miserez et al., 1997).

Miles et al. (2006) developed PCR assays that will detect Mmm and differentiate European and African/Australian isolates. More recent technological advances have resulted in the use of real-time PCR assays. Vilei and Frey, (2010) describe a TaqMan real-time PCR which they designed specifically to target the LppQ gene, which may be useful if a LppQ devoid vaccine was successfully developed. Schnee et al. (2011) describe a novel multiplex real-time PCR which they demonstrated to be specific and sensitive when assessed using experimentally infected cattle.

Table 5: Molecular identification of Mycoplasma of Ruminants in Africa from 2010-2015

Molecular technique	Mycoplasma strain	% identification	Source of sample	Country	Remarks	Reference
PCR & DGGE	M. ovipneumoniae, M. argini, M.m capri	25.8% (135/508) in sheep & goats	Sheep & goats (nasal swabs)	Nigeria (North-central)	High isolation rate of mycoplasma in sick & apparently healthy sheep & goats indicates carrier statue which indicates serious problem in disease control.	Akwuobu et al., 2014
PCR & REA	M. capripneumoniae	32.3% (82/251)	Goats	Tanzania (Central zone)	The use of PCR/REA demonstrate its usefulness in the investigation of field outbreaks of CCPP even when isolation is not possible.	Noah et al., 2011
PCR, cloning & gene sequencing	M. argini, M. bovigenitalium, MmmLC, M. canadense, M. spp ovine/ caprine gp II	50% (17/34)	Dooper sheep (genital, tract)	South Africa	Ulcerative vulvitis and balanitis of sheep in South Africa is a multifactorial disease with involvement of different Mycoplamsa species	Kalshingi et al., 2015
PCR	Mmm	3,4% (13/384)	Cattle	Cameroun	The uses of PCR in CBPP surveillance especially test and slaughter process of disease eradication programme is recommended.	Wade et al., 2015
PCR	Mmm	1.88% (9/480)	Cattle	Nigeria (Plateau)	PCR be used as a molecular identification tool for diagnosis of CBPP.	Ankeli, 2015

NB: PCR: polymerase chain reaction; REA: restriction enzyme analysis; DGGE: denaturing gradient gel electrophoresis
Mmm: Mycoplasma mycoides subsp. mycoides

Until recently, isolation was the only way to confirm the presence of CCPP. A DNA probe which differentiates M.c. capripneumoniae from other members of the M. mycoides cluster was developed (Taylor *et al.,* 1992). Diagnostic systems based on PCR have been developed for the rapid detection, identification and differentiation of members of the M. mycoides cluster and the specific identification of M.c. capripneumoniae (Bashiruddin *et al.,* 1994a; Hotzel *et al.,* 1996; Woubit *et al.,* 2004). The sequence of the gene for 16S rRNA has also been used to develop a PCR-based test where the final identification of M.c. capripneumoniae is made dependant on the pattern of the products after digestion of the PCR product with the restriction enzyme Pst1 (Bascuñana *et al.,* 1994; Bölske *et al.,* 1996). More recently specific real-time PCR assays have been developed (Lorenzon *et al.,* 2008) and Schnee et al. (2011) describe a microarray for Mycoplasma species that can also differentiate members of the M. mycoides cluster.

Polymerase chain reaction and restriction enzyme analysis (REA) was employed to confirm CCPP infection in central zone of Tanzania (Noah *et al.,* 2011). Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) was employed in the identification of M. ovipneumoniae, M. arginini and M. mycoides subspecies Capri in North-central Nigeria (Akwuobu *et al.,* 2014). Table 5 shows various molecular techniques used in identifying mycoplasmas of ruminants and remarks of each method used in different parts of Africa from 2010-2015.

Table 6: Serological tests carried out on Mycoplasma of Ruminants in Africa from 2010-2015

Type of test kit	Mycoplasma spp.	Source of sample	Prevalence	Country	Reference
c-ELISA,	Mmm	Camel	9.62%	Sudan	Elghazali et al., 2011
Slide agglutination	Mmm	Camel	8.81%	Sudan	Elghazali et al., 2011
c-ELISA	Mmm	Cattle	0.4% (13/3187)	Ethiopia	Gezahegn et al., 2014
c-ELISA	Mmm	Cattle	10.65% (18/169)	Nigeria (Kwara)	Olabode et al., 2013
CHECKIT ELISA	M. bovis	Cattle	66% (41/62)	Nigeria (Sokoto)	Tambuwal et al., 2011
Latex Agglutination Test	Mmm	Cattle	47% (20/43)	Nigeria (Kaduna)	Danbirni et al., 2010
c-ELISA	Mmm	Cattle	0.63% (1/160)	Nigeria (Borno)	Musa et al., 2015
ELISA	Mmm	Cattle	54.7%	Nigeria (Kaduna)	Suleiman et al., 2015
Bio-X ELISA	M. bovis	Cattle	19.5% (78/400)	Nigeria (Adamawa)	Francis et al., 2015a
CFT	M.c. capripneumoniae	Goats	18.1% (219/1152)	Ethiopia	Fasil et al., 2015
c-ELISA	Mmm	Cattle	14.39% (76/528)	Nigeria (Plateau)	Ankeli, 2015

NB: c-ELISA: competitive Enzyme-linked Immunosorbent Assay; CFT: compliment fixation test; Mmm: Mycoplasma mycoides subsp. mycoides; Mccp: Mycoplasma capricolum subsp. capripneumoniae

Conclusion

Mycoplasma is endemic in Africa as reported by various studies in ruminants and detected by various techniques used. More molecular methods need to be exposed in order to determine the strain in each part of Africa. Also indigenous vaccines need to be produce using the strain in that area rather than depending on a particular strain that does not give adequate protection to the infection.

References

Abbas SI, Harbi MSMA, Gameel AA, 2013. First reported case of pathological manifestations of natural cases of Contagious Caprine Pleuropneumonia in the Sudan from which M.capricolum subsp.capripneumoniae; M. mycoides subsp. mycoides (LC type); M. ovipneumoniae and M. arginini were isolated. Journal of Pharmaceutical and Biomedical Science, 35(35): 1739-1746.

Abdo EM, Nicolet J, Frey J, 2000. Antigenic and genetic characterization of lipoprotein LppQ from Mycoplasma mycoides subsp. mycoides SC. Clinical Diagnostic Laboratory Immunology, 7(4): 588-595.

Abdo EM, Nicolet J, Miserez R, Gonçalves R., Regalla J, Griot C, Bensaide A, Krampe M, Frey J, 1998. Humoral and bronchial immune responses in cattle experimentally infected with Mycoplasma mycoides subsp. mycoides small colony type. Veterinary Microbiology, 59(2): 109-122.

African Union-Interafrican Bureau for Animal Resources, 2011. Panafrican Animal Health Yearbook 2011, Contagious Caprine Pleuropneumonia, 1-90. http://www.au-ibar.org/pan-african-animal-health-yearbook

African Union-Interafrican Bureau for Animal Resources, 2013a. Panafrican Animal Health Yearbook 2013a Contagious Bovine Pleuropneumonia, 1-45.

African Union-Interafrican Bureau for Animal Resources, 2013b. Panafrican Animal Health Yearbook 2013b. Contagious Caprine Pleuropneumonia. 1-14. http://www.au-ibar.org/pan-african-animal-health-yearbook

Agbakoba NR, Adetosoye AI, Ikechebelu JT, 2007. Genital mycoplasmas in semen smples of males attending a tertiary care Hospital in Nigeria. Nigerian Journal of Clinical Science, 10(2): 160-173.

Ajuwape ATP, Ikheloa JO, Ojo MO, Alaka OO, Adatosoye AJ, 2003. Biochemical and serological identification of mycoplasmas isolated from pneumonic bovine lungs in Nigeria. Israel J. Veterinary Medicine Microbiology and Parasitology, 58: 2-3.

Akwuobu CA, Ayling RD, Chah, KF, Oboegbulem SI, 2014. Studies into the prevalence of Mycoplasma species in small ruminants in Benue State, North-central Nigeria. Tropical Animal Health and Production, 46(6): 1087-1092.

Amanfu W, Masupu KV, Adom EK, Raborokgwe MV, Bashiruddin JB, 1998. An outbreak of contagious bovine pleuropneumonia in Ngamiland district of north-western Botswana. Veterinary Record, 143(2): 46-48.

Amin S, Rahman SU, Hussain I, Muhammad G, 2013. Seroprevalence of Mycoplasma ovipneumoniae among sheep from different districts of Baluchistan. Pakistan Int. Journal of Agric Biology, 15: 1043-1046.

Ankeli PI, 2015. Seroprevalence, isolation and molecular characterization of Mycoplasma mycoides subspecies mycoides SC in cattle in Plateau State, Nigeria. Unpublished MSc Thesis, Ahmadu Bello University Zaria, Nigeria, 1-106.

Ayling RD, Godinho K, Nicholas RAJ, 2007. Comparative studies on the in vitro antimicrobial sensitivities of Mycoplasma mycoides subsp. mycoides SC type and Mycoplasma bovis. In: CBPP Control: antibiotics to the rescue. FAO-OIE-AU/IBAR-IAEA Consultative Group Meeting on CBPP in Africa, 2006. Rome, Italy: FAO, 51-61.

Bascunana CR, Mattsson JG, Bölske G, Johansson KE, 1994. Characterization of the 16S rRNA genes from Mycoplasma sp. strain F38 and development of an identification system based on PCR. Journal of Bacteriology, 176(9): 2577-2586.

Bashiruddin JB, Nicholas RAJ, Santini FG, Ready RA, Woodward MJ, Taylor TK, 1994a. Use of the polymerase chain reaction to detect Mycoplasma DNA in cattle with contagious pleuropneumonia.

Veterinary Record, 134: 240-241.

Bashiruddin JB, Taylor TK, Gould AR, 1994b. A PCR-based test for the specific identification of Mycoplasma mycoides subspecies mycoides SC. Journal of Veterinary Diagnostic Investigation, 6(4): 428-434.

Bashiruddin JB, De Santis P, Vacciana A, Santini FG, 1999a. Detection of Mycoplasma mycoides subsp. mycoides SC in clinical material by rapid colorimetric PCR. Molecular Cellular Probe, 13: 23-28.

Bashiruddin JB, Santini FG, Santis P, Visaggio MC, Francesco G, D'Angelo A, Nicholas RAJ, 1999b. Detection of Mycoplasma mycoides subspecies mycoides SC in tissues from an outbreak of contagious bovine pleuropneumonia by culture, immunohistochemistry and polymerase chain reaction. Veterinary Record, 145(10): 271-274.

Bizarro CV, Schuck DC, 2007. Purine and pyrimidine nucleotide metabolism in Mollicutes, Genetic and Molecular Biology, 30(1): 190-201.

Bölske G, Johansson KE, Heinonen R, Panvuga PA, Twinamasiko E, 1995. Contagious caprine pleuropneumonia in Uganda and isolation of Mycoplasma capricolum subspecies capripneumoniae from goats and sheep. Veterinary Record, 137(23): 594-599.

Bölske G, Mattsson JG, Bascunmacronana CR, Bergström K, Wesonga H, Johansson KE, 1996. Diagnosis of contagious caprine pleuropneumonia by detection and identification of Mycoplasma capricolum subsp. capripneumoniae by PCR and restriction enzyme analysis. Journal of Clinical Microbiology, 34(4): 785-791.

Brandao E, 1995. Isolation and identification of Mycoplasma mycoides subspecies mycoides SC strains in sheep and goats. Veterinary Record, 136(4): 98-99.

Brocchi E, Gamba D, Poumarat F, Martel JL, De Simone F, 1993. Improvements in the diagnosis of contagious bovine pleuropneumonia through the use of monoclonal antibodies. Rev. Sci. Tech – Office des International Epizootics 12: 559-570.

Chazel M, Tardy F, Le Grand D, Calavas D, Poumarat F, 2010. Mycoplasmoses of ruminants in France: recent data from the national surveillance network. BMC Veterinary Research, 6: 32- 40.

Churchward CP, Flint JP, Danks C, Humbert I, Hashem E, Nicholas RAJ, Ayling RD, 2007. Development and initial evaluation of a lateral flow device for the rapid detection of contagious bovine pleuropneumonia. In: Proceedings of the World Association of Veterinary Laboratory Diagnosticians, 13th International Symposium, Melbourne, Australia, 12-14 November 2007. Melbourne, Australia: World Association of Veterinary Laboratory Diagnosticians.

Cottew GS, Breard A, DaMassa AJ, Erno H, Leach RH, 1987. Taxonomy of the Mycoplasma mycoides cluster. Israel Journal of Medical Science, 23: 632-635.

Danbirni S, Okaiyeto SO, Pewan SB, Kudi CA, 2010. Concurrent infection of contagious bovine pleuropneumonia and bovine tuberculosis in Bunaji nomadic cows. Research Journal of Animal Science, 4(1): 23-25.

Dedieu L, Mady V, Lefevre PC, 1994. Development of a selective polymerase chain reaction assay for the detection of Mycoplasma mycoides subsp. mycoides SC. (contagious bovine pleuropneumonia agent). Veterinary Microbiology, 42(4): 327-339.

Egwu GO, Ameh JA, Aliyu MM, Mohammed FD, 1999. Caprine mycoplasmal mastitis in Nigeria. Veterinary Arhive, 69(5): 241-250.

Egwu GO, Adamu M, Mshelia GD, Bukar-Kolo YM, 2012. Isolates of Mycoplasma mycoides subspecies mycoides (SC) in small ruminants in Sahel zone of Nigeria and its implications on disease control. African Journal of Biotechnology, 11(23): 6396-6401.

Elghazali F, Hamid FM, Amal MMA, Shallali AAO, 2011. Isolation of Mycoplasmas and Sero-surveillance for Mycoplasma mycoides subsp. mycoides in the Dromedary camel in some areas of Sudan. Sudan Journal of Veterinary Research, 26: 13-22.

Fasil A, Yilkal A, Maximillan B, Getnet A, 2015. Epidemiological study of contagious caprine pleuropneumonia (CCPP) in selected Districts of Gambella region, Western Ethiopia. African Journal of Agric Research, 10(24): 2470-2479.

Fischer A, Shapiro B, Muriuki C, Heller M, Sachse C, 2012. The origin of the Mycoplasma mycoides clusters coincides with domestication of ruminants. PLOS ONE, 7(4) :e36150.doi:10.1371/journal.pone.0036150

Fisher J, 2006. The origin, spread and disappearance of contagious bovine pleuropneumonia in New Zealand. Australian Veterinary Journal, 84: 434-444.

Francis MI, Ejeh EF, Raji, MA, Kazeem HM, Musa JA, Ankeli PI, Akalusi Y, 2014. Isolation and biochemical identification of Mycoplasma bovis in cattle pneumonic lungs in Adamawa State, Nigeria. Sahel Journal of Veterinary Science, 13: 1-5.

Francis MI, Raji MA, Kazeem HM, Suleiman MM, 2015. ELISA-based serological survey of Mycoplasma bovis in cattle in three local government areas in Adamawa State, Nigeria. Journal of Advance Veterinary and Animal Research, 2(2): 170-174.

Freundt EA, Erno H, Lemcke RM, 1979. Identification of mycoplasmas. In: Methods in Microbiology, Vol. 13, Bergen T. and Norris J.R.(Eds). Academic Press, London, UK, 377-434.

Freundt EA, 1983. Culture media for classic mycoplasmas. In: Razin S, Tully JG., editors. Methods in Mycoplasmology Vol. 1. Mycoplasma characterization. New York, USA: Academic Press, 127-135.

Gezahegn A, Samson L, Berhuta H, 2014. Low seroprevalence of contagious bovine pleuropneumonia in Bulls originating from Borena pastoral area of Southern Ethiopia. Animal and Veterinary Science, 2(6): 213-217.

Goff C, Thiaucourt F, 1998. A competitive ELISA for the specific diagnosis of contagious bovine pleuropneumonia (CBPP). Veterinary Microbiology, 60(2): 179-191.

Gonçalves R, Regalla J, Nicolet J, Frey J, Nicholas RAJ, Bashiruddin JB, Santis P, Gonçalves AP, 1998. Antigen heterogeneity among Mycoplasma mycoides subsp. mycoides SC isolates: discrimination of major surface proteins. Veterinary Microbiology, 63(1): 13-28.

Gonçalves R, Regalla J, Ayling RD, Nicholas RAJ, 2008. Impact of Mycoplasma bovis infection on serosurveillance for contagious bovine pleuropneumonia. Veterinary Record, 163(21): 632-633.

Harbi MSMA, El-Tahir MS, 1981. Mycoplasma strain F38 and contagious caprine pleuropneumonia in the Sudan. Veterinary Record, 108: 261-265.

Hotzel H, Sachse K, Pfützner H, 1996. A PCR scheme for differentiation of organisms belonging to the Mycoplasma mycoides cluster. Veterinary Microbiology, 49(1): 31-43.

Hutcheon D, 1989. Contagious pleuropneumonia in goats at Cape Colony, South Africa. Veterinary Journal, 29: 399-404.

Joachim F, 2002. Mycoplasma of animals. In; Razin, S., Hermann, R. (Eds). Molecular biology and pathogenicity of mycoplasmas. Springer, 73- 90.

Jones GE, Wood AR, 1988. Microbiological and serological studies on caprine pneumonias in Oman. Research Veterinary Science, 44(1): 125-131.

Kalshingi HA, Bosman A, Gouws J, van Vuuren M, 2005. Molecular characterization of Mycoplasma species isolate from the genital tract of Dooper sheep in South Africa. Journal of South African Veterinary Association, 86(1): 1-11.

Kusiluka LJM, Semuguruka WD, Kazwala RR, Ojeniy B, Friis NF, 2000. Demonstration of Mycoplasma capricolum subsp. capripneumoniae and Mycoplasma mycoides subsp. mycoides Small Colony type in outbreaks of caprine pleuropneumonia in eastern Tanzania. Acta Veterinaria Scandinavica 41(3): 311-319.

Lefevre PC, Breard A, Farouk I, Buron S, 1987. Mycoplasma species F38 isolated in Chad. Veterinary Record, 121(24): 575-576.

Lorenzon S, Manso-Silván L, Thiaucourt F, 2008. Specific real-time PCR assays for the detection and quantification of Mycoplasma mycoides subsp. mycoides SC and Mycoplasma capricolum subsp. capripneumoniae. Molecular and Cellular Probe, 22: 324-328.

MacOwan KJ, 1976. A Mycoplasma from chronic caprine pleuropneumonia in Kenya. Tropical Animal Health Production, 8: 28-36.

MacOwan KJ, Minette JE, 1976. A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. Tropical Animal Health and Production, 8: 91-95.

MacOwan KJ, Minette JE, 1977. The role of Mycoplasma strain F38 in contagious caprine pleuropneumonia (CCPP) in Kenya. Veterinary Record, 101: 380-381.

Manso-Silvan L, Vilei EM, Sachse K, Djordjevic SP, Thiaucourt F, Frey J, 2009. Mycoplasma leachii sp. nov. as a new species designation for Mycoplasma sp. bovine group 7 of Leach, and reclassification of Mycoplasma mycoides subsp. mycoides LC as a serovar of Mycoplasma mycoides subsp. capri. International Journal of Systemic and Evolutional Microbiology, 59: 1353-1358.

Marouf SA, Mohammed KF, El-Jakee J, 2011. Detection of Mycoplasma bovis and Mycoplasma bovigenitalium in cattle and buffalo in Egypt using Dot ELISA and PCR with Anti- Microbial Trials. European Journal of Biological Science, 3(1): 01- 08.

McMartin DA, MacOwan KJ, Swift LL, 1980. A century of classical contagious pleuropneumonia: from original description to aetiology. British Veterinary Journal, 136: 507-515.

Miles K, Churchward CP, McAuliffe L, Ayling RD, Nicholas RAJ, 2006. Identification and differentiation of European and African/Australian strains of Mycoplasma mycoides subspecies mycoides small-colony type using polymerase chain reaction analysis. Journal of Veterinary Diagnostic Investigation, 18: 168-171.

Miles RJ, Wadher BJ, Henderson CL, Mohan K, 1988. Increased growth of yields of Mycoplasma species in the presence of pyruvate. Letters of Applied Microbiology, 7: 149-151.

Miles RJ, Taylor RR, Varsani H, 1991. Oxygen uptake and hydrogen peroxide production by fermentative Mycoplasma species. Journal of Medical Microbiology, 34: 219-223.

Miles RJ, 1992. Catabolism of mollicutes. Journal of Gen. Microbiology, 7: 149-151.

Miles RJ, Agbanyim CD, 1998. Determination of substrate utilization rates by mycoplasmas. In: Miles RJ, Nicholas RAJ, Editors. Mycoplasma Protocols. Humana Press, Totowa, New Jersey, 95-104.

Miserez R, Pilloud T, Cheng X, Nicolet J, Griot C, Frey J, 1997. Development of a sensitive nested PCR method for the specific detection of Mycoplasma mycoides subsp. mycoides SC. Molecular Cellular Probe, 11: 103-111.

Musa JA, Ikpa LT, Lawan FA, Ejeh EJ, Tizhe JQ, 2015. Detection of antibodies to Mycoplasma mycoides subsp. mycoides in cattle using competitive enzyme-linked immunosorbent assay. International Journal of Current Microbiology and Applied Science, 4(8): 770-777.

Muthomi EK, Rurangirwa F, 1983. Passive haemagglutination and complement-fixation as diagnostic tests for contagious caprine pleuropneumonia caused by F-38 strain of mycoplasma. Research in Veterinary Science, 35: 1-4.

Muuka G, Hang'ombe BM, Nalubamba KS, Kabilika S, Mwambazi L, Muma JB, 2011. Comparison of complement fixation test, competitive ELISA and LppQ ELISA with post-mortem findings in the diagnosis of contagious bovine pleuropneumonia (CBPP). Tropical Animal Health and Production, 43(5): 1057-1062.

Niang M, Sery A, Cisse O, Diallo M, Doucoure M, Kone MCF, Simbe W, Amanfu W, Thiaucourt F, 2007. Effect of antibiotic therapy on the pathogenesis of CBPP. In: CBPP Control: antibiotics to the rescue. FAO-OIE-AU/IBAR-IAEA Consultative Group Meeting on CBPP in Africa, 2006. Rome, Italy: FAO, 25-32.

Nicholas RAJ, Bashiruddin JB, Santini FG, Regalla J, Taylor TK, 1994. Evaluation of a polymerase chain reaction for contagious bovine pleuropneumonia in naturally infected cattle. IOM Letters, 3: 15-16.

Nicholas RAJ, Baker SE, 1998. Recovery of mycoplasmas from animals. In: R. J. Miles, R. J. and Nicholas, R. A. J., Editors. Mycoplasma Protocols. Totowa, USA: Humana Press, 37-44.

Nicholas RAJ, 2002. Contagious caprine pleuropneumonia. In: Tempesta, M., (Eds). Recent Advances in Goat Diseases. International Veterinary Information Service, Ithaca, New York, USA. 1-8.

Nicholas RAJ, Ayling RD, 2003. Mycoplasma bovis: disease, diagnosis, and control. Research Veterinary Science, 74: 105-112.

Nicholas RAJ, Aschenborn HKO, Ayling RD, Loria GR, Lukhele O, Tijpura-Zaire G, Godinho K, Hubschle OJB, 2007. Effect of Advocin on the elimination of CBPP from the Caprivi Region of Namibia. In: CBPP Control: antibiotics to the rescue. FAO-OIE-AU/IBAR-IAEA Consultative Group Meeting on CBPP in Africa, 2006. Rome, Italy: FAO, 33-40.

Nicholas RAJ, Ayling RD, McAuliffe L, 2008. Isolation and Growth of Mycoplasma of Ruminants. In: Nicholas RAJ, Ayling RD, McAuliffe L., Editors. Mycoplasma Diseases of Ruminants. Cab International, 3-14.

Noah EY, Kusiluka LMJ, Wambura P, Kimera SI, 2011. Field Isolation of Mycoplasma capripneumoniae in Central Zone of Tanzania. International Journal of Animal and Veterinary Advances, 3(6): 434-442.

Nocard R, Roux ER, 1898. Le microbe de la péripneumonie. Annals de l'Institut Pasteur, 12: 240-262.

Office International des Epizooties, 2014. Contagious bovine pleuropneumonia. OIE Terrestrial Manual, 1-16.

Olabode HOK, Mailafia S, Adah BMJ, Nafarnda WD, Ikpa LT, Jambalang AR, Bello RH, 2013. Seroprevalence of contagious bovine pleuropneumonia antibodies in trade cattle sold in Kwara State-Nigeria. Online International Journal of Microbiology Research, 1(1): 14-19.

Perreau P, Breard A, Goff C, 1984. Experimental infection of goats with type F.38 Mycoplasma strains (CCPP). Annals of Microbiology, 135(1): 119-124.

Persson A, Pettersson B, Bölske B, Johansson KE, 1999. Diagnosis of contagious bovine pleuropneumonia by PCR-laser-induced fluorescence and PCR-restriction endonuclease analysis based on the 16S rRNA genes of Mycoplasma mycoides subsp. mycoides SC. Journal of Clinical Microbiology, 37(12): 3815-3821.

Provost A, 1972. Recherches immunologiques sur la péripneumonie. XIV. Description de deux techniques applicables sur le terrain pour le diagnostic de la maladie. Rev Elev. Med. Vet. Pays Tropicaux, 25: 475-496.

Rawadi G, Lemercier B, Roulland-Dussoix D, 1995. Application of an arbitrarily-primed polymerase chain reaction to mycoplasma identification and typing within the Mycoplasma mycoides cluster. Journal of Applied Bacteriology, 78(6): 586-592.

Razin S, 1991. The genera Mycoplasma, Ureaplasma, Acholeplasma, Anaeroplasma, and Asteroplasma. In: A. Balows, H. G. Truper, M. Dwarkin, W, Harder, and K. H. Schleifer, (Eds). The Prokaryotes. Volume, 2, Second Edition. Springer-Verlag, New York, 1937-1959.

Razin S, 1994. DNA probes and PCR in diagnosis of Mycoplasma infections. Molecular and Cellular Probes, 8: 497-511.

Razin S, Yogev D, Naot Y, 1998. Molecular biology and pathogenicity of mycoplasmas, Microbiol. Mol. Biol. Review, 62: 1094-1156.

Rodriguez JL, Ermel RW, Kenny TP, Brooks DL, DaMassa AJ, 1997. Polymerase chain reaction and restriction endonuclease digestion for selected members of the "Mycoplasma mycoides cluster" and Mycoplasma putrefaciens. Journal of Veterinary Diagnostic Investigation, 9(2): 186-190.

Rottem S, 2003. Interaction of mycoplasmas with host cells, Physiol. Review, 83: 417-432.

Rurangirwa FR, McGuire TC, Kibor A, Chema S, 1987. A latex agglutination test for field diagnosis of contagious caprine pleuropneumonia. Veterinary Record, 121(9): 191-193.

Rurangirwa FR, Kouyate B, Niang M, McGuire TC, 1990. Contagious Caprine Pleuropneumonia: antibodies to F38 polysaccharide in Mali goats. Veterinary Record, 127(14): 353-357.

Schnee C, Heller M, Jores J, Tomaso H, Neubauer H, 2011. Assessment of a novel multiplex real-time PCR assay for the detection of the CBPP agent Mycoplasma mycoides subsp. mycoides SC through experimental infection in cattle. BMC Veterinary Research, 7(47): 23-32.

Srivastava NC, Thiaucourt F, Sunder J, Singh VP, 2000. Isolation of Mycoplasma mycoides subsp. mycoides small colony type from contagious caprine

pleuropneumonia in India. Veterinary Record, 147(18): 520-521.

Srivastava AK, Meenowa D, Barden G, Salguero FJ, Churchward C, Nicholas RAJ, 2010. Contagious caprine pleuropneumonia in Mauritius. Veterinary Record, 167(8): 304-305.

Stradaioli G, Sylla L, Mazzarelli F, Zelli R, Rawadi G, Monaci M, 1999. Mycoplasma mycoides subsp. mycoides SC identification by PCR in sperm of seminal vesiculitis-affected bulls. Veterinary Research, 30(5): 457-466.

Suleiman A, Bello M, Dzikwi A, Talba AM, Grema H, Geidam Y, 2015. Serological prevalence of contagious bovine pleuropneumonia in agro-pastoral areas of Nigeria. Tropical Animal Health Production 47(6):Doi:10,1007/S11250-015-0824-5.

Tambi NE, Maina WO, Ndi C, 2006. An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. Rev. Sci. Tech – Office des International Epizootics, 25(3): 999-1011.

Tambuwal FM, Stipkovits L, Egwu GO, Junaidu AU, Abubakar MB, Turaki UA, 2011. Enzyme-Linked Immunosorbent Assay (ELISA) based detection of antibodies to Mycoplasma bovis in Cattle naturally infected with haemoparasites in Institutional farms in Sokoto State, Nigeria. Current Research Journal of Biological Science, 3(1): 12- 16.

Taylor TK, Bashiruddin JB, Gould AR, 1992. Relationships between members of the Mycoplasma mycoides cluster as shown by DNA probes and sequence analysis. International Journal of Systemic Bacteriology, 42(4): 593-601.

Teshale S, Aschalew Z, Esayas G, Fikru R, 2005. Isolation and identification of Mycoplasma mycoides subsp. mycoides bovine biotype in Eastern Ethiopia. International Journal of Applied Research Veterinary Medicine, 3(1): 1-5.

Thiaucourt F, Bölske G, 1990. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. Sci. Tech – Office des International Epizootics, 15(4): 1397-1414.

Thiaucourt F, Breard A, Lefèvre PC, Mebratu GY, 1992. Contagious caprine pleuropneumonia in Ethiopia. Veterinary Record, 131(25) 585-589.

Thiaucourt F, Bölske G, Libeau G, Goff C, Lefèvre PC, 1994. The use of monoclonal antibodies in the diagnosis of contagious caprine pleuropneumonia (CCPP). Veterinary Microbiology, 41(3): 191-203.

Thiaucourt F, Bolske G, Leneguersh B, Smith D, Wesonga H, 1996. Diagnosis and control of contagious caprine pleuropneumonia. Office Intl. Epiz. Rev. Sci. Tech, 15(4): 1415-1429.

Thomas P, 1873. Rapport medical sur le Bou Frida. In: Jourdan, A, editions. Publication du gouvernement general civil de l'Algerie. Algiers.

Ugoh SC, Nneji LM, Samuel S, 2014. Prevalence of Mycoplasma species in urine samples collected from females attending University of Abuja Teaching Hospital, Gwagwalada, FCT-Nigeria. Researcher, 6(2): 99-104.

Vilei EM, Frey J, 2010. Detection of Mycoplasma mycoides subsp. mycoides SC in bronchoalveolar lavage fluids of cows based on a TaqMan real-time PCR discriminating wild type strains from an lppQ-mutant vaccine strain used for DIVA-strategies. Journal of Microbiology Methods, 81(3): 211-218.

Wade A, Yaya A, El-Yuguda AD, Nafarnda HU, Daniel W, Ikechukwu ES, Egwu GO, 2015. The Prevalence of Contagious Bovine Pleuropneumonia in Cameroon: A Case Study Garoua Central Abattoir, Cameroun. Journal of Veterinary Medical Research, 2(4): 10-24.

Walker LR, 1999. Mollicutes: In Hirsh, D. C. and Zee, Y. C. Veterinary Microbiology. Blackwell Science, Inc, 165-172.

Wamwayi HM, Wafula JS, Litamoi JK, Nandokha EN, 1989. Detection of antibody to Mycoplasma F38 in goat sera by an enzyme-linked immunosorbent assay. Tropical Animal Health Production, 21(1): 43-49.

Woubit S, Lorenzon S, Peyraud A, Manso-Silván L, Thiaucourt F, 2004. A specific PCR for the identification of Mycoplasma capricolum subsp. capripneumoniae the causative agent of contagious caprine pleuropneumonia (CCPP). Veterinary Microbiology, 104(1):125-132.

Yassim MH, Al-Humiany AA, Mansour AMA, 2012. Colistin sulphate as a substitute, of Thalium acetate in culture media for isolation of mycoplasma from cattle diseased by mycoplasmosis. International Journal of Microbiologiy Research, 3(2): 128-132.

ACUTE TOXICITY STUDY AND EFFECT OF ETHANOLIC LEAF EXTRACT OF GUIERA SENEGALENSIS J. F. GMEL (COMBRETACEAE) ON *TRYPANOSOMA BRUCEI* BRUCEI INDUCED PATHOLOGY IN ALBINO RATS

Badau S J[1], *Luka J[2,3], Igwenagu E[1] and Suleiman I E[1].
[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, P. M. B 1069, Maiduguri, Nigeria
[2]Parasitology Research Unit, Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, P.M. B 1069, Maiduguri, Nigeria
[3]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria

Abstract

The current study evaluated the acute toxicity and the effect of crude ethanolic leaf extract of Guiera senegalensis J. F. Gmel (Combretaceae) on *Trypanosoma brucei* brucei induced pathology in albino rats. A total of sixty five (65) rats of both sexes and varying weights were used in all; 35 for acute toxicity study and 30 for the study of effect of extract on *Trypanosoma brucei* brucei induced pathology. In the acute toxicity testing, the thirty five (35) rats were randomly divided into seven groups (A-G) of five each and administered graded concentrations (100, 200, 400,800, 1600, 3200 and 6400 mg/kg) of the extract intraperitoneally. They were monitored for signs of toxicity including mortality in the 24 hours following administration. Following the determination of LD50 and safe dose, the rats for the study of effect of extract on pathology were similarly divided into six groups (A-F) of five rats each. Rats in groups A-E were individually infected with 4×10^6 of *Trypanosoma brucei* brucei intraperitoneally, while those in group F remained uninfected control. The infected rats were later treated on day 4 post infection. Groups A, B and C were treated orally with 100 mg, 300 and 600 mg/kg of the extract of Guiera senegalensis respectively for four consecutive days, while group D was treated intramuscularly with Diminazine aceturate at 3.5 mg/kg once. Rats in groups E and F were not treated during the experiment. The infected rats were monitored for the establishment of parasitaemia and subsequently; gross and histopathological changes. The extract produced an intraperitoneal LD50 of 2130 mg/kg. Similarly, milder lesions were observed at histopathology in some extract treated groups compared to the infected untreated group, suggesting a dose dependent extract activity.

Conclusively, the extract at the dosages tested appeared to be less or non-toxic and resulted in mild improvement in pathology induced by Trypanasoma brucei brucei.

Key words: Pathology, Toxicity, Guiera senegalensis, Extract, Effect, Trypanosoma

ÉTUDE DE DETOXICITÉ ET DE L'EFFET DE L'EXTRAIT ETHANOLIQUE BRUT DE FEUILLES DE GUIERA SENEGALENSIS J. F. GMEL (COMBRETACEAE) SUR LA PATHOLOGIE INDUITE PAR *TRYPANOSOMA BRUCEI* BRUCEI CHEZ LES RATS ALBINOS

Résumé

La présente étude évalue le degré de toxicité et l'effet de l'extrait éthanolique brut de feuilles de de Guiera Senegalensis J. F. Gmel (Combretaceae) sur la pathologie induite par *Trypanosoma brucei* brucei chez les rats albinos. Au total, soixante-cinq (65) rats des deux sexes et de poids différents ont été utilisés : 35 rats ont été utilises pour l'étude du degré de toxicité, et 30 autres l'ont été pour l'étude de l'effet de l'extrait éthanolique sur la pathologie induite par *Trypanosoma brucei* brucei. Dans l'étude du degré de toxicité, les trente-cinq (35) rats ont été répartis de manière aléatoire en sept groupes (A-G) de cinq

*Corresponding author email:joshuadiriki@yahoo.com

rats chacun et ont reçu des concentrations croissantes échelonnées (100, 200, 400, 800, 1600, 3200 et 6400 mg / kg) de l'extrait par voie intrapéritonéale. Ils ont été suivis en vue de rechercher des signes de toxicité, dont la mortalité, dans les 24 heures suivant l'administration de l'extrait. Après détermination de la DL50 et de la dose sure, les rats soumis à l'étude de l'effet de l'extrait sur la pathologie ont été répartis de manière similaire en six groupes (A-F) de cinq rats chacun. Les rats des groupes A à E ont été infectés individuellement avec 4×10^6 de *Trypanosoma brucei* brucei par voie intrapéritonéale, tandis que ceux du groupe F ont servi de témoins non infectés. Les rats infectés ont ensuite été traités au jour 4 post-infection. Les groupes A, B et C ont reçu par voie orale 100 mg, 300 et 600 mg / kg de l'extrait de Guiera senegalensis pendant quatre jours consécutifs, tandis que le groupe D a été traité une seule fois par voie intramusculaire avec l'acéturate de diminazène à la dose de 3,5 mg / kg. Les rats des groupes E et F n'ont pas été traités au cours de l'expérience. Les rats infectés ont été suivis afin de déterminer la parasitémie et les changements généraux et histopathologiques. L'extrait a produit une dose intrapéritonéale DL50 de 2130 mg / kg. De même, des lésions modérées ont été observées à l'histopathologie chez certains groupes traités avec l'extrait par rapport au groupe infecté non traité, ce qui porte à croire que l'activité de l'extrait dépend de la dose.

En conclusion, l'extrait aux doses testées semble être peu ou non toxique et a donné lieu à une légère amélioration de la pathologie induite par Trypanasoma brucei brucei.

Mots-clés : pathologie, toxicité, Guiera senegalensis, extrait, effet, trypanosome

Introduction

Trypanosomes are flagellated haemoprotozoan parasites that cause infection in humans and animals (Igbokwe et al., 2009), which adversely affect the economic and social well being of sub-Saharan Africans (Adeyemi et al., 2009, Samdi et al., 2010). Trypanosomiasis, the disease caused by Trypanasoma spp is still endemic in Nigeria (Samdi et al., 2011), causing an estimated annual loss of about 4.5 billion US Dollars in agricultural products of African countries alone (Adamu et al., 2009). The disease is transmitted, mainly; cyclically by Tsetse flies of the genus Glossina, and mechanically by several biting flies. In animals that die during the chronic phase of the disease, the lymphoid organs are usually not enlarged and severe myocarditis is a common finding (Ukpai and Nwabuko, 2014). Associated histopathological changes include mild congestion of the spleen, liver, and the lung, acute bronchopneumonia, severe pulmonary emphysema and focal centrilobular necrosis with peri-portal mononuclear cell aggregation in the liver, in addition to myocarditis (Morrison et al., 1981).

The use of anti-trypanosomes such as suramin, melarsoprol, eflornithine and pentamidine for the treatment of trypanosomosis has been the main control approach (Adeiza et al., 2010). These drugs are however, beset by various limitations such as inadequate supply to the rural areas and toxicity (Asuzu and Chineme, 1990), development of resistance by the parasites due to antigenic variation (Kuzoe, 1993; Doua and Yapo, 1993), thereby resulting in persistence of the disease in an area.

Several African medicinal plants have shown some promising anti-trypanosomal effects (Ibrahim et al., 2014). The plant; Guiera senegalensis has been reported to have a wide range of applications in folkloric medicine as well as in experimental trials. Such applications include the use by Fulani herdsmen for the treatment of several disorders including venereal diseases (Silva et al., 1997), ulcer-protective properties against ethanol-induced ulceration in rats (Aniangu et al. 2005) and topical application for wound healing (Bosisio et al., 1997). Similarly, macerated leaves of the plant have been administered, orally for the treatment of fever as well as for hyperglycaemia and hypertension (Shettime et al., 2012). These reports coupled with the recent report by Akuodor et al. (2013) necessitated our quest to investigate the effect of the ethanolic extract of Guiera senegalensis on *Trypanosoma brucei* brucei induced pathology in albino rats.

Materials and Methods

Collection and Authentication of Plant Material

Leaves of Guiera senegalensis and the leaf powder (The form sold and used by the populace) were obtained from traditional medicine market, opposite NEPA house, in April 2014, in Maiduguri, Borno State, Nigeria. The plant was authenticated by a Botanist from the Department of Biological Sciences, University of Maiduguri. A voucher specimen (Vet 220 A4) has been deposited at the herbarium of the Department of Veterinary Physiology, Pharmacology and Biochemistry of the Faculty of Veterinary Medicine, University of Maiduguri. Five hundred (500) grams of the powder was exhaustively extracted using soxhlet extractor and condenser utilizing absolute (l00%) ethanol as solvent at the Department of Chemistry, University of Maiduguri, according to standard method (WHO, 1992). The extracted was concentrated in-vacuo to remove traces of ethanol and yielded dark green viscous extract at the bottom of the flask. The concentration of the extract was determined and the stock solution stored at refrigerator temperature (4°C) until used.

Phytochemical screening of the plant extract

The crude ethanolic extract of the leaves was subjected to qualitative chemical screening for the identification of the various classes of its chemical constituents. The analyses were carried out following the method described by Sofowora et al. (2006). Since this is a reaction between metabolites within an extract with some chemical compounds, colour changes indicate reaction, and the degree of the colour changes determines the concentrations of the extract.

Experimental animals

A total of sixty-five adult albino rats of both sexes and varying weights, used for this study were obtained from the University of Maiduguri staff (quarters NH-3), where they have been bred. They were kept in clean netted plastic cages under favourable condition (temperature 30 ± 2 oC and a 12 hour daylight/night cycle) at the Postgraduate Laboratory, Department of Veterinary Microbiology and Parasitology. They were fed on commercial poultry grower pelleted feeds (Vital feeds®, Jos, Plateau State, Nigeria) ad-libitum throughout the experiment.

The experiment was approved by Animal Welfare Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, and carried out according to International guiding principles for biochemical research involving animals (C. I. O. M. S 1985)

Source of Trypanosome

Trypanosoma brucei brucei (Federe strain) used for the study was obtained from donor rats maintained at the postgraduate laboratory of the Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary medicine, University of Maiduguri. The parasite was earlier sourced from the stabilate maintained at Nigerian Institute of Trypanosomosis and Research, (NITR), Vom, Nigeria, for a previous experiment. It was maintained by serial passages in rats, which produced acute and sub-acute infections with a pre-patent period of 2-3 days.

Acute toxicity study and safe dose determination

The thirty-five albino rats used for this study were fasted overnight and randomly divided into seven groups (A, B, C, D, E, F, and G) of five rats each. Rats in the groups were, respectively, administered intraperitoneally (i.p) graded doses (100, 200, 400, 800, 1600, 3200 and 6400 mg/kg) of crude ethanolic extract of Guiera senegalensis resuspended in normal saline (Aliu and Nwude, 1982). The rats were monitored for clinical signs of toxicity and mortality for a period of 24 hours. The LD50 was calculated using the arithmetic method of Karber as described previously (Aliu and Nwude, 1982)

Study of effect of extract on Trypanosoma brucei *brucei induced pathology*

A total of thirty adult albino rats of both sexes were used for this experiment. They were randomly divided into six groups

(A, B, C, D, E and F) of five rats each. Rats in groups (A-E) were individually inoculated, intraperitoneally with 0.5 ml of glucose saline diluted with blood containing approximately 4x106 *Trypanosoma brucei* brucei from donor rats, while those in group F remained uninfected control. The number of parasites in the diluted blood was estimated as previously described (Herbert and Lumsden, 1976). Infected rats were monitored for parasitaemia two days post-inoculation and later on day 3 post-infection. Following establishment of parasitaemia on day 3 post infection, groups (A-C), were treated with graded doses of the extract of 100 mg/kg, 300 mg/kg, and 600 mg/kg orally, respectively for 4 days, while group D, was treated with Diminazine aceturate at a single dose of 3.5 mg/kg intramuscularly. Group E remains infected untreated, while group F is the uninfected untreated. All treatments commenced on day 3 post-infection. Clinical signs and mortalities were monitored after infection up to the end of the experiment. Rats that died or those humanely sacrificed were subjected to detailed necropsy and collected tissues processed for histopathology according to standard procedure (Akpavie, 2004).

Statistical Analysis

The data generated were expressed using descriptive statistics by means of tables and figures

Results

Phytochemical analysis

The yield of the extract was 95.05% w/w, dark green in colour and soluble in water. Phytoconstituents found include; reducing sugars, tannins, cardiac glycosides, terpenoids, flavonoids, saponin glycosides and carbohydrates (Table 1).

Acute toxicity study

No clinical sign attributable to the administered extract was observed in any of the groups. The calculated median lethal dose (LD50) of the ethanolic extract was 2130 mg/kg body weight (Table 2).

Study of effect of extract on Trypanosoma brucei *brucei induced pathology*

Clinical signs attributable to trypanosomiasis started manifesting on day 3 post infection in all the infected groups. They all presented with the signs of depression, rough hair coat and bulging of the eyes. These signs however, became less severe and later abated in group D, from day 20 post infection.

Mortality

Mortality started on day 5 post infection in the infected groups, with 60%, 40%, 10% and 60% mortalities in groups A, B, C, and E, respectively, whereas groups D and F had no mortality. At the end of this experiment, 100% mortality was recorded in groups A, B, C, and E between days 5 and 13 pi (Figure 1).

Gross pathology

No clearly distinct gross lesions were observed in the infected groups. However, lesions generally presented as congestion and oedema of the lungs, enlargement of the liver with various degrees of pale streaked areas on the surface, splenic congestion with enlargement, and pale kidneys were observed.

Histopathology

Rats in groups A, B, C, and E showed hepatocellular necrosis and widespread moderate to marked vacuolar degeneration of the hepatocytes, which are mostly centrilobular. Groups B and C showed moderate periportal lymphocytic aggregation. In group A, there were multiple areas of local extensive sinusoidal congestion, in addition to vacuolation, while group E showed marked centrilobular hepatocellular necrosis (Figure 2). Kidney showed marked congestion in groups B and D with mild tubular necrosis in group A. Groups B, C, and E, had marked degenerative changes and necrosis with a mild haemorrhage in group B. There were moderate to marked deposits of hyaline materials in the capsular space of the glomeruli of groups B and C accompanied by hypocellularity of the glomeruli, with glomerular atrophy in group C (Figure 3). Group D did not show any histological lesion. Spleen was

Table 1: Phytochemical screening of the ethanolic extract of the leaf of Guiera senegalensis J. F. Gmel (Combretaceae)

Phytochemical constituents	Tests	Inferences
Carbohydrate	Molisch's	+
	Ketoses	-
	Pentoses	-
	Fehling's	+
	Combined reducing sugars	+
	Soluble starch	-
Tannins	Ferric chloride	+
	Lead acetate test	
Phlobannins	Hydrochloride acid	-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinines	-
Cardiac glycosides	Salkowski	+
	Liebermann-Burchard test	+
Terpenoids	Terpenoids	+
Saponin glycosides	Frothing	+
Flavonoids	Shinoda's test	+
	Ferric chloride	+
	Lead acetate	+
	Sodium hydroxide	-
Alkaloids	Dragendorff's reagent	-
	Mayer's test	-

Key: (+) = Present in low concentration, (-) = Absent.

Table 2: Intraperitoneal LD50 of the ethanolic root extract of Guiera senegalensis in albino rats.

Groups (n=5)	Dose	Dose diff. (DD)	Dead(D)	Mean dead (MD)	Dose × Mean diff.
1	100	-	0	-	-
2	200	100	1	0.5	50
3	400	200	0	0.5	100
4	800	400	2	1.0	400
5	1600	800	2	2.0	1600
6	3200	1600	4	3.0	4800
7	6400	3200	5	4.5	14400

LD50= Least dose that killed all animals in a group – $\frac{(DD \times MD)}{No.\ animals/group}$

LD50= 6400 - $\frac{21350}{5}$

LD50 = 6400 – 4270

LD50 = 2130 mg/kg.

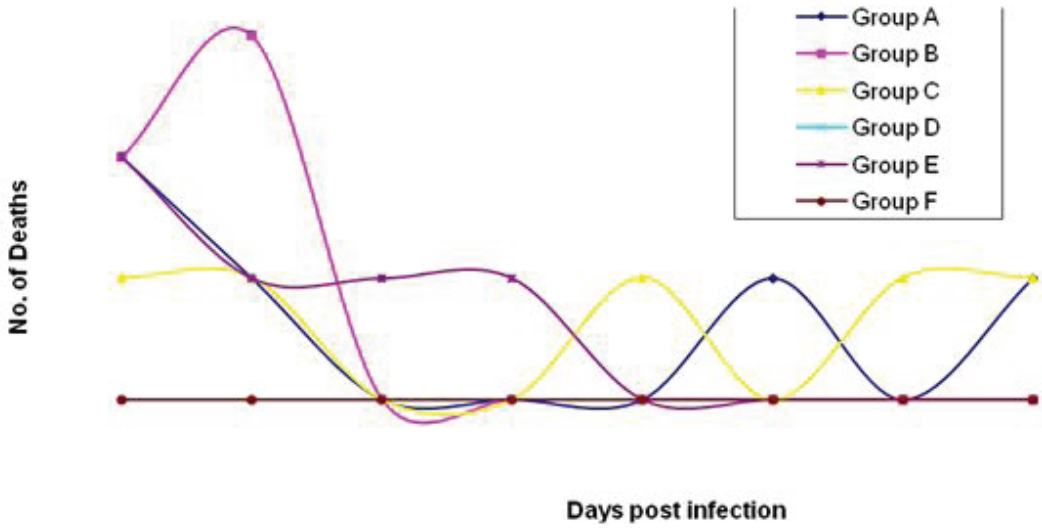


Figure 1: Mortality pattern of rats during the experiment

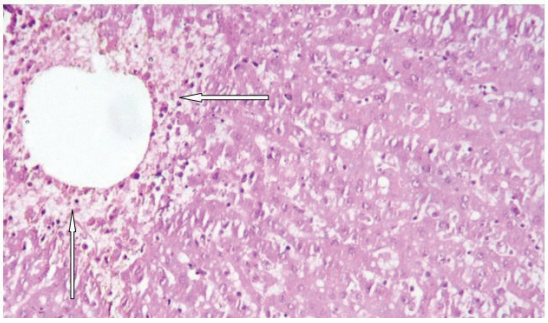


Figure 2: Photomicrograph of a liver showing marked centrilobular necrosis (arrows) following experimental infection with *T. brucei*. (Group E). H&E, X100.

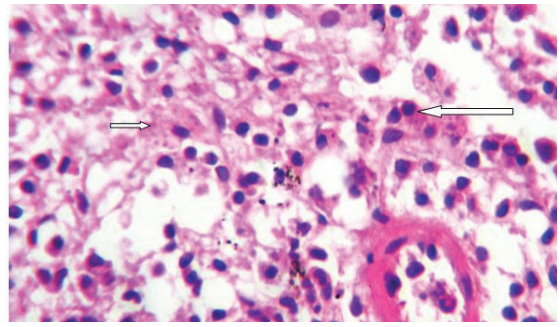


Figure 4: Photomicrograph of a lung showing interstitial thickening by oedema fluid (short arrow) and lymphocytic infiltration (long arrow) following experimental infection by *T. brucei*. (Group E). H&E, X400.

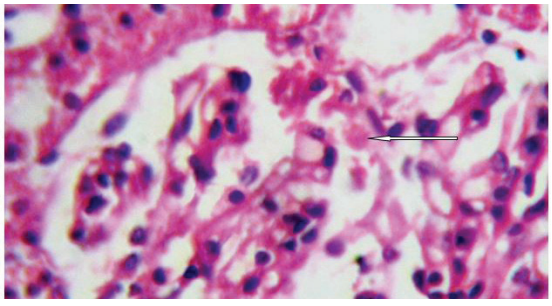


Figure 3. Photomicrograph of a kidney showing deposits of hyaline materials in the capsular space (arrow) with hypocellularity of the glomerulus following experimental infection with *T. brucei* and treated with ethanolic leaf extract of Guiera senegalensis. (Group C). H&E, X400.

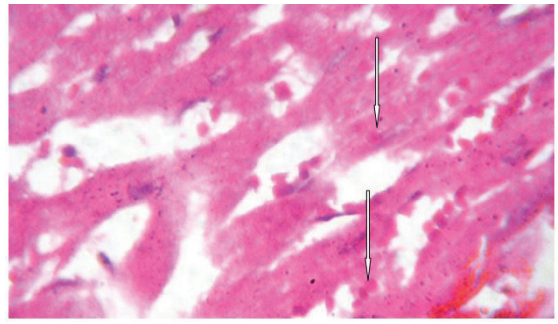


Figure 5: Photomicrograph of a myocardium showing multifocal areas of haemorrhages (arrows) following experimental infection with *T. brucei* and treated with ethanolic leaf extract of Guiera senegalensis. (Group B). H&E, X100.

characterized by multiple areas of mild to moderate lymphocyte depopulation from the white pulp in groups A, B, C and E. Group D did not show any visible lesion. Congestion, emphysema, and interstitial thickening generally characterized lung lesions by oedema fluid. The reactions appeared to be mild in groups A, B, and C, while it was marked in E, which showed interstitial thickening that, occurred mostly due to lymphocytic infiltration (consolidation) (Figure 4), in addition to the oedema fluid. There were no visible histopathology in group D. Heart showed moderate congestion and haemorrhage in group B, with a moderate myocardial necrosis and lymphocytic infiltration (non-suppurative myocarditis) (Figure 5).

Discussion

This study reports on the effects of ethanolic leaf extract of Guiera senegalensis on *Trypanosoma brucei* brucei induced pathology in albino rats. Phytochemical metabolites found in this study are similar to those previously reported (Onwuliri et al., 2009; Shettima et al., 2012), with minor variations. Alkaloids and terpenes earlier reported by Onwuliri et al. (2009), were not detected in the present study, while Onwuliri et al. (2009) did not report flavonoids, ketoses and reducing sugars detected in the present study. However, Shettima et al. (2012) reported all these metabolites.

The intraperitoneal LD50 of 2130 mg/kg reported for this plant extract indicate a plant with slight or no toxicity. Clarke and Clarke (1977) reported that any substance with an intraperitoneal LD50 of above 1000 mg/kg should be regarded as safe. This might have been responsible for the lack of clinical signs observed during the acute toxicity testing, even at the highest dosage tested. The safety of this plant has long been established, as previous workers (Akuodor et al., 2013; Bako et al., 2014], did not report mortality or toxic signs that could be attributed to the plant, when administered even up to 5000 mg/kg orally. Contrarily, Traore et al. (2014) in Burkina Faso, reported an intraperitoneal LD50 of 273 mg/kg in mice, using the extract of the leaves. The geographical sites of plant's collection as well as method of preparation of the plant material are likely factors determining uneven distribution of the secondary metabolites, which in turn determine the toxicity or safety of such material.

The clinical signs observed in this study are consistent with the findings of Anene (2006), which are typical signs in animal trypanosomosis. Morbidity period was longer in group C (treated with 600 mg/kg), compared to groups A and B treated with lower dosages of the extract and the infected untreated (Group E). The prolonged survival of the rats in group C indicate that, the extract contain metabolites that could have acted on the parasite and produced a dose-dependent effects.

On the study of effect of extract on Trypanasoma brucei brucei induced pathology, the degenerative changes and tubular cell necrosis observed in the kidneys of all infected groups and the marked centrilobular hepatocellular necrosis observed in group E are consistent with previous histopathological changes associated with trypanosomiasis (Itard et al., 1989; Takeet et al. 2009). Centrilobular necrosis was however absent in the extract treated groups (A, B, and C), suggesting amelioration of pathological lesions. Surprisingly, lesions in the kidney were more severe in groups (B and C) treated with higher dosages of the extract, than in group A treated with 100 mg/kg and the infected untreated (group E). Secondary metabolites such as flavonoids are known to be free radical scavengers, super antioxidants and potent water-soluble vitamins, thus preventing oxidative damage and having strong anti-cancer activity (Salah et al. 1995) Both flavonoids and saponins are known to possess potent anti-inflammatory effects (Igile et al. 1995)

Glomerular atrophy and hypocellularity with hyaline deposits in the capsular space were observed in groups B and C. Similarly, the mild to moderate depopulation of lymphocytes in the spleen is associated with trypanosome infection and may be related to the specific protective anti-parasite antibody response

(Radwanska et al., 2008). The inability of most of these lesions to abate in the extract treated groups may mean that metabolites capable of counteracting these effects may be absent or present in low concentration.

The lesions (congestion, oedema and emphysema) observed in the lungs are consistent with previous report (Takeet and Fagbemi, 2009). However, nonsuppurative pneumonia observed in group E (infected untreated), was absent in both the extract and Diminazene aceturate treated groups, suggesting an improved extract activity, probably due to saponins and flavonoids. In addition, nonsuppurative myocarditis observed in this experiment is in agreement with the report of Morrison et al. (1981). However, the haemorrhage observed was not previously documented.

Conclusion

The extract at the dosages tested appear to be relatively non-toxic. Similarly, the extract ameliorated some pathological changes in a dose-dependent pattern, while no definite pattern was noticed regarding some lesions.

References

Adamu S, Barde N, Abenga JN, Useh NM, Ibrahim NDG, Esievo KAN, 2009. Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the clinical implication in pigs. Journal of Cell and Animal Biology, 3(2), 015-020.

Adeiza W, Patrick ML, Myburg DP, 2010. Human trypanosomiasis in southern Africa. South African Medical Journal, 51,453-457.

Adeyemi OS, Akanji MA, Oguntoye S, 2009. Ethanolic leaf extract of Psidium guajava: Phytochemical and trypanocidal activity in rats infected with *Trypanosoma brucei* brucei. Journal of Medicinal Plants Research, 3(5), 420-423.

Akpavie SO, 2004. General Veterinary Pathology (Notes for undergraduates) 1st ed. pp. 9-10.

Onwuliri F, Ndako J, Olabode AO, Echeonwu GON, Onwuliri EA, Nwankiti PP, Salisu G, Ohaeri MC, Amona GE, 2009. Phytochemical screening and antibacterial activity of Guiera senegalensis leaf extracts. International Journal Trop Agric Food Sys, 3(2).

Akuodor, GC, Essien AD, David-Oku E, Chilaka KC, Akpan JL, Ezeokpo B, Ezeonwumelu JO C, 2013. Gastroprotective effect of the aqueous leaf extract of Guiera senegalensis in Albino rats. Asian Pac J Trop Med. (2013), 771-775. doi: 10.1016/S1995-7645(13)60136-4

Aliu YO, Nwude N, 1982. Veterinary Pharmacology and Toxicology Experiments. 1st ed. ABU Press, Zaria, Nigeria.

Anene BM, Ezeokonkwo RC, Mmesirionye TI, Tettey JNA, Brock JM, Barrett MP, De Koning HP, 2006. A Diminazene-resistant strain of *Trypanosoma brucei* brucei isolated from a Dog is cross-resistant to Pentamidine in experimentally infected albino rats. Parasitology, 132(01), 127-133.

Aniagu SO, Binda LG, Nwinyi FC, Orisadipe A, 250 Amos S, Wambebe C, Gamaniel K, 2005. Anti-diarrhoeal and ulcer-protective effects of the aqueous root extract of Guiera senegalensis in rodents. Journal of Ethnopharmacology, 97(3), 549-554

Asuzu IU, Chineme CN, 1990. Effects of Morinda lucida leaf extract on *Trypanosoma brucei* infection in mice. Journal of Ethnopharmacology, 30(3), 307-313.

Bako HY, Ibrahim M, Mohammad JS, Zubairu M, Bulus T, 2014. Toxicity studies of aqueous, methanolic and hexane leaf extracts of Guiera senegalensis in rats. International Journal of Science and Engineering Research, (10), 1338-1347.

Bosisio E, Mascetti D, Verotta L, Zanni F, Mazza P, Talbot M, 1997. Guiera senegalensis J. F. Gmelin (Combretaceae): Biological activities and chemical investigation. Phytomedicine, 3(4), 339-348. doi: 10.1016/S0944-7113(97)80007-4.

C. I. O. M. S, 1985. International guiding principles for biomedical research involving animals, Developed by Council for International Organization of Medical Sciences in collaboration with World Health Organization (WHO), 1211 Geneva, 27, Switzerland.

Clarke ECG, Clarke ML, 1977. Veterinary Toxicology:

Poisonous plants. Cassel and Collier, Macmillan Publisheers, London.pp 268-277.

Doua F, Boa Yapo F, 1993. Human trypanosomosis in the Ivory Coast: Therapy and problems. Acta Tropica, 54(3-4), 163-168.

Herbert WJ, Lumsden WHR, 1976. *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitaemia. Experimental Parasitology, 40, 427-431.

Ibrahim MA, Mohammed A, Isah MB, Aliyu AB, 2014. Anti-trypanosomal activity of African medicinal plants: A review update. Journal of Ethnopharmacology, 154, 26-54.

Igbokwe IO, Buratai LB, Ubah UL, Amomnde A, Igbokwe NA, 2009. Serum and hepatic lipid levels in rats infected with *T. brucei*.Comparative Clinical Pathology, 18,191-195.

Igile GO, 1995. Phytochemical and biological studies on some constituents of Vernonia amygdalina (Compositae) leaves. PhD Thesis, Department of Biochemistry, University of Ibadan, Nigeria.

Itard J, 1989.African animal trypanosomosis. In: Manual of Tropical Veterinary Parasitology. C. A. B. International. pp. 176-290.

Kuzoe F, 1993. Current situation of African trypanosomiasis. Acta Tropica, 54,153-162.

Morrison WI, Max M, Sayer PD, Preston JM, 1981. The pathogenesis of experimental induced *T. brucei* infection in dog tissue and organ damage. American Journal of Pathology, 102:168.

Salah W, Miller N, Pagauga G, Tybury G, Bolwell E, Rice E, Evans C, 1995. Polyphenolic
flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants. Arch.
Biochem, 2, 239-346.

Radwanska M, Guirnalda P, De Trez C, 2008. Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. PLoS Pathog 2008; 4:e 1000078.

Samdi SM, Abenga JN, Attahir A, Haruna MK, Wayo BM, Fajinmi AO, Sumayin HM, Usman AO, Hussaina JZ, Muhammad H, Yarnap JE, Ovbagbedia RP, Abdullahi RA, 2010. Impact of Trypanosomosis on food security in Nigeria-A review. International Journal of Animal and Veterinary Advances, 2(2), 47-50.

Samdi SM, Fajinmi AO, Kalejaye JO, Wayo B, Haruna MK, Yamap JE, Mshelia WP, Usman AO, Hamra SM, Jijitar A, Ogunwole R, Ovbagbedia RP, Bizi R, 2011. Prevalence of Trypanosomosis in cattle slaughtered in Kaduna central abattoir. Asian Journal of Animal Science, 5, 162-165.

Shettima AY, Karumi Y, Tijjani MA, Sodipo OA, Idris M, 2012. Phytochemical and anti-diarrhoeal properties of ethyl acetate root extract of Guiera Senegalensis J. F. Gmelin via oral route. Journal of Chemical and Pharmaceutical Research, 4(10), 4604-4612.

Silva O, Ferreira E, Pato MF, Gomes E, 1997. Guiera senegalensis Plant: In vitro susceptibility studies on Neisseria gonorrhea. International Journal of Pharmacology, (35), 323.

Sofowora EA, 2006.Medicinal plants and traditional use in Africa. Spectrum Books Ltd, Ibadan.

Takeet MI, Fagbemi BO. Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with Trypanosoma congolense. Science World Journal, (2):29-36.

Traore A, Ouedraogo S, Kabore A, Tamboura HH, Guissou IP, 2014. The acute toxicity in mice and the in vitro anthelminthic effects on Haemonchus contortus of the extracts from three plants (Cassia sieberiana, Guiera senegalensis and Sapium grahamii) used in traditional medicine in Burkina Faso. Annals of Biological Research, 5, 41-46.

Ukpai OM, Nwabuko OP, 2014. Effects on haematological parameters and pathology of internal organs of *Trypanosoma brucei* brucei infected albino rats. Nigerian Journal of Biotechnology,
27,8-13.

World Health Organization, 1992. Quality control methods for medicinal plants. World Health Organization, Geneva; 1992.

RESPONSE OF FINISHING BROILER CHICKENS TO SUPPLEMENTAL NEEM (AZADIRACHTA INDICA) AND GARLIC (ALLIUM SATIVUM) ON OOCYST COUNT, BACTERIA COUNT AND GUT MORPHOLOGY.

Sobayo R A[1], Muhammad S B[1], Sogunle O M, Adegbenjo A A[1], Adejola A Y[2,3], Oso A O[1], Adeyemi O A[3]

[1]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria
[2]Federal College of Agric. Moor Plantation Ibadan. 3Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria

Abstract

An eight weeks feeding trial was conducted to investigate the effects of feeding diets containing Neem Leaf Meal (NLM), Garlic Meal (GM) and their combinations (NLM +GM) on oocyst count, bacteria count and gut morphology of finishing broiler chickens. A total of 180 day-old Cobb broiler chickens were divided into twelve groups of fifteen chicks with three replicate of five chicks each. The diet contained NLM, GM and NLM + GM at four levels of inclusion (0mg/kg, 500mg/kg, 1000mg/kg and 1500mg/kg). The experiment was arranged in a 3 × 4 factorial layout in a completely randomized design (CRD). Additives did not significantly ($P>0.05$) affect oocyst and bacteria count. There was significant ($P<0.05$) decrease in oocyst and bacteria count of birds fed 1000mg/kg levels of inclusion than those of 0mg/kg levels of inclusion. Significant ($P<0.05$) reduction in oocyst and bacteria count was achieved in birds fed NLM at 1500mg/kg levels of inclusion and GM at 1000mg/kg levels of inclusion. There was significant ($P<0.05$) increase in duodenum villi height, apical width and lamina propria depth of birds fed NLM + GM compared to that of NLM. Jejunal villi height in birds fed GM (686.67µm) and NLM + GM (686.42µm) was statistically similar but superior ($P<0.05$) to that obtained in NLM (622.67µm). Birds fed diets containing NLM (103.42µm) affected ($P<0.05$) jejunum apical width compared to GM (72.67µm). Ileum villi height and basal width of birds fed NLM + GM was significantly ($P<0.05$) improved than those of NLM. However, ileum apical width was influenced in birds fed NLM + GM (61.33µm) but statistically similar to that of NLM (60.75µm) compared to GM (55.17µm). Gut morphology parameters were significantly ($P<0.05$) improved by all the levels of inclusion but more at 1500mg/kg inclusion levels compared to 0mg/kg which was lowered. Significant ($P<0.05$) improvement on gut morphology parameters were recorded by added additives at various inclusion levels except duodenum villi height which was not affected.

It was evident that additives employed most especially NLM and GM at 1500mg/kg and 1000mg/kg reduced oocyst and bacteria count and NLM + GM at 1500mg/kg improved gut morphology.

Keywords: Day-old broiler, neem, garlic, broilers, oocyst count, bacteria count, gut morphology.

LES EFFETS DE LA SUPPLEMENTATION EN MARGOUSIER (AZADIRACHTA INDICA) ET AIL SUR LE NOMBRE D'OEUFS, LE NOMBRE DE BACTERIES ET LA MORPHOLOGIE DE L'INTESTIN DES POULETS DE CHAIR EN STADE DE FINITION

Résumé

Un essai alimentaire de huit semaines a été réalisé dans le but d'étudier les effets des régimes alimentaires contenant de la farine de feuilles de margousier (NLM), la farine d'ail (GM) et leur association (NLM + GM) sur le nombre d'oocystes, le nombre de bactéries et la morphologie de l'intestin des poulets de chair en stade de finition. Au total, 180 poulets de chair Cobb âgés d'un jour ont été répartis en douze groupes de quinze poussins avec trois répétitions de cinq poussins chacun. Le régime alimentaire contenait la NLM, la GM, et l'association NLM + GM à quatre niveaux d'inclusion (0mg / kg ; 500 mg / kg ; 1000mg /

*Corresponding author email: rasobayo@gmail.com

kg ; et 1500mg / kg). L'expérience a prévu un arrangement factoriel 3 × 4 selon un dispositif complètement aléatoire (CRD). Les additifs n'ont pas affecté de manière significative (P> 0,05) le nombre d'oocystes et de bactéries. On a noté une diminution significative (P <0,05) des oocystes et des bactéries chez les oiseaux nourris aux niveaux d'inclusion de 1000mg / kg par rapport à ceux des niveaux d'inclusion de 0mg / kg. Une réduction significative (P <0,05) du nombre d'oocystes et de bactéries a été enregistrée chez les oiseaux nourris avec la NLM à des niveaux d'inclusion de1500mg / kg et la GM à des niveaux d'inclusion de1000mg / kg. L'on a observé une augmentation significative (P <0,05) de la hauteur des villosités du duodénum, la largeur apicale et la profondeur de la lamina propria des oiseaux nourris avec l'association NLM + GM par rapport à ceux recevant la NLM. La hauteur des villosités du jéjunum chez les oiseaux nourris avec la GM (686.67µm) et l'association NLM + GM (686.42µm) était statistiquement similaire, mais supérieure (P <0,05) à celle notée chez ceux recevant la NLM (622.67µm). Chez les oiseaux soumis aux régimes alimentaires contenant la NLM (103.42µm) le régime a affecté (P <0,05) la largeur apicale du jéjunum par rapport à ceux soumis à la GM (72.67µm). La hauteur des villosités et la largeur de base de l'iléon des oiseaux recevant l'association MNL + GM ont été significativement (P <0,05) améliorées par rapport à ceux nourris avec la NLM. Cependant, la largeur apicale de l'iléon a été influencée chez les oiseaux nourris avec l'association MNL + GM (61.33µm), mais elle était statistiquement similaire à celle des oiseaux sous la NLM (60.75µm) par rapport à ceux sous la GM (55.17µm). Les paramètres de la morphologie de l'intestin étaient significativement (P <0,05) améliorés à tous les niveaux d'inclusion, mais ils étaient meilleurs aux niveaux d'inclusion de 1500mg / kg par rapport au niveau 0mg / kg qui les a fait baisser. Une amélioration significative (P <0,05) des paramètres de la morphologie de l'intestin a été enregistrée à la suite des additifs ajoutés à différents niveaux d'inclusion, à l'exception de la hauteur des villosités du duodénum qui n'a pas été affectée.

L'on a constaté que les additifs utilisés, surtout la NLM et la GM aux niveaux d'inclusion de 1500mg / kg et 1000mg / kg ont réduit le nombre d'oocystes et de bactéries et que l'association NLM + GM aux niveaux d'inclusion de 1500mg / kg a amélioré la morphologie des intestins.

Mots-clés : poussin de chair d'un jour, margousier, ail, poulet de chair, nombre d'oocystes, nombre de bactéries, morphologie de l'intestin.

Introduction

Antibiotic feed additives as growth promoters have long been supplemented to poultry feed to stabilize the intestinal microbial flora and improve the general performances and prevent some specific intestinal pathology (Waldroup et al., 1985). However, antibiotics used as growth promoters in animal feeds have been banned recently due to potential development of antibiotic resistant in human (Patterson et al., 2003). Alternatives to antibiotics as growth stimulators are numerous, amounting to finding an adequate non-pharmacological products from the group prebiotics, probiotics, organic acids and other essential oils, medicinal plants or parts of plants such as thyme, basil, oregano and others reported by Simon (2005). Phytogenics additives, as natural substances, have been recognised as a very promising alternative as they meet the requirements of consumers in terms of food safety and solve the problem of bacteria resistance that occurs as a result of using antibiotics as growth promoters (Silva Cardoso et al., 2012).Some positive changes in digestive enzymes, gut morphology and immune system were noticed in birds given phytogenic-supplemented feed (Windischet al., 2008).Awad et al.(2008) demonstrated that an improvement of gut morphology is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area and nutrient transport systems. There are evidences in the literature of the beneficial effects of using leaf meal from different sources in poultry production (Iheukwumere et al., 2008). D'Mello et al. (1987) observed that leaf meals do not only serve as protein source but also provide some necessary vitamins, minerals and oxy-carotenoids which cause yellow colour of broiler skin, shank and egg yolk. Neem (Azadirachta indica) has been identified among the tropical plants that have been used as livestock feed resource (Ogbuewu et al., 2009). Garlic (Allium sativum) has been used

as a spice and a native medicine since long ago (Rivlin, 2001). Its medicinal effects on intestinal disorders, abdominal distension and treatment of intestinal worms and respiratory infections have been observed (Amagase et al., 2001).

Materials and Methods

Experimental site

This experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State. The area lies on latitude 7010'N and longitude 30 2'E. It is 76m above sea level and located in the tropical rain forest vegetation zone with an average temperature of 34.70C and relative humidity of 82% (Google Earth, 2012).

Processing of test ingredients

Allium sativum (garlic) powder was prepared by cutting garlic bulbs into small pieces, followed by sun-drying for 14 days (≤ 90% DM) and pulverised using laboratory mill (1mm sieve) while Azadirachta indica(neem) leaves were removed from the stalk and air dried under a shed (29±20C) until they are crispy to touch, while still retaining their greenish colouration, milled using a laboratory mill (1mm).

Experimental birds and dietary treatment

A total of 180 unsexed day old Cobb broilers were used for the experiment. They were divided into twelve treatment groups of fifteen birds. Each treatment group was replicated thrice with five birds per replicate in a 3×4 factorial experimental design. Brooding of birds was done for three weeks using charcoal and bulbs as source of heat. The birds were fed ad libitum and managed intensively throughout the duration of the experiment. Twelve experimental diets were formulated with the inclusion of neem, garlic and their combinations for finishing broiler. Diet 1 - 4 contains inclusion of NLM at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, Diet 5 - 8 contains inclusion of GM at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, Diet 9 - 12 contains inclusion of NLM + GM each at 0 mg/kg, 250 mg/kg, 500mg/kg and 750mg/kg.

Feacal bacteria counts

Fresh feaces of the finishing broiler chickens were taken to Veterinary Microbiology laboratory, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta for bacterial count using the procedures of estimation of bacterial total count according to (Hedge, 2002).

Feacal egg worm counts

Fresh feaces of the finishing broiler chickens were taken to the Veterinary Microbiology laboratory, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta to check for microbial count using (Urquhart et al., 2008).

Gut morphology

At eight weeks of the experiment, three birds per replicate were randomly selected for gut morphology. The gastro intestinal tract were removed and separated into intestinal segments: duodenum, jejunum and ileum. The duodenum was taken distally from the gizzard to the end of the pancreatic loop to meckels diverticulum, the ileum distally from meckels diverticulum to the ileo-caecal junction. A sample portion (0.5 cm in length) was taken at the medium part of each of the three intestinal segments (duodenum, jejunum and ileum) for histological observation. Intestinal villi with their crypts were individually separated under a dissecting microscope as described by Goodlad et al., (1991). The length and width of the villi were measured according to the procedure described by Hampson (1986) using an optical microscope and a camera.

Statistical analysis

Data collected were subjected to 3×4 factorial arrangement in a completely randomized design. Significant ($p<0.05$) differences among treatment means were determined using Duncan Multiple Range Test (Duncan 1955) as contained in Statistical Analysis Software (SAS 2000) package.

Table 1: Gross Composition % of experimental Diets (4-8 weeks)

	GLM				NLM				GLM+NLM			
Ingredients	0	500	1000	1500	0	500	1000	1500	0	500	1000	1500
Maize	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Wheat offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy meal	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
PKC	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
GNC	9.30	9.30	9.30	9.30	9.30	9.30	9.30	9.30	9.30	9.30	9.30	9.30
Fish meal (72%CP)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
GM	-	+	++	+++	-	-	-	-	-	-	-	-
NLM	-	-	-	-	-	+	++	+++	-	-	-	-
GM+NLM	-	-	-	-	-	-	-	-	-	+	++	+++
TOTAL	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Analysis (%)												
ME(kcal/kg)	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33	2875.33
Crude Protein	20.24	20.24	20.24	20.24	20.24	20.24	20.24	20.24	20.24	20.24	20.24	20.24
Crude Fibre	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03
Fat	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27	4.27
Calcium	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Phosphorus	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82

**Vitamin, mineral, and additive contributions per kilogram of feed: vitamin A, 9,000 IU; vitamin D3, 2,500 IU; vitamin E, 20 IU; vitamin K3, 2.5 mg; vitamin B1, 1.5 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; pantothenic acid, 1.2 mg; biotin, 0.06 mg; folic acid, 0.8 mg; niacin, 25 mg; vitamin B12, 12 µg; I, 2 mg; Se, 0.25 mg; Cu, 20 mg; Mn, 160 mg; Zn, 100 mg; Fe, 100 mg.*

Table 2: Main effects of NLM, GM, NLM + GM and levels of inclusion on oocyst and bacteria count of finishing broiler chickens (0-8weeks)

	Additives				Levels of Inclusion (mg/kg)				
Parameters	**NLM**	**GM**	**NLM + GM**	**SEM**	**0**	**500**	**1000**	**1500**	**SEM**
Oocyst count (opg) $\times 10^4$	75.00	63.25	66.00	3.402	173.33^{a}	84.33^{b}	22.00^{d}	33.33^{c}	3.443
Bacteria count (cfu/ml)	63.78	81.75	78.00	2.981	82.33^{a}	66.04^{b}	54.11^{b}	54.89^{b}	3.928

^{abcd} means on the same row having different superscript were significantly different (P<0.05)
OPG (Oocyst per gramme), CFU/ml (Colony Forming Unit per ml) GM: Garlic Meal NLM: Neem Leaf Meal

Table 3: Interaction effect of NLM, GM, NLM + GM and levels of inclusion on oocyst and bacteria count of finishing broiler chickens (0- 8weeks)

Additives													
NLM	**GM**				**NLM + GM**								
Levels of Inclusion	**0**	**500**	**1000**	**1500**	**0**	**500**	**1000**	**1500**	**0**	**500**	**1000**	**1500**	**SEM**
Oocyst count (opg) $\times 10^4$	200.00^{a}	100.00^{e}	0.00^{h}	0.00^{h}	140.00^{c}	120.00^{d}	0.00^{h}	67.00^{f}	180.00^{b}	33.00^{g}	66.00^{f}	33.00^{g}	3.901
Bacteria count (cfu/ml)	80.00^{a}	74.00^{ab}	55.00^{cde}	46.00^{e}	82.00^{a}	59.00^{cd}	46.00^{e}	66.00^{bc}	85.00^{a}	65.00^{bc}	61.00^{cd}	53.00^{de}	11.272

^{abcdef} means on the same row having different superscript were significantly different (P<0.05)
OPG (Oocyst per gramme), CFU/ml (Colony Forming Unit per ml) GM: Garlic Meal NLM: Neem Leaf Meal

Table 4: Main effect of NLM, GM, NLM + GM and Levels of inclusion on Gut Morphology of broiler chickens at finisher phase (0-8 weeks)

	Additives				Levels of inclusion (mg/kg)				
Parameters	**NLM**	**GM**	**NLM + GM**	**SEM**	**0**	**500**	**1000**	**1500**	**SEM**
Duodenum									
VH (µm)	1002.50b	1072.50ab	1115.33a	30.777	985.11b	1070.00ab	1098.67a	1100.00a	35.538
AW (µm)	105.00b	113.83b	129.50a	3.407	91.33c	126.67a	131.78a	114.67b	3.934
BW (µm)	196.00	200.92	213.83	5.824	200.78	205.11	205.11	205.11	6.725
LPD (µm)	226.33b	250.17a	269.75a	7.213	228.44b	260.00a	249.44ab	257.11a	8.329
Jejunum									
VH (µm)	622.67b	686.67a	686.42a	19.434	586.11c	650.22ab	725.11a	699.56b	22.451
AW (µm)	103.42a	72.67c	87.67b	2.610	68.44d	77.89c	93.56b	111.78a	3.014
BW (µm)	120.75b	126.33b	141.33a	3.747	116.11b	131.67a	137.44a	132.67a	4.327
LPD (µm)	155.00	163.83	168.83	4.724	138.44c	160.00b	171.78ab	180.00a	5.455
Ileum									
VH (µm)	374.75b	391.50ab	418.25a	11.444	329.33b	413.00a	425.33a	411.67a	13.214
AW (µm)	60.75a	55.17b	61.33a	1.722	54.11b	60.22a	60.22a	61.78a	1.988
BW (µm)	69.33b	71.83b	81.50a	2.126	69.78	76.44	74.00	76.67	2.455
LPD (µm)	95.25	94.75	100.83	2.824	87.11b	100.00a	98.89a	101.78a	3.261

[abcd] means on the same row having different superscript were significantly different (P<0.05)
VH: (Villi Height) AW: (Apical Width) BW: (Basal Width) LPD: (Lamina Propria Depth). µm: (micrometer)
GM: Garlic Meal NLM: Neem Leaf Meal

Table 5: Interaction effect of NLM, GM, NLM + GM and Levels of inclusion on Gut Morphology of broiler chickens at finisher phase (0-8 weeks)

Additives	**NLM**				**GM**				**NLM + GM**				
Levels (mg/kg)	**0**	**500**	**1000**	**1500**	**0**	**500**	**1000**	**1500**	**0**	**500**	**1000**	**1500**	**SEM**
Duodenum													
VH (µm)	980.00	1000.00	1010.00	1020.00	990.00	1080.00	1120.00	1100.00	985.00	1130.00	1166.00	1180.00	61.554
AW (µm)	60.00^{d}	100.00^{bc}	140.00^{a}	120.00^{ab}	80.00^{c}	140.00^{a}	135.00^{a}	100.00^{bc}	134.00^{a}	140.00^{a}	120.00^{ab}	124.00^{b}	6.814
BW (µm)	194.00^{ab}	210.00^{ab}	200.00^{ab}	180.00^{b}	198.00^{ab}	190.00^{ab}	205^{ab}	210.00^{ab}	210.00^{ab}	215.00^{ab}	210.00^{ab}	220.00^{a}	11.649
LPD (µm)	210.00^{d}	250.00^{abcd}	220.00^{cd}	225.00^{bcd}	215.00^{cd}	260.00^{abc}	255.00^{abcd}	270.00^{ab}	260.00^{abc}	270.00^{ab}	273.00^{ab}	276.00^{a}	14.426
Jejunum													
VH (µm)	585.00^{b}	630.00^{ab}	675.00^{ab}	600.00^{b}	576.00^{b}	675.00^{ab}	750.00^{a}	745.00^{a}	597.00^{b}	645.00^{ab}	750.00^{a}	753.00^{a}	38.886
AW (µm)	75.00^{bc}	83.00^{b}	120.00^{a}	135.00^{a}	60.00^{c}	75.00^{bc}	75.00^{bc}	80.00^{b}	70.00^{bc}	75.00^{bc}	85.00^{b}	120.00^{a}	5.220
BW (µm)	113.00^{cd}	120.00^{bcd}	130.00^{abcd}	120.00^{bcd}	110.00^{d}	132.00^{abcd}	135.00^{abc}	128.00^{abcd}	125.00^{abcd}	143.00^{ab}	147.00^{a}	150.00^{a}	7.494
LPD (µm)	140.00^{de}	150.00^{bcde}	170.00^{abcd}	160.00^{bcde}	130.00^{e}	150.00^{bcde}	175.00^{abc}	200.00^{a}	145.00^{cde}	180.00^{ab}	170.00^{abcd}	180.00^{ab}	9.448
Ileum													
VH (µm)	320.00^{e}	443.00^{ab}	353.00^{cde}	383.00^{bcde}	330.00^{de}	383.00^{bcde}	453.00^{ab}	400.00^{abcd}	338.00^{de}	413.00^{abc}	470.00^{a}	452.00^{ab}	22.887
AW (µm)	55.00^{bcd}	65.00^{ab}	67.00^{a}	55.00^{bcd}	45.00^{d}	67.00^{a}	60.00^{abc}	60.00^{abc}	50.00^{cd}	60.00^{abc}	65.00^{ab}	70.00^{a}	3.444
BW (µm)	60.00^{c}	75.00^{ab}	70.00^{abc}	72.00^{abc}	65.00^{bc}	74.00^{ab}	70.00^{abc}	78.00^{ab}	84.00^{a}	80.00^{a}	82.00^{a}	80.00^{a}	2.251
LPD (µm)	88.00^{bc}	100.00^{abc}	93.00^{abc}	100.00^{abc}	85.00^{c}	90.00^{bc}	98.00^{abc}	105.00^{ab}	88.00^{bc}	110.00^{a}	105.00^{ab}	100.00^{abc}	5.648

^{abcde} *means on the same row having different superscript were significantly different (P<0.05)*
VH: (Villi Height) AW: (Apical Width) BW: (Basal Width) LPD: (Lamina Propria Depth). µm: (micrometer)
I Levels: Inclusion levels GM: Garlic Meal NLM: Neem Leaf Meal

Results

Data on main effects of additives and levels of inclusion on oocyst and bacteria count of finishing broiler chickens is presented in Table 2. There was no significant (P>0.05) effect on oocyst and bacteria count of chickens fed additives. Levels of inclusion revealed significant (P<0.05) effect on oocyst and bacteria count of chickens. Oocyst count (173.33opg) at 0mg/kg levels of inclusion was elevated (P<0.05) compared to that obtained at 1000 levels ofmg/kg inclusion which was depressed (33.33opg). Increased bacteria count (82.33cfu/ml) was observed at 0mg/kg levels of inclusion compared to other inclusion levels which were lowered.

The interaction effects of additives and levels of inclusion on oocyst and bacteria count of finishing broiler chickens is presented in Table 3. Oocyst and bacteria count of birds was significantly (P<0.05) affected. Highest oocyst count (200opg) was recorded at 0mg/kg inclusion levels of NLM compared to those of 1000 and 1500mg/kg inclusion of NLM and 1000mg/kg inclusion of GM which was decreased. Increased values (80cfu/ml, 82cfu/ml and 85cfu/ml) in bacteria count were recorded at 0mg/kg inclusion of added additives compared to those obtained at 1500mg/kg inclusion of NLM and 1000mg/kg inclusion of GM (46cfu/ml)

Table 4 shows the effect of additives and levels of inclusion on gut morphology of finishing broiler chickens. There was significant (P<0.05) effect on duodenum, jejunum and ileum parts of finishing broiler chickens birds fed additives. Duodenum villi height, apical width and lamina propria depth of birds fed NLM + GM was significantly (P<0.05) influenced compared to that of NLM. Increased (P<0.05) Jejunal villi height was recorded in chickens fed GM (686.67µm) but was statistically similar to that of NLM + GM(686.42µm) compared to NLM (622.67µm). Jejunal apical width in birds fed NLM was significantly (P<0.05) affected (103.42µm) compared to that obtained in GM (72.67µm). Improved (P<0.05) jejunum basal width was observed in birds fed NLM + GM compared to those of NLM and GM. Ilea villi height and basal width in birds fed NLM + GM were significantly (P<0.05) influenced compared to NLM. Ilea apical width in birds fed NLM + GM was better (P<0.05) than that of GM but within comparable range to that of NLM. Levels of inclusion revealed significant (P<0.05) effect on Duodenum, Jejunum and Ileum parts of finishing broiler chickens. Duodenal villi height (1100.00µm) of birds at 1500mg/kg inclusion levels was significantly (P<0.05) influenced compared to 0mg/kg inclusion levels (985.11µm). Duodenal apical width (131.78µm) of birds at 1000mg/kg inclusion levels was better than that of 0mg/kg levels of inclusion (91.33µm). Also improved (P<0.05) duodenum lamina propria depth (260.00µm) of birds at 500ppm inclusion levels was observed compared to 0mg/kg inclusion levels (228.44µm). Jejunal villi height and basal width at 1000mg/kg were significantly (P<0.05) increased compared to that obtained at 0mg/kg inclusion levels. Jejunal apical width(111.78µm) at 1500mg/kg inclusion levels was significantly (P<0.05) affected compared to 0mg/kg inclusion levels (68.44µm). However,jejunal lamina propria depth of birds at 1500mg/kg inclusion levels was increased (180.00µm) compared to 0mg/kg inclusion levels (138.44µm). Depressed (P<0.05) Ilea villi height of birds was recorded at 0mg/kg inclusion levels (329.33µm) compared to 1000mg/kg inclusion levels (425.33µm). Ilea apical width and lamina propria depth of birds at 1500mg/kg was improved compared to those of 0ppm inclusion levels.

Data on interaction effect of finishing broiler chickens fed additives and levels of inclusion is presented in Table 5. There was significant (P<0.05) effect on duodenum, jejunum and ileum parts of bird. Duodenal apical width in birds fed 1000mg/kg inclusion of NLM, GM inclusion at 500mg/kg and NLM + GM inclusion at 500mg/kg was significantly (P<0.05) superior (140µm) compared to that of 0mg/kg inclusion of NLM (60µm). Duodenal basal width of birds at 1500mg/kg inclusion of NLM + GM was significantly (P<0.05) better (220µm) than that of 1500mg/kg inclusion of NLM (180µm). Also, duodenal lamina propria

depth at 1500mg/kg inclusion of NLM + GM was significantly (P<0.05) increased (276µm) compared to that obtained at 0ppm inclusion of NLM (210µm). Jejunal villi height, apical width and basal width at 1500mg/kg inclusion of NLM + GM was significantly (P<0.05) influenced compared to those of 0mg/kg inclusion of GM. Jejunal lamina propria depth of birds at 1500mg/kg inclusion of GM was significantly (P<0.05) better (200µm) compared to that of 0mg/kg inclusion of GM (130µm). Ilea villi height of birds at 1000mg/kg inclusion of NLM + GM was significantly (P<0.05) improved (470µm) compared to 0mg/kg inclusion of NLM (320µm). Decreased (P<0.05) Ilea apical width of birds was recorded at 0mg/kg inclusion of GM (45µm) compared to that of 1500mg/kg inclusion of NLM + GM(70µm). However, ilea basal widths of birds at inclusion levels of 1000mg/kg of NLM + GM were significantly (P<0.05) improved than that of 0ppm inclusion of NLM. Ilea lamina propria depth of birds at 500mg/kg inclusion of NLM + GM was significantly (P<0.05) improved (110µm) compared to that of 0mg/kg inclusion of GM (85µm).

Discussion

The non-significant differences in birds fed additives on oocyst and bacteria count was against the findings of Allen et al. (1998) who reported that herbs reduces oocyst yield. Reduction in oocysts and bacteria count recorded at 1000ppm levels of inclusion was in line with report of Murtaza et al. (2002) who fed neem fruit to broilers and observed reduction in coccidialoocysts count per gram of feaces and also Mushtaq and Durrani (2007) observed significant reduction in coccidial oocysts count by supplementing aqueous extract of Withanias omnifera in broilers production. A reduced amount of oocyst and bacteria count recorded in birds fed NLM at 1500ppm and GM at 1000ppm levels of inclusion was in line with Guo et al., (2004a, 2004b, and 2004c) who demonstrated that plants and their extracts could improve the growth performance, reduce the populations of coliforms and/or C. perfringens, and enhance both cellular and humoral immune responses of chickens. The results were also supported with Misra et al. (1993), who reported that herbal anti-coccidial is effective to reduce faecal oocyst output.

Improvement on gut morphology of birds achieved by NLM+GM could be due to greater efficiency in the utilization of feed, resulting in enhanced development of intestinal morphology of poultry (Apajalahti et al., 2004). These finding was in line with report of Montagne (2003) who studied that villus: crypt ratio is an indicator of the likely digestive capacity of small intestine. The morphological changes observed in all the parts of the intestine could also be as a result of increase in villus height which suggests an increase in surface area capable of greater absorption of available nutrients (Awad et al., 2008).

The increase in villi height, apical width, basal width and laminapropria depth achieved by levels of inclusion in duodenum, jejunum and ileum was supported by Iji et al. (2001) who registered deeper jejunal and ileal crypts when feeding 0.1 to 0.5% of MOS to broilers (Loddi, 2003).

Interactive effects of additives and levels of inclusion on intestinal morphology of broiler chickens was in line with Loddi et al. (2004) who stated that the described changes in intestinal morphology can explain the observed beneficial effects on productivity. On the other hand, a compensatory mechanism reflected in Ileum villi height has been reported by (Choi et al., 1994). Similarly, the increase in villi height of the intestine across the dietary treatment was in line with Samanya and Yamauchi (2002) where they reported that villus height in duodenum and ileum significantly increased in 28-day old chicks fed Bacillus subtilis. The observed increase in jejunum and ileum villus height at 56 days of age compared to non-supplemented basal diet confirmed the fact that the additives in the diets increase villus height.

Conclusion

It was evident that additives employed most especially NLM and GM at 1500mg/kg and 1000mg/kg reduced oocyst and bacteria count and NLM + GM at 1500mg/kg improved gut morphology.

References

Allen, P. C. Danforth, H. D. Augustine. P. C. 1998. Dietary modulation of avian coccidiosis. International Journal of Parasitology. 28:1131-1140.

Amagase, H. Petesch, B.L. Matsuura, H. Kasuga, S. Itakura Y. 2001. Recentadvances on the nutritional effects associated with the use of garlic as a supplement: Intake ofgarlic and its bioactive components. Journal of Nutrition. 131 (Supplement. 3):955–962.

Apajalahti, J. Kettunen, A. Graham H.2004. Characteristics of the gastrointestinalmicrobial communities, with special reference to the chicken World's Poultry Science Journal ,60:223–232.

Awad, W.A. Ghareeb, K. A. Bohm J. 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and Oligosaccharides. International Journal of Molecular Science. 9: 2205-2216.

Choi, K.H. Namkung H. Paik, I.K. 1994.Effects of dietary fructooligosaccharides on the suppresion of intestinal colonization of Salmonella typhimuriumin broiler chickens.KoreanJournal ofAnimal Science 36, 271-284.

D'mello, J.P.E. Acamovic, T. Walker A.G. 1987. Evaluation of leucaena leaf meal for broiler growth and pigmentation.Tropical. Agriculture (Trinidad), 64: 33-35

Gibson, G.R. Fuller R. 2000.Aspects of in vitro and in vivo Research ApproachesDirected toward Identifying Probiotics andPrebiotics for Human Use. Journal of Animal Nutrition.2000; 130:391 – 5.

Google Earth. 2012.http://www.google.earth.

Duncan, D.B. 1955.Multiple range and multiple F tests.Biometerics11:1-42.

Guo, F.C. Kwakkel, R.P.Williams, B.A. 2004A.Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on growth performance of broilers. British Poultry Science 45: 684-694.

Guo, F.C. Kwakkel, R.P.Williams, B.A. 2004b. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the caecal microbial ecosystem in broiler chickens.Poultry Science 83: 175-182.

Guo, F.C. Kwakkel, R.P. Williams, B.A. 2004C.Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of Eimeria tenella-infected chickens. Poultry Science 83: 1124-1132.

Hedges, A.J 2002.Estimating the precision of serial dilutions and viable bacterial counts. International Journal of Food Microbiology76 (3): 207–14.

Iheukwumere,F.C.NdubuisiE.C.MaziE.A.Onyekwere, M.U. 2008. Performance, Nutrient utilization and organ characteristics of Broilers fed cassava. Leaf meal (ManihotesculentaCrantz). Pakistan. Journal.Nutrition. 7:13-16.

Iji, P.A. Saki, A.A. Tivey, D.R. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Animal. Feed Science.Technology.v.89, p.175-188, 2001.

Loddi, M.M.Moraes, V.M.B. Nakaghi, L.S.O. Tuca, F.M. Hannas, M.I. Ariki, J. 2004. Mannan oligosaccharide, organic acids on performance and intestinal morphometric characteristic of broilers chickens.Abstract of Alltechs20th Annual Symposium, Nicholasville, Kentucky, USA, Pp 45.

Loddi, M.M. 2003.Probióticos, prebióticos e acidificanteorgânicoemdietasparafrangos de corte [tese].Jaboticabal: FCAV, UNESP.

Misra, S.K. 1993.Zycox – A new generation herbal anti-coccidial system for higher profitability. Pashudhan 8:4-5.

Montagne, L. 2003.A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals".Animal FeedScience andTechnology, 108: 95-117.

Murtaza, A. T. Pasha, T. N. Zulfiqar. A. 2002. Comparative Effeicacy of Salincomycin and Neem Fruit (Azadirechta indica) as feed Additive and Anticoccidial in broilers. International.Journal of Poultry.Science. 1(4): 91-93.

Musthaq,M. Durrani,F.R. 2007. Heamatological HypolipidemicHypoglycemicAnticoccidail HepatoprotectiveImmunostimulating and Growth promoting role of withaniaSomnifera in broiler production. 2007. MSc (Hons) Thesis. NWFP Agricultural University, Peshawar.

Ogbuewu, I.P. Okoli, I.C. Iloeje, M.U. 2009. Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed neemAzadirachtaindica) leaf meal based diets. Iranian Journal of Reproductive Medicine 7(1): 23-28.

Patterson, J.A. Burkholder, K.M. 2003.Application of Prebiotics and Probiotics in Poultry Production. Poultry.Science.; 82: 627 – 31

Rivlin, R.S. 2001.Historical perspective on the use of garlic.Journal of Nutrition.131 (3S), 951S–954S.

Samanya, M. Yamauchi, K. 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comparative Biochemistry and Physiology Part A, 133: 95-104.

Silva Cardoso, V. Ribeiro De Lima C.A. Freire De Lima, M.E. Gomes Dorneles, L.E. Gracas Miranda Danelli, M. 2012.Piperine as a Phytogenic Additive in Broiler Chickens. Pesquisa Agropecuária Brasileira, 47, 4.

Simon, O. 2005. Micro-organisms as feed additives-probiotics, Advanced Pork Production., 39: 161-167.

Urquhart,G.M. Armar, J. Duncan, J.L. Jennings, F.W. 1997. Veterinary Parasitology (2nd Edition), The faculty of Veterinary Medicine, The University of Glassgow, Scotland. Book Power Publisher. Pp. 276-277.

Windisch, W. Schedle, K. Plitzner, C. Kroismayr. A. 2008.Useof phytogenic products as feed additives for swine and poultry. Journal of Animal Science.86:140- 148.

Waldroup, P.W. Spencer, G.K. Waibeal, P.E. Quarles, C.L. Grant, R.J. 1985.The use of bambemycins (flavomycin) and halofuginone (stenorol) in diets for growing turkey. Poultry science 64.1296-1301.

ARTERIAL BLOOD PRESSURE CHANGES IN ACUTE *T. BRUCEI* INFECTION OF DOGS

Ajibola Eyitayo Solomon[1], Oyewale Johnson Oluwayemisi[2], Oke Bankole Olusiji[2]
[1]College of Veterinary Medicine, Federal University of Agriculture, Abeokuta
[2]Faculty of Veterinary Medicine, University of Ibadan, Ibadan

Summary

The aim of this study is to find out the usefulness of serial arterial blood pressure measurements in predicting severity and outcome of acute *Trypanosoma brucei* infection in dogs.

Twenty adult dogs of mixed sexes and aged between 2 and 5 years were used for this study. The dogs were of good cardiac health and were divided into two groups of infected and uninfected. The infected groups were inoculated with 1×106 of PBS lafenwa strain of *T. brucei* intraperitoneally. The systolic arterial pressure (SAP), Diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were recorded before and serially at days eight, sixteen and twenty four post infection.

The SAP of control dogs (119.62±3.77) was not different from the SAP on days 8(118.83±0.75), 16(120.33 ± 4.04) and 24(123.14±6.84). The DAP of the infected dogs on day8 (52.87±7.90), day 16(56.00±8.54) and day24 (48.42±7.97) is not different from the control value of 52.87±7.90. The MAP of the infected dogs on day 8 (77.00±1.41), day 16(72.66±2.88) and day24 (73.51± 3.53) is not significantly different from the control value of 76.00±6.80.

A value of two standard deviation below the control mean was defined as hypotension and therefore a SAP<112mmhg, DAP <36mmhg, and MAP <62mmhg was regarded as cutoff point for hypotension in the breed of dog studied. All the control and the infected dogs were therefore not considered hypotensive.

The result of the present study has shown that acute T .brucei infection does not alter the blood pressure of infected dogs and arterial blood pressure does not predict severity and outcome of the infection.

MODIFICATIONS IMPORTANTES DE LA PRESSION ARTERIELLE CHEZ LES CHIENS SOUFFRANT D'INFECTION AIGUE A *T. BRUCEI*

Résumé

Le but de cette étude est de déterminer l'utilité de mesures sériées de la pression sanguine artérielle pour prédire la gravité et l'issue de l'infection aiguë à *Trypanosoma brucei* chez les chiens.

Vingt chiens adultes des deux sexes et âgés de 2 à 5 ans ont été utilisés dans cette étude. Les chiens avaient une bonne santé cardiaque, et ils ont été répartis en deux groupes de chiens infectés et chiens non infectés. Les groupes infectés ont été inoculés avec 1 x 106 de PBS souche lafenwa de *T. brucei* par voie intrapéritonéale. La pression artérielle systolique (PAS), la pression artérielle diastolique (PAD) et la pression artérielle moyenne (PAM) ont été enregistrées avant l'infection et en série à huit, seize et vingt-quatre jours post-infection.

La PAS des chiens témoins (119.62 ± 3.77) n'était pas différente de la PAS des chiens infectés aux jours 8 (118,83 ± 0,75) ; 16 (120,33 ± 4,04) ; et 24 (123,14 ± 6,84). La PAD des chiens infectés aux jours 8 (52,87 ± 7.90) ; 16 (56,00 ± 8,54) ; et 24 (48,42 ± 7,97) n'était pas différente de la valeur 52,87 ± 7,90 notée chez les chiens témoins. La PAM des chiens infectés aux jours 8 (77,00 ± 1,41) ; 16 (72,66 ± 2,88) ; et 24 (73,51 ± 3,53) n'était pas significativement différente de la valeur témoin de 76,00 ± 6,80.

Une valeur de deux écart-type en dessous de la moyenne de contrôle a été définie comme étant une hypotension, par conséquent une PAS<112mmhg, PAD <36mmhg et PAM <62mmhg a été considérée comme la valeur limite de l'hypotension dans la race de chiens étudiés. Les chiens témoins et les chiens infectés n'ont donc pas été tous considérés comme hypotendus.

Corresponding author email esajibola@yahoo.com

Le résultat de la présente étude a montré que l'infection aiguë à T .brucei ne modifie pas la pression artérielle des chiens infectés, et que la pression artérielle ne prédit pas la gravité et l'issue de l'infection.

Introduction

Acute *T. brucei* infection in dogs is a protozoan sepsis that produce cardiac and multiple organ damage (Morrison et al., 1981). This infection is of immense public health importance and could cause sleeping sickness in man. Of late, there have been reports of human mortality resulting from the cardiac form of human African trypanosomiasis.

On the whole not much is known about the pathophysiology of the heart in both human and canine trypanosomiasis. Previous attempts at using cardiac biomarkers, and electrocardiographic biomarkers at understanding the pathogenesis of the disease have been reported in both humans and dogs (Ndungu et al., 1991; Ajibola & Oyewale, 2014; Blum et al., 2007; Blum et al., 2008).

So far there is no report detailing the usefulness of serial monitoring of blood pressure in predicting severity and outcome of either canine African trypanosomiasis or human African trypanosomiasis. This is very important because Jacobson et al (2000) have reported the occurrence of hypotension in canine babesiosis, another protozoan infection with massive systemic inflammatory response syndrome.

In the therapeutic management of canine trypanosomiasis, diminazene aceturate, a drug known to cause hypotension have been widely used(Joubert et al.,2003) This is of immense concern as complication could arise from using diamidines for therapeutic intervention in both human African trypanosomiasis and canine African trypanosomiasis. This agent has been reported to cause hypotension and could therefore aggravate the existing systemic hypotension caused by the infection.

This study is therefore aimed at understanding the serial changes in SAP, DAP, and MAP of *T. brucei* infected dogs and to investigate whether blood pressure changes is associated with disease severity and outcome.

Materials and Methods

Institutional approval for this study was granted by the animal ethics committee of the college of veterinary medicine, Federal university of Agriculture, Abeokuta. Twenty adult dogs sourced randomly from local breeders in Abeokuta were used for this study. The dogs were of mixed sexes, ranging in weight between 6 and 15 kg. They were acclimatized to laboratory conditions for two weeks and screened against trypanosome species and other hemoparasites. Each of the dogs was marked on the head with an indelible marker for ease of identification. The dogs received all routine vaccinations against common dog diseases like canine distemper, leptospirosis, hepatitis, parvo virus enteritis and were all certified free from heart worm. Cardiac health of the dogs were assessed by auscultation and ECG examination. Animals with cardiac abnormalities were excluded from the study. They were all kept be in a netted room of 100m2 measuring 10mx10m and fed twice daily on cooked local foods and fish diet.

The twenty dogs was divided into two major groups of infected and uninfected control. The infected group comprised of 15 dogs and the uninfected control group has 5 dogs.

Each infected dogs received 1ml of 1x106 of the parasite in phosphate buffered saline (PBS) intra-peritoneally, while the uninfected groups received 1ml of normal saline through the same route.

The blood pressure of the dogs was determined non-invasively by oscillometric technique using Omron health care blood pressure monitor (model; HEM-7322-E) and neonatal cuffs. Each dog was positioned in right lateral recumbency and the cuff placed on the left distal forelimb immediately proximal to the carpus, over the radial artery. In order to avoid error of measurements, cuff bladder width

was always about 40% of the circumference of the limb at the level of application (Bodey et al., 1994). Five to six recordings of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) was recorded over a five minute period. The readings was then be averaged. The blood pressure monitor device measures SAP, DAP, and pulse rate. The MAP is usually calculated using the formula; 1/3SAP + 2/3 DAP. The mean arterial pressure (MAP), systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) was recorded from each of the dogs before and serially on days 8, 16 and 24 after the experimental infection.

Statistics

All data were expressed as mean ± standard deviation. Differences within parameters during the course of the disease were evaluated by repeated measure ANOVA. When $P< 0.05$, the infection is adjudged to have affected the parameter significantly. The association between any two parameters were evaluated using regression analysis. The strength of association, i.e. correlation coefficient (r) was considered significant when $p <0.05$. The control mean±2SD was used to determine the cutoff point for hypotension. Hypotension was defined as falling below 2SD of control values. All statistical tests were done using SPSS version 16.

Results

Table 1 revealed that the systolic arterial pressure, diastolic arterial pressure and the mean aortic pressure of the uninfected dogs is not significantly different from the values obtained in days 8, 16, and 24 post infection. The SAP of control dogs (119.62±3.77) was not different from the SAP on days 8(118.83±0.75), 16(120.33 ± 4.04) and 24(123.14±6.84). The DAP of the infected dogs on day8 (52.87±7.90), day 16(56.00±8.54) and day24 (48.42±7.97) is not different from the control value of 52.87±7.90. The MAP of the infected dogs on day 8 (77.00±1.41), day 16(72.66±2.88) and day24 (73.51± 3.53) is not significantly different from the control value of 76.00±6.80.

There was a significant difference between the mean heart rate value of infected and non-infected dogs ($p<0.001$). The mean heart rate of the dogs before infection (77.80± 18.18) was significantly lower than the mean heart rate obtained from infected dogs on day 8(160.75±12.94) and day 16(142.25±22.14) but it is not significantly different from the values obtained at 24 days post infection. At 24 days post infection, the mean heart rate of the dogs (98.50±3.53) was not significantly different from the pre-infection control and at 16 days post infection.

Values are expressed as mean±SD, values along the same row with different superscript are significantly different ($p<0.05$). SAP; systolic arterial pressure, DAP; diastolic arterial pressure, MAP; mean aortic pressure, HR; Heart rate 8dpi; eight day post infection, 16dpi; sixteen day post infection, 24dpi; twenty four day post infection

Table 2 showed that the correlation coefficient obtained between MAP, SAP and DAP and HR, were not significant ($p<0.05$).

Discussion

The result of this study have further confirmed that blood pressure is highly breed dependent and that since standardization of

Table 1: Hemodynamic indices of T.Brucei infected dogs

Parameters	Control	8dpi	16dpi	24dpi	p-values
SAP(mmHg)	119.62±3.77	118.83±0.75	120.33±4.04	123.14±6.84	NS
DAP(mmHg)	52.87±7.90	56.33±1.63	56.00±8.54	48.42±7.97	NS
MAP(mmHg)	76.00±6.80	77.00±1.41	72.66±2.88	73.51±3.53	NS
HR(b/min)	$77.80±18.18^{a}$	$160.75±12^{ab}$	$142.25±22.14^{ab}$	$98.50±3.53^{a}$	<0.05

Table 2: Coefficient of correlation between the heart rate and arterial blood pressure indices

Correlation	Regression coefficient(r)	P values
SAP and HR	0.04	NS
DAP and HR	0.39	NS
MAP and HR	0.34	NS

SAP; systolic arterial pressure (mmHg), DAP; diastolic arterial pressure (mmHg), MAP; mean aortic pressure (mmHg), HR; Heart rate (beats/minute), NS; not significant

value is yet to be achieved (Bodey and Michell, 1996), efforts should be made to establish breed specific values, if rational clinical judgment is to be made.

The range of SAP of both infected and control dogs are within the normal reference values for dogs (Wadell, 2000). The range of DAP of control and infected dogs was however below the reported reference range for most other healthy breeds previously studied (Bodey & Michell 1996).

The highly breed specific nature of blood pressure makes it difficult to define pathological derangement in blood pressure. In this work, hypertension is defined as SAP, DAP and MAP above 128mmhg, 60mmhg and 90mmhg respectively. SAP, DAP and MAP below 112mmhg 36mmhg and 62mmhg is defined as hypotension. Systolic and diastolic blood pressure ranging from 112/37mmhg to 127/69mmhg and MAP ranging from 62-90mmhg is regarded as normotensive in the breed studied. On the basis of clinical definition, and the cutoff points established in this study, all the control and infected dogs were not hypotensive.

The definition of hypotension in this study is not as conservative as that of Littman & Drobatz (1993) (SAP<80mmhg, MAP <60mmhg), but definitely more conservative than the report of Jacobson et al (2000) who defined hypotension as SBP<118mmHg, DAP <65mmhg and MAP <76mmhg. Many authors have also defined hypotension as MAP, SAP, and DAP of below 60mmhg, 90mmhg and 50mmhg respectively.

The cutoff point obtained for systolic and diastolic and MAP hypertension is lower and less conservative than values obtained in other breeds of dogs. Jacobson et al., (2000) defined cutoff point for hypertension in dogs as SAP<184mmHg, DAP<113mmHg and MAP<138mmHg. Hypertension of MAP, SAP and DAP have been defined by many authors as values above 145mmhg, 163mmhg and 90mmhg respectively.

In contrast to findings of low blood pressure in human African trypanosomiasis (WHO, 1998), T.brucei infection in this current work did not affect SAP, DAP, and MAP.

In this study, SAP, DAP and MAP did not reflect disease severity and these indices in control dogs were not different in infected dogs. Even in canine babesiosis, where complications involving other organs could cause systemic hypotension, there was no difference in the arterial blood pressure of survivors and non survivors (Jacobson et al., 2000). As in canine babesiosis, blood pressure is therefore not a predictor of mortality in acute T.brucei infection of dogs (Jacobson et al., 2000).

The micro-vascular disturbances which have been reported in T.brucei infection could cause lesion centrally in the autonomic nervous system (Morrison et al., 1983), and peripherally in the chemoreceptor and baroreceptor areas of the carotid sinus and aortic arch resulting in poor baroreceptor sensitivity and autonomic imbalances. This could thus lead to a derangement in the homeostatic regulation of blood pressure in the infected dogs as blood pressure changes would no longer be detected by the baroreceptors. In the presence of hypotension as would be expected in this study, baroreceptor response would be to increase heart rate. Although increased heart rate was reported in the infected dogs, their blood pressure were not altered. Similarly to the observation in canine babesiosis, a correlation between heart rate and blood pressure was not seen (Jacobson et al., 2000), it therefore means that the heart rate response is not reflexly mediated.

Renal mechanisms involved in homeostatic blood pressure regulation could also be involved in stabilizing the blood pressure during acute T.brucei infection of dogs. In the

presence of low blood pressure as could occur in sepsis, renin is released by the kidney. This would then stimulate the renin angiotensin aldosterone system (RAAS) pathway leading to the production of angiotensin and aldosterone, each of which could cause vasoconstriction and increased sodium retention respectively (Guyton & Hall 2000; De Laforcade & Rozansky, 2001). Either way, the outcome is an elevated blood pressure which counteracted the hypotension.

The mechanism of hypotension development in canine babesiosis is related to sepsis and have been linked to vasodilation, increased vascular permeability and myocardial depression (Hardie, 1995). Although inflammatory mediators like (TNF)-α, nitric oxide have been implicated in HAT (McLean & Odiit, 2001; Daulouede et al., 2001), the extent of their involvement in T.brucei infection was not investigated in the present study. The severity of the systemic inflammatory response elicited by T.brucei and the breed susceptibility of the dogs used for the study are factors that could play a role in the development of hypotension in the infected dogs.

Blood pressure monitoring, although, of importance in routine clinical work ups, may not be of immediate importance in predicting severity and outcome of acute canine African trypanosomiasis and human African trypanosomiasis as result of several cardiovascular and renal compensatory mechanism operational during this phase of the disease.

References

Ajibola, E.S & Oyewale, J.O. 2014. Relationship between some serum electrolytes and electrocardiographic indices of Trypanosoma brucei infected dogs. Sokoto. J.Vet. Sci 14(1): 36-44

Bodey, A.R.; Young, L.E.; Bartram, B.H.; Diamond, M.J. & Michell, A.R. A comparison of the direct and indirect (Oscillometric) measurements of arterial blood pressure in anaesthetized dogs using tail and limb cuffs. Res. Vet. Sci, 57: 265-269, 1994.

Bodey, A.R & Michell, A.R. Epidemiological study of blood pressure in domestic dogs. J. Small Anim. Pract, 37: 116-125, 1996.

Blum, J.A.; Zellweger, M.J.; Burri, C. & Hatz, C. Cardiac involvement in African and American trypanosomiasis. Lancet Infect. Dis, 8(10):631-641, 2008.

Blum, J.A.; Burri, C.; Hatz, C., Kazumba, I.; Mangoni, P & Zellweger, M.J. Sleeping Hearts: the role of the heart in sleeping sickness (human African trypanosomiasis). Trop. Med. Int. Health, 12; 1422□1432, 2007

Daulouede, S,; Bouteille, B.; Moynet, D.; De Baetselier, P.; Courtois, P.; Lemesre, J.L.; Buguet, A,;Cespuglio, R & Vincendeau, P. Human macrophages Tumour Necrosis Factor(TNF)-□ Production 183:988-991, 2001

De Laforcade, A.M & Rozanski, E.A. Central venous pressure and Arterial blood pressure measurements. Vet. Clin. North Am. Small Anim Pract, 31(6): 1163-1174, 2001.

Guyton, A.C. & Hall, J.E. Pocket Companion to Textbook of Medical Physiology.10th ed . Philadelphia, WB Saunders Company, 2005, 213-233

Hardie, E.M. Life threatening bacterial infection. Compendium on continuing education for the practicing veterinarian, 17:763-778, 1995.

Jacobson, L.S.; Lobetti, R.G & Vaughan-Scott, T. Blood pressure changes in dogs with Babesiosis. J.S.Afr.Vet. Assoc, 71: 14□20, 2000

Joubert, K.E.; Kettner, F; Lobetti, R.G & Miller, M.D. The effects of Diminazene aceturate in clinically healthy adult dogs. J. S. Afr.Vet. Assoc, 74(3):69-71, 2003

Littman, M.P & Drobatz, K.J. Hypertensive and hypotensive disorders. In: Ettinger, S.J & Feldman, E.C(ed). Textbook of veterinary internal medicine. Philadelphia, W.B Saunders, 1995. Pp 93-100.

MacLean, L.; Odiit, M & Sternberg, J.M. Nitric oxide and cytokime synthesis in Human African Trypanosomiasis. J. Infect Dis, 184: 1086-1090, 2001

Morrison, W.I.; Max, M., Sayer, P.D & Preston, J.M. The Pathogenesis of experimentally induced *Trypanosoma brucei* infection in the dog: Tissue and Organ Damage. Am. J. Pathol, 102:168□181, 1981

Ndungu, J.M.; McEwan, N.A.; Jennings, F.W & Murray, M. Cardiac damage in dogs infected with T.brucei: Clinical and Electrocardiographic features. J. Small. Anim. Pract. 32(11): 579□584, 1991

Waddel, L.S. Direct blood pressure monitoring. Clin. Tech. Small Anim. Pract, 15(3):111-118, 2000.

World health Organisation (WHO). Control and surveillance of African trypanosomiasis: report of a WHO expert committee, 1998

INTAKE AND DIGESTIBILITY OF NUTRIENTS BY WAD GOATS FED DIETS CONTAINING VARYING LEVELS OF CASHEW NUT SHELL LIQUID INCLUSION

Olagoke K O[1]*, Aderinboye R Y[1], Onwuka C F I[1] and Ojo V O A[2]
[1]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria
[2]Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria

Abstract

A complete concentrate (14%CP) was formulated and cashew nut shell liquid (CNSL) was included at four levels (0, 5, 10 and 15ml/kg DM). The concentrates in combination with Panicum maximum (40% concentrate and 60% grass) was fed to WAD goats at 5% of their metabolic weight. 12 WAD goats weighing 8.5 ± 0.25kg were randomly allotted into the four dietary treatments, each treatment was replicated thrice. The experiment was carried out for 21 days and samples of faeces were collected during the last 7days to evaluate digestibility. Intake and digestibility of nutrients by WAD goats in this experiment were not significantly ($p>0.05$) affected by the dietary inclusion of CNSL up to 15ml/kg DM feed except CP digestibility which decreased ($p<0.05$) with increasing levels of CNSL inclusion. The inclusion of CNSL in the diets showed no adverse effect on the intake and digestibility of nutrients by WAD goats.

Keywords: CNSL, WAD goats, Intake, Digestibility.

CONSOMMATION ET DIGESTIBILITE DES NUTRIMENTS DES REGIMES CONTENANT DIFFERENTS NIVEAUX D'INCLUSION DU LIQUIDE DE COQUES DE NOIX DE CAJOU

Résumé

Un concentré complet (14% de CP) a été formulé, et du liquide de coques de noix de cajou (CNSL) a été inclus à quatre niveaux (0 ; 5 ; 10 ; et 15ml / kg DM). Les concentrés en combinaison avec Panicum maximum (40% de concentré et 60% d'herbe) ont été donnés aux chèvres WAD à 5% de leur poids métabolique. Douze (12) chèvres WAD pesant 8,5 ± 0,25 kg ont été réparties de manière aléatoire à quatre traitements alimentaires, chaque traitement étant répété trois fois. L'expérience a été réalisée pendant 21 jours et des échantillons de matières fécales ont été recueillis au cours des derniers sept jours afin d'évaluer la digestibilité. La consommation et la digestibilité des nutriments par les chèvres WAD dans cette expérience n'ont pas été significativement ($p> 0,05$) affectées par l'inclusion alimentaire du CNSL jusqu'à 15ml / kg DM d'aliments sauf la digestibilité de la PB qui a diminué ($p <0,05$) avec l'augmentation des niveaux d'inclusion du CNSL. L'inclusion du CNSL dans les régimes alimentaires n'a montré aucun effet néfaste sur la consommation et la digestibilité des nutriments par les chèvres WAD.

Mots-clés : CNSL, chèvres naines d'Afrique de l'Ouest, consommation, digestibilité.

*Corresponding author email: kenolag23@gmail.com

Introduction

Rapid increase in human population coupled with low protein intake constitutes is a major problem facing developing countries. Perhaps as a result of the increasing population and dismal productivity of livestock in developing countries like Nigeria, the demand for protein of animal origin has far exceeded the supplies. Animal protein is one of the most important components of human diet and its consumption varies from country to country (Okai et al., 2005).

Small ruminant production though have the potential to boost animal protein availability, requires adequate nutrition to enhance the productivity of these animals. Most feed resources i.e grasses, crop residues, byproducts, and processing waste; available for these groups of animals are characterized by nutrient digestibilities below 50% (Taiwo, 1995).

Various methods had earlier been use to manipulate the ruminal microbial ecosystem to improve the efficiency of converting feed to produce consumable products by humans. There are various methods of rumen manipulation that have been employed which include increasing feed intake, proportion of concentrate in the diet, feeding high-quality forages or dietary supplements of plant and marine oils, oilseeds or specific fatty acids and ionophores (Boadi et al., 2004).

This present study seeks to determine the effect of dietary inclusion of cashew nut shell liquid on nutrient intake and apparent total tract digestibility in West African dwarf goats.

Materials and Methods

Extraction of CNSL

The extraction of CNSL was carried out using a Soxhlet extractor and petroleum ether (laboratory reagent grade with boiling range of 40 – 60oC) as solvent. The procedure outlined by Edoga et al., (2006) was followed.

Preparation of Diets and experimental design

A complete concentrate feed was formulated to contain 14% crude protein and cashew nut shell liquid was included as a feed additive at four (4) levels of 0, 5ml, 10ml and 15ml /kg DM of the complete concentrate feed. Panicum maximum grass was daily harvested and chopped into small sizes. The grass was then allowed to wilt over the night and thereafter used as a basal diet. Animals were fed 5% of their metabolic weight (60%grass and 40%concentrate). 12 WAD goats weighing 8.5 ± 0.25kg were sourced and housed in individual pen for 7days for treatment with antibiotics and dewormer before been transferred into the metabolic cage for digestibility trial. The experimental animals were balanced for weight and randomly allocated to four dietary groups with 3 animals in each group, in a completely randomized design.

Digestibility studies

The digestibility experiment was carried out for a 21-days period. The animals were transferred into individual metabolic cages for 14days of adaptation and adjustment and the last seven (7) days served for the collection of samples. Daily feed intake was determined. Total faeces voided by the animals were collected during the last 7 days. Faeces voided were collected in the morning before feeding and watering. The faeces were weighed fresh, dried and bulked for each animal. A sub sample of faecal sample from each animal was dried in oven at 100-105°C for 48h for DM determination. Another sample was dried at 65 for 48-72 hfor determination of proximate composition." with "A sub sample of the faecal sample from each animal was dried in the oven at 100-105°C for 3-4h for DM determination. Another sub sample was dried in the oven at 65°C for 48-72h for determination of proximate composition.

Analytical procedure: All feed and faecal samples were analyzed for proximate components using AOAC (1990) method.

Statistical analysis: Data obtained were analyzed as a one-way ANOVA in a complete randomized design using the compare mean procedure of SPSS (IBM SPSS Statistics 20 (2013)). Significant differences among means

were compared using Duncan multiple range test (Steel and Torrie, 1980).

Results and Discussion

The proximate and fibre composition of the experimental diets is presented in Table 1. The ether extract of the experimental concentrates increased ($p<0.05$) with the increasing level of CNSL inclusion. This increase may be due to the effect of volatile oil reported to be present in most essential oils (Calsamiglia et al., 2007 and Benchaar and Greathead, 2011).

Intake of nutrients by WAD goats in this experiment were not significantly ($p>0.05$) affected by the dietary inclusion of CNSL up to 15ml/kg DM feed. Similar to this result are reports of many authors. For instance, Cortinho et al. (2014) investigated intake, digestibility of nutrients, milk production and composition in dairy cows on diets containing cashew nut shell liquid (CNSL) reported that feeding up to 7g/day of CNSL did not affect intake dairy cows. In another study by Benchaar et al. (2006), a product containing a mixture of EO (Crina Ruminants, Akzo Nobel Surface Chemistry Ltd) was fed at 2g/cow/day in the diet of lactating cows. They found no effect on dry matter and nutrient intake. Benchaar et al. (2007), Yang et al. (2007) and Tager and Krause (2011) also confirmed this result in their respective studies.

Apparent digestibility of crude protein (CP) was significantly different ($p<0.05$) among treatments, while those of DM, EE, Ash, OM, NDF and ADF were not (Table 3). Percentage CP digestibility was least in diet containing the highest CNSL inclusion recording 73.85% compared to the control. The result of apparent digestibility reported by Coutinho et al. (2014) in their experiment showed that CNSL inclusion had no effect on digestibility. Also, Shinkai et al. (2012) conducted two experiments and verified the occurrence of variability in the effect on DM digestibility when CNSL was included in the diet. On the other hand, lower digestibility values were observed by Baiden et al. (2007) when growing WAD goats were fed graded levels of cashew nut shell. The authors

Table 1: Physical and chemical composition of concentrate feeds and P. maximum

Ingredient of concentrate					
Particulars			**g/kg on DM basis**		
Rice Bran			710		
Wheat Offal			140		
Brewers Dried Grain			100		
Bone Meal			30		
Common Salt			20		
Chemical constituents of concentrate at different levels of CNSL inclusion and P. maximum					
Constituents (g/kg)	**0ml**	**5ml**	**10ml**	**15ml**	**P. maximum**
DM	863.3	865.0	866.3	868.0	477.5
CP	127.4	124.1	123.1	127.8	81.2
EE	63.3^{c}	68.3^{bc}	73.3^{b}	93.3^{a}	3.14
OM	783.3	781.7	779.7	776.8	485.9
NFC	338.7	318.0	283.2	246.9	196.6
NDF	263.9	281.2	282.0	281.9	585.9
ADF	410.7	392.9	424.8	491.2	391.8

^{abc} Means on the same row having different superscripts are significantly different ($P < 0.05$)
DM= Dry Matter, CP= Crude Protein, EE= Ether Extract, OM= Organic Matter, NFC= Non-fibre Carbohydrates, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre

Table 2: Nutrient intake of WAD goats fed diets containing varying levels of CNSL inclusion

	Levels of CNSL inclusions				
Parameters	**0ml**	**5ml**	**10ml**	**15ml**	**SEM**
Mean body weight (kg)	8.46	8.62	8.48	8.52	0.25
Dry matter Intake (g/day)	302.13	288.24	288.68	289.48	8.18
Crude protein Intake (g/day)	26.62	24.49	24.77	21.20	0.99
Ether extract Intake (g/day)	10.96	10.17	10.49	8.39	0.54
Ash Intake (g/day)	36.48	34.67	34.48	27.77	1.89
Organic matter Intake (g/day)	161.10	149.02	149.64	127.15	6.30
Neutral detergent fibre Intake (g/day)	162.37	158.87	159.37	150.85	0.38
Acid detergent fibre Intake (g/day)	119.34	112.92	114.16	102.07	3.32

[ab] Means on the same row having different superscripts are significantly different (P < 0.05)
0ml - control diets, 5ml – contains 5ml of CNSL/kg DM feed, 10ml - contains 10ml of CNSL/kg DM feed, 15ml - contains 15ml of CNSL/kg DM feed.

Table 3: Apparent digestibility of nutrients by WAD goats fed diets containing varying levels of CNSL inclusion

	Levels of CNSL inclusions				
Nutrients (%)	**0ml**	**5ml**	**10ml**	**15ml**	**SEM**
Dry matter (DM)	88.29	80.93	82.98	87.11	1.62
Crude protein (CP)	85.69a	83.85a	78.76ab	73.85b	1.72
Ether extract (EE)	81.69	63.98	73.11	75.49	3.34
Ash	78.02	66.75	68.19	73.59	3.08
Organic matter (OM)	80.17	66.51	71.29	75.73	2.73
Neutral detergent fibre (NDF)	42.28	39.12	41.27	37.95	0.98
Acid detergent fibre (ADF)	61.69	53.90	56.25	55.88	1.62

[ab] Means on the same row having different superscripts are significantly different (P < 0.05)
0ml - control diets, 5ml – contains 5ml of CNSL/kg DM feed, 10ml - contains 10ml of CNSL/kg DM feed, 15ml - contains 15ml of CNSL/kg DM feed.

reported that digestibility were lowered due to the increasing levels of cashew nut shell leading to higher fibre and ether extract content of the treatment diets and submitted that this could have inhibited digestibility. Though the CP digestibility values of this present study are higher compared to those obtained from previous studies mentioned earlier, a decreased trend in CP digestibility (85.69 to 73.83%) was similar to the report of Shinkai et al. (2012). The inclusion of CNSL in the diets showed no adverse effect on the intake and digestibility of nutrients by WAD goats.

References

Okai, D.B., Abora, P.K.B., Davis, T. and Martin, A. (2005). Nutrient composition, availability, current and potential uses of "Dusa": A cereal by-product obtained from "koko" (porridge) production. Journal of Science and Technology. 25:33-38.

Boadi, D.A., K. M. Wittenberg, S. L. Scott, D. Burton, K. Buckley, J. A. Small, and K. H. Ominski 2004. Effect of low and high forage diet on enteric and manure pack greenhouse gas emissions from a feedlot. Canadian Journal of Animal Science.

Edeoga H. O, Omobuna G. and L. C. Uche 2006.

Chemical composition of Hyotis suaveoleus and Ocimum gratissium hybrids from Nigeria. Africa Journal of Biotechnology. 5 (910): 892-895.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists International, 17th edition. 2: 2000.
Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw Hill Book Co., New York, NY, USA

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castilejos, L., Ferret, A. and Fandino, I. 2007. The Use of Essential Oils in Ruminants as Modifiers of Rumen Microbial Fermentation. Penn State Dairy Cattle Nutrition Workshop

Benchaar, C. and H. Greathead 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology. 166-167: 338-355.

Cortinho, D. A., Branco, A. F., Santos, G. T., Osmari, M. P., Teodoro, A. L. and T. G. Diaz. 2014. Intake, digestibility of nutrients, milk production and composition in dairy cows fed on diets containing cashew nut shell liquid. Acta Scientiarum. Animal Sciences, Maringá, 36 (3) 311-316.

Benchaar, C.; Petit, H. V.; Berthiaume, R.; Whyte, T. D.; Chouinard, P. Y. 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. Journal of Dairy Science, 89 (11) 4352-4364.

Benchaar, C., A.V. Chaves, G.R. Fraser, Y. Yang, KA. Beauchemin and T.A. McAllister, 2007. Effects of essential oils and their components on in vitro rumen microbial fermentation. Canadian Journal of Animal Science, 87: 413-419.

Yang, W. Z.; Benchaar, C.; Ametaj, B. N.; Chaves, A. V.; He, M. L.; Mcallister, T. A. 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. Journal of Dairy Science, 90 (12): 5671 - 5681.

Tager, L. R. and K. M. Krause, 2011. Effects of essential oils on rumen fermentation, milk production, and feeding behavior in lactating dairy cows. Journal of Dairy Science, 94:2455-2464.

Baiden, R.Y., Rhule, S.W.A., Otsyina, H.R., Sottie E.T. and Ameleke G. 2007. Performance of West African Dwarf sheep and goats fed varying levels of cassava pulp as a replacement for cassava peels. Livestock Research for Rural Development. 19 (3): 118-124.

Shinkai, T., Enishi, O., Mitsumori, M., Higuchi, K., Kobayashi, Y., Takenaka, A., Nagashima, K. and Mochizuki, M. 2012. Mitigation of methane production from cattle by feeding cashew nut shell liquid. Journal of Dairy Science, 95 (9): 5308-5316.

KOBS (KOBUS KOB ERXLEBER, 1777) ACTIVITIES AT SALT-LICK SPOTS IN KAINJI LAKE NATIONAL PARK, NIGERIA

Ogunjobi J A[1]* and Ayodele I A[2]
[1]Dept. of Biological Sciences, Ondo State University of Sci. and Tech., Okitipupa, Ondo State.
[2]Dept. of Wildlife and Ecotourism, University of Ibadan, Oyo Staate.

Abstract

Activities (foraging, ruminating, resting, salt-licking, walking, playing, agonistic, mating, vigilant and standing) of kobs (Kobus kob Erxleber, 1777) visiting two different sizes of salt-lick spots (Larger: 100.00m2 and Smaller: 15.10m2) located on (Latitude N09^{0}54'76", longitude E03^{0}57'17") and (Latitude N09^{0}54'33", longitude E03^{0}56'34"), respectively within Gilbert-child track, Kainji Lake National Park, Nigeria were investigated during dry season between January to April, 2012. It was observed that kobs visits to the larger salt-lick spot 158(58.70%) were more frequent compared to the smaller salt-lick spot 111(41.30%). The sizes of the salt-lick spots had significant effects on sex (χ^2 = 23.20, df = 1, P<0.01), age (χ^2 = 189.67, df = 5, P<0.01) and group size (r = 0.10, P < 0.01) of the visiting kobs. Highest percentage of the kobs (30.20%) was noticed ruminating at the salt-lick spots. Activities of the animal at the spots were not significantly affected (χ^2 = 43.85, df =9, P >0.01). Evidently, sizes of salt-lick spots influenced kobs composition and their visiting motive was beyond salt-licking.

Keywords: Kobs, salt-lick, animal activities, eco-nutrition.

LES ACTIVITES DES KOBS (KOBUS KOB ERXLEBER, 1777) AUX AFFLEUREMENTS SALINS DANS LE PARC NATIONAL DU LAC KAINJI AU NIGERIA

Résumé

Les activités (butinage, rumination, repos, léchage de sel, marche, jeu, agonistique, accouplement, vigilance, position debout) des kobs (Kobus kob Erxleber 1777) fréquentant deux affleurements salins de tailles différentes (grande dimension 100,00m2 et petite dimension15,10m2) situées respectivement aux latitude N09054'76" & longitude E03057'17" et latitude N09054'33" & longitude E03056'34", à l'intérieur du Gilbert child track et du parc national du lac Kainji au Nigeria, ont fait l'objet d'une étude pendant la saison sèche, entre janvier et avril 2012. L'on a constaté que les visites des kobs au grand affleurement salin 158 (58,70%) étaient plus fréquentes par rapport au petit affleurement 111 (41,30%). Les tailles des affleurements salins avaient des effets significatifs sur le sexe (χ^2 = 23,20, df = 1, P<0,01), l'âge (χ^2 = 189,67, df = 5, P<0,01) et la taille du groupe (r = 0,10 ; P <0,01) des kobs qui les fréquentaient. Le pourcentage le plus élevé des kobs (30,20%) a été remarqué pendant la rumination aux affleurements salins. Les activités des animaux aux affleurements n'ont pas été affectées de manière significative (χ^2 = 43,85, df =9, P >0,01). De toute évidence, les dimensions des affleurements salins ont influencé la composition des kobs, et les raisons de leurs visites allaient au-delà du simple léchage du sel.

Mots-clés : kobs, affleurements salins, activités animales, éco-nutrition.

*Corresponding author email: ja.ogunjobi@osustech.edu.ng; ogunjonson02@gmail.com

Introduction

Kainji Lake National Park (KLNP) since its creation in 1979 by the merger of Borgu and Zuguruma game reserves in the north central Nigeria remains a home for diverse of flora and fauna resources despites some anthropogenic challenges. One of such fauna resources enjoying protection in this park is kob (Kobus kob). Kob is one of the ungulates with reddish-brown coat cover (Stuart and Stuart, 1992) standing approximately 92cm at shoulder height with only the males carrying two horns (Smith, 1985). In Nigeria, KLNP remains one of the three nation's park with stable population of kob (Jayeola et al., 2012). According to Jayeola et al. (2012) a more acceptable reason for high population of kobs inside KLNP is linked with optimal conditions that enhance the survival and protection of the animal therein. Judging by the guideline and standard given, kobs remain one of the most easily sighted animals inside KLNP (Happlod, 1978).

Animal habitat is the sum total of all the environmental conditions such as food, cover, water and special factors present at a specific place for the survival and reproductive success of organisms (Garshelis, 2000). The ways wild animal uses the physical and biological resources in any environment however varies (Krausman, 1999). Niemuth et al. (2005) submitted that animal distribution in any environment is closely related to availability of favourable habitat. According to Rose and Robert (2013), animal behaviour can be classified into three major classes, i.e. foraging, reproducing and predation avoidance and these three broad behavioural classes cover most of the activities performed by animal.

Lameed and Adetola (2012) informed that salt-lick occur naturally in certain locations in the forest where mineral salt are found on the ground surface. In KLNP, one of such locations supporting kobs is the salt-licks spots. Ayotte et al. (2006) informed that areas where animals actively ingest soil have commonly been referred to as salt-licks. Measuring activity levels in animals provides important information about behavioral ecology which gives relevant information in their management and conservation (Kilgo et al.,1998; Gervasi et al., 2006). Holistic behavioural studies of animals irrespective of the habitat cannot be over emphasized for sustainable wildlife conservation. Information regarding the activities of kobs at salt-lick spots inside KLNP is rare. This study investigates the sex composition, age composition, group size, and activities of Kobus kob visiting larger and smaller salt-lick spots within Gilbert Child track inside Kainji Lake National Park, Nigeria.

Materials and Methods

Kainji Lake National Park (KLNP) is one of the seven national parks in Nigeria, West Africa. The park was established in 1979 by the merger of Borgu game reserve (located in Niger and Kwara States) and Zurguma game reserve (located in Niger State). The location of the park is approximately between latitude 09o 40'N - 10o 30'N and longitude 03o 30'E - 05o50'E covering a total area of 5,340.82km2 (Amusa et al., 2010). Fauna species in the park include Buffalo (Syncerus caffer), Roan antelope (Hippotragus equines), Kob (Kobus kob), Western hartebeest (Alcelaphus buselaphus), Hippopotamus (Hippopotamus amphibious), Olive baboon (Papio anubis), Bushbuck (Tragelaphus scriptus), Red flanked duiker (Cephalopus rufilatus), Oribi (Ourebia ourebia), Lion (Panthera leo) among others (Amusa et al., 2010). This study was conducted at salt-lick spots within Gilbert Child track (latitude N09^{0}54'76", longitude E03^{0}57'17") occupying 15.10m2 and (N09^{0}54'33", longitude E03^{0}56'34") occupying 100.00m^2.

Study approach

The study was conducted on three visits per week during the day time (7:00hr – 11:00hr and 14:00hr to 18:00hr) for four months between January to April, 2012 (dry season). In the first study, direct observation of kobs visiting the two salt-lick spots was done to determine whether sizes of the salt-lick spots had any influenced on the numbers of visiting kobs. This

was done in accordance with MacHutchon (2001) and Gelatt et al. (2002) guidelines. Since no animals were radio tagged or marked during the study period, all sightings were considered as independent observations. The implication of this was that an animal may be observed more than once. Second study determined the effects salt-lick spots on sex composition, age composition and group size of the visiting kobs. Kobs on their arrival at the spots were categorized into four group sizes (Group 1 = one animal; Group 2 = two animals; Group 3= three animals and Group 4= four animals and above).

It was followed by the identification on their sex. Sex composition was classified as 1= males and 2= females. Identified kobs were then grouped into age composition. (1= adults males; 2 = adult females; 3 =sub adult males; 4 = sub-adult females; 5 = young males and 6 = young females) as recommended by Prater (1980). In the third study, we observed the behaviour and activities of the animals in relation to the two salt-lick spots. This was done using scan sampling technique as described by Altmann (1974). The studied animals at the sites were scanned at intervals (8:00hr, 10:00hr, 15:00hr, 17:00hr). Their behaviour was first classified either as active (when the animals remain on its feet) or passive (when the animal goes on the ground) five minutes after its arrival at the sites. Classification of on animal activities (1 = foraging; 2 = ruminating; 3 = resting; 4 = salt licking; 5 = walking; 6 = playing; 7 = agonistic; 8 = mating; 9 = vigilant and 10 = standing) were done in accordance with Owen-Smith (1984) guidelines. Global Positioning System (Model GPS Map 76C GARMIN) was used to determine the coordinates of the salt lick sites while measuring tape was used to measure the length and breadth of the sites to calculate their sizes. During the study, nearby trees of about 200meters away from each of the spots were used as hide out. Data collected were subjected to descriptive statistics, Pearson's correlations, and chi-square analysis.

Results

It was observed from this study that the number of kobs visiting larger or smaller salt-lick spots varies. Pooled data in this study indicates that kobs visits were made to the two salt-lick spots in 269 times. Higher proportion 158(58.70%) of the visits were observed at the largest salt-lick spots while the remaining animal 111(41.30%) were observed at the smaller salt-lick spot (Figure 1). Frequency of kobs visitation as shown (Table 1) revealed that more of the female kobs moved towards these salt-lick (64.70%) compared to their male counterpart. Mean sex composition of the visiting animal was 1.73±0.04. Significant differences occurred in the sex of the visiting kobs ($\chi 2$= 23.20, df = 1, P< 0.01) at the two salt-lick spots. Majority of the kobs (45.40%) are adult females followed by the adult males (21.90%) while the least (3.70%) of them are in the category of sub-adult males. Age composition of the visiting kobs were significantly affected ($\chi 2$= 189.67, df = 5, P< 0.01). Highest percentage (47.2%) of the visiting kobs was recorded among group size of two followed by group size of three (25.70%) while the least (8.90%), was recorded among the animal with group size of four. Animal group size shows significant variation (r = 0.10, P < 0.01) with respect to the spots.

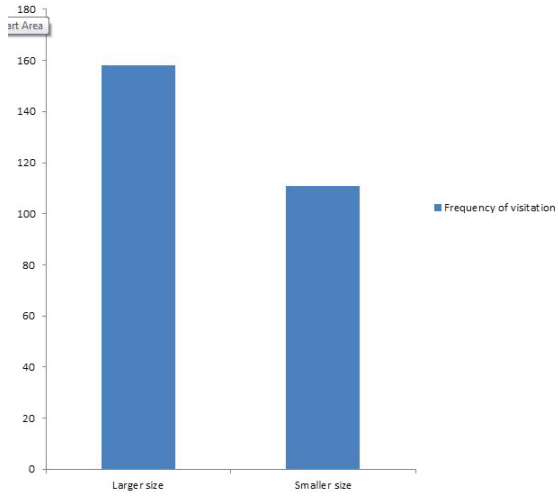


Figure 1: Kobs visitation to salt-lick spots

Table 1: Composition of kobs at salt-lick spots

Variables	Frequency	Percentages	Mode
Sex composition			
Males	95	35.3	Females
Females	174	64.7	
Total	**269**	**100**	
Age composition			
Adult males	59	21.9	
Adult females	122	45.4	Adult females
Sub adult males	10	3.7	
Sub adult females	32	11.90	
Young males	26	9.70	
Young females	20	7.40	
Group size of			
One	49	18.20	
Two	127	47.20	Group size of two
Three	69	25.70	
Four	24	8.90	
Total	**269**	**100**	

Kobs activities were recorded in 520 occasions. Of the observed activities, percentage ruminating (30.20%) was the highest followed by percentage salt-licking (23.80%) while the percentage animals sighted mating (0.40%) was the least (Figure 3). Statistically, no significant difference was observed in the animal behaviour and as well as its activities ($p \geq 0.01$).

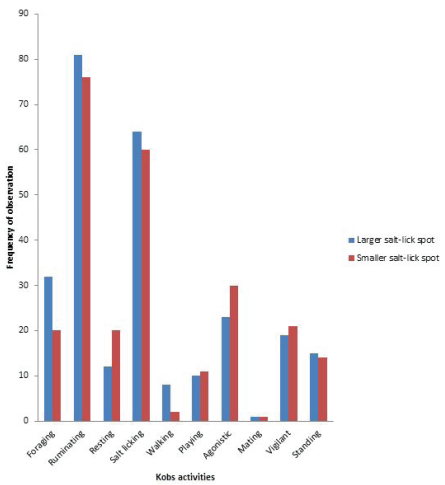


Figure 3: Kobs activities at salt-lick spots

Discussion

Kobs at Kainji Lake National Park, Nigeria are visiting salt-lick sites for different motive. The manner in which the studied animal visited the salt-lick spots may be connected with the factors such as season, reproductive stage of the animal, or the quality of the food consumed reported by Van Shaik and Hrdy (1991), environmental variation and predation mentioned by Milner-Gulland et al. (2003); Owen-Smith and Mason, 2005) or the slope and soil quality (Kushwaha et. al., 2004; Zarri, 2005). These factors were reported to affects composition of animal utilizing any habitat as well as ungulates movements between habitat patches (Andersen, 1991). Variation in the composition of kobs visiting the salt-lick spot in the present study agreed with the report of Atwood and Weeks (2002) where it was reported that the visitation frequency of moose (Alces alces) and white-tailed deer (Odocoileus virginianus) at natural licks increased during late gestation and/or at

the peak of the birth season. Present study also confirmed the report of Atwood et al (2002); Atwood et al. (2003) and Bravo et al. (2008) where they emphasized that reproductive activity is a huge factor for animals patronizing salt lick sites. It was reported that in most adult ungulate populations, sex ratios tend to be female biased exhibiting attributes typical for polygynous mating systems due to higher male mortality (Fischer & Linsernmair, 2002; Holland et al., 2002). Kruuk et al. (1999) stressed that density dependent factor affects number of survived male after birth in any habitat. It has been reported that animals in late pregnancy and during lactation require large quantities of calcium for their infants and newborn (Kovacs, 2005). This observation may be connected with the submission of Newing (2001) where it was reported that ungulate of different size displays different metabolic rates even as they have specific demands in terms of nutrition.

Our observation on kob's group size disagreed with the findings of Sharma et al. (2005) where they reported group size of one for the antelopes at the Panna National Park, India. This may be linked to differences in studied species and the environmental condition of the two areas. It was reported that species and animal behaviour changes to a great extent with different levels of anthropogenic pressures (Kilgo et. al., 1998; Bolhuis and Giraldeau, 2005; Rabin, 2003). Activities of the animal partly agreed with the findings of Linsernmair (2002) and Sharma et al. (2007). Less vigilant activity displayed by these animals' shows that they still enjoy some level of adequate protection especially at this spot that is very close to the center of the park at Oli camp. Vigilant activities displayed in this study are in agreement with the report of Reby et. al., (1999); Bergstrom and Lachmann, (2001) and Blumstein, 2001 where they reported that animals makes alarm calls to escape.

References

Andersen, R. (1991). Habitat deterioration and the migratory behaviour of moose Alces alces in Norway. Journal of Applied Ecology 28: 102-108.

Altmann, J. (1974). Observational study of behaviour: Sampling methods. Behaviour 49: 227-267.

Amusa, T.O., Jimoh, S.O., Aridanzi, P and Haruna, M. (2010). Ethnobotany and Conservation of Plant Resources of Kainji Lake National Park, Nigeria. Ethnobotany Research and Applications 8: 181-194.

Atwood, T.C and Weeks, H. P., Jr. (2002). Sex- and age-specific patterns of mineral lick use by white-tailed deer (Odocoileus virginianus). American. Midl. Nature 148: 289-296.

Atwood, T.C and Weeks, H. P., Jr. (2003). Sex-specific patterns of mineral lick preference in white-tailed deer. Northeast Nature 10: 409-414.

Ayotte, J. B., Parker, K. L., Arocena, J. M and Gillingham, M. P. (2006). Chemical composition of lick soils: Functions of soil ingestion by four ungulate species. Journal of Mammalogy 87(5): 878–888.

Bergstrom, C. T and Lachmann, M. (2001). Alarm calls as costly signals of antipredator vigilance: the watchful babbler game. Animal Behaviour 61: 535-543.

Blumstein, D. T. (2001). The evolution of functionally referential alarm communication: Multiple adaptations, multiple constraints. Evolution of Communication 3(2): 135-147.

Bolhuis, J. J and Giraldeau, L.A. (2005). The behaviour of animals. Mechanisms, function,and evolution. Oxford: Blackwell Publishing.

Bravo, A.; Harms, K.E.; Stevens, R.S.; Emmons and L.H. Collpas (2008). Activity hotspots for frugivorous bats (Phyllostomidae) in the Peruvian Amazon. Biotropica 40: 203-210.

Fisher, F., and Linsenmair, K. E. (2002). Demography of a West African Kob (Kobus kob) population. African Journal of Ecology 40: 130-137.

Garshelis, D. L. (2000). Delusion in habitat evaluation: measuring use, selection and importance. In: Research techniques in animal ecology: controversies and consequences, eds. Boitani, L. and Fuller, T. K. Columbia University Press. Pp 111-164.

Gelatt, T. S., D. B. Siniff, and J. A. Estes. (2002). Activity patterns and time budgets of the declining sea otter

population at Amchitka Island, Alaska. Journal of Wildlife Management 66: 29–39.

Gervasi, V., Brunberg, S and Swenson , J. E. (2006). An individual-based method to measure animal activity levels: a test on brown bears. Wildlife society bulletin 34(5), 1314–1319.

Holland, O., Roed, H.K., Mysterud, A., Kumpula, J., Nieminen, M and Smith, E.M. (2002). The effect of sex ratio and male age structure on Reindeer Calving. Journal of Wildlife Management 67: 25-33.

Jayeola O. A., Onadeko S. A., Mafiana C. F., Inah E. I and Okeyoyin O. A. (2012). Past and present status of kob (Kobus (Adenota) kob (Erxleben) in Nigeria. International Journal of Biodiversity and Conservation 4(5): 197-205.

Kruuk, L.E.B., Clutton-Brock, T.H., Albon, S.D., Pemberton, J.M and Guinness, F.E. (1999). Population Density Affects Sex Ratio Variation In Red Deer. Nature (Lond.) 399: 459-461.

Happold, D.C. D (1978). Large Mammals of West Africa. Longman Publishers Singapore, Pp. 105.

Kligo, J. C., R. F. Labisky and D. E. Fritzen. (1998). Influences of hunting on the behaviour of white-tailed deer: implications for conservation of the Florida panther. Conservation Biology 12: 1359 -1364.

Kovacs, C.S. (2005). Calcium and bone metabolism during pregnancy and lactation. Journal of Mammary Gland Biology and Neoplasia 10: 105-118.

Krausman, P. R. (1999). Some basic principles of habitat use. Paper presented in: Grazing Behavior of Livestock and Wildlife. Idaho Forest. Wildlife and Range Exp. Sta. Bull. 70: 85-90.

Kushwaha, S. P. S., Khan, A., Habib, B., Quadri, A and Singh, A (2004). Evaluation of sambar and muntjak habitats using geostatistical modeling. Current Science 86 (10): 1390-1400.

Lameed, G. A and Adetola, J. (2012). Species-Diversity utilization of salt lick sites at Borgu sector of Kainji lake national park. Biodiversity Enrichment in a Diverse World. Pp 35-62.

MacHutchon, A. G. (2001). Grizzly bear activity budget and pattern in the Firth River Valley, Yukon. Ursus 12:189 -198.

Milner-Gulland, E.J., Bukreeva, O.M., Coulson, T., Lushchekina, A.A., Kholodova, M.V., Bekenov, A.B and Grachev, I.A. (2003). Conservation: Reproductive Collapse In Saiga Antelope Harems. Nature (Lond.) 422: 135-136.

Newing, H. (2001). Bushmeat hunting and management: implications of duiker ecology and interspecific competition. Biodiversity and Conservation 10 (1): 99-108.

Niemuth, N. D. Estey, M. E. and Loesch, C. R. (2005). Developing spatially explicit habitat models for grassland bird conservation planning in the Prairie Pothole region of North Dakota. In USDA Forest Service General Technical Report. PSW-GTR-191. Pp. 469-477.

Owen-Smith, N and Mason, D.R. (2005). Comparative Changes In Adult Vs. Juvenile Survival Affecting Population Trends Of African Ungulates. Journal of Animal Ecology 74: 762-773.

Owen-Smith,. N (1984). Spatial and temporal components of the mating systems of kudu bulls and red deer stags. Animal Behaviour 32: 321– 332.

Prater, S. H. (1980). The book of Indian animals. 3rd edition (reprint). Bombay Natural History Society, Mumbai. Pp. 324.

Rabin, L. A. (2003). Maintaining behavioural diversity in captivity for conservation: Natural behavioural management. Animal Welfare 12: 85-94.

Reby, D., Cargnelutti, B and Hewison, A. J. M. (1999). Contexts and possible functions of barking in roe deer. Animal Behaviour 57: 1121-1128.

Rose, P and Robert, R (2013). Evaluating the activity patterns and enclosure usage of a little-studied zoo species, the sitatunga (Tragelaphus spekii). Journal of Zoo and Aquarium Research 1(1), 14-19

Sharma, K., Rahmani, A.R and Chundawat, R. S. (2007). Ecological Studies on the Four-horned Antelope Tetracerus quadricornis in a Tropical Dry Forest. Final Report. Bombay Natural History Society. Pp. 153

Smith, S. (1985). The altas of Africa's principal Mammals. San Antonio: National History Books.

Stuart, C and Stuart, T (1992). Southern, Central and East African Mammals: A photographic guide. Cape Town, South Africa: Struik Publisher.

Zarri, A. A. (2005). Ecology of Nilgiri Laughingthrush (Garrulax cachinnans) in Nilgiris, Western Ghats. Ph.D. Thesis. University of Mumbai. Pp. 156.

ANTIBIOTICS PROFILING OF PROTEUS MIRABILIS AND PSEUDOMONAS AERUGINOSA ISOLATED FROM SOME COMMERCIAL POULTRY FARMS IN NSUKKA, ENUGU STATE, NIGERIA

Ogunleye A O[1]* Oyeleye Oluwatosin A[1]. and Ajuwape A T P[1]
[1]Department of Veterinary Microbiology and Parasitology, University of Ibadan.

Abstract:

Proteus mirabilis and Pseudomonas aeruginosa are attracting attention as pathogenic organisms of public health importance, not only as agents of various infections in human and food animals(poultry inclusive), but also due to their ability to carry multidrug resistant traits that are often transferable to other pathogens of both human and animal. Constant tracking of the antibiotic susceptibilities of these organisms at different region within each country is of great epidemiological value to formulate well informed and scientific based preventive measures to curtail the spread of drug resistant pathogens through the food chain.

We screened 19 Proteus mirabilis and 35 Pseudomonas aeruginosa isolated from the cloaca swab of some commercial poultry from Nsukka, Enugu State, Nigeria; for their antibiotics susceptibilities to 10 commonly used antibiotics for food animals and humans in Nigeria; using standard methods. The fluoroquinolone resistant isolates were subsequently studied for point mutation at the GyrA of the quinolone resistant determining region by PCR assay. The Proteus mirabilis isolates had 100% resistance to: ceftazidime, ceftriaxone, amikacin, sulfamethoxazole, chloramphenicol, and streptomycin and between 78.95% - 94.7% resistance to other antibiotics used. The fluoroquinolone resistant isolates possessed high MICs ranges between 16µg/mL ->128 µg/mL. The Pseudomonas aeruginosa isolates likewise displayed 100% resistance to: cefepime, sulfamethoxazole, chloramphenicol, ampicillin and streptomycin and 71.43% - 97 14% resistance to the other antibiotics tested. The levofloxacin resistant isolates also exhibited a high MICs ranges between 16µg/mL ->128 µg/mL and 5/21(23.8%) of the levofloxacin resistant Pseudomonas aeruginosa isolates exhibited 4-23 point mutations at the GyrA region of the quinolone resistant determining region. The study showed that the poultry in the studied area carries multidrug resistant pathogens that can serve as a source of drug resistant pathogen through the food chain to the public. Proper public health education on the potential health risk associated with the findings is advocated in the region.

ETABLISSEMENT DU PROFIL DE LA RESISTANCE AUX ANTIBIOTIQUES DES BACTERIES PROTEUS MIRABILIS ET PSEUDOMONAS AERUGINOSA ISOLEES DANS CERTAINES FERMES AVICOLES DE NSUKKA DANS L'ETAT D'ENUGU AU NIGERIA

Résumé:

Les bactéries Proteus mirabilis et Pseudomonas aeruginosa attirent de l'attention comme organismes pathogènes d'importance pour la santé publique, non seulement comme agents étiologiques de diverses infections chez les humains et les animaux destinés à la consommation (y compris les volailles) mais aussi en raison de leur capacité à porter des traits de résistance à plusieurs médicaments, lesquels traits sont souvent transférables à d'autres agents pathogènes des humains et des animaux. Le suivi constant des sensibilités de ces organismes aux antibiotiques dans chaque pays est d'une grande valeur épidémiologique car il permet de formuler des mesures préventives bien éclairées et fondées sur la science afin de freiner la propagation de pathogènes résistants aux médicaments par le biais de la chaîne alimentaire.

Corresponding author email: peculiarj@yahoo.com; ao.ogunleye@mail.ui.edu.ng.

Nous avons examiné 19 Proteus mirabilis et 35 Pseudomonas aeruginosa isolées dans des écouvillons cloacaux de certaines volailles commerciales de Nsukka dans l'État d'Enugu au Nigeria pour déterminer leurs sensibilités à 10 antibiotiques couramment utilisés chez les animaux destinés à la consommation et les humains au Nigeria, en utilisant des méthodes standard. Les isolats résistants aux fluoroquinolones ont ensuite été étudiés afin de déterminer la mutation ponctuelle au GyrA de la région déterminant la résistance à la quinolone en utilisant la méthode PCR. Les isolats de Proteus mirabilis ont montré une résistance de 100% à la ceftazidime, à la ceftriaxone, à l'amikacine, au sulfaméthoxazole, au chloramphénicol et à la streptomycine, et une résistance de 78,95% à 94,7% aux autres antibiotiques utilisés. Les isolats résistant aux fluoroquinolones possédaient des CMI élevées variant entre 16µg/mL ->128 µg/mL. Les isolats de Pseudomonas aeruginosa ont également montré une résistance de 100% à la céfépime, au sulfaméthoxazole, au chloramphenicol, à l'ampicilline et à la streptomycine, et une résistance variant entre 71,43% et 97,14% aux autres antibiotiques testés. Les isolats résistant à la lévofloxacine ont également montré des CMI très variables se situant entre 16µg / mL -> 128 pg / ml, et 5/21 (23,8%) des isolats de Pseudomonas aeruginosa résistants à la lévofloxacine ont montré 4-23 mutations ponctuelles au GyrA de la région déterminant la résistance à la quinolone. L'étude a montré que les volailles de la zone étudiée sont porteuses d'agents pathogènes multirésistants qui peuvent servir de source d'agents pathogènes résistant aux médicaments, transférables au public à travers la chaîne alimentaire. L'éducation appropriée à la santé publique sur le risque sanitaire potentiel associé aux résultats est préconisée dans la région.

Introduction

The roles of Proteus mirabilis and Pseudomonas aeruginosa as opportunistic/ nosocomial infectious agents in human is well known (Morrison et al., 1984; NNISS, 2004). They have been associated with infections like pneumonia, central nervous system and urolithiasis among many others in human (Unger et al., 1973; Lysy et al., 1985; kassim et al., 2003; Tavichakorntrakool et al., 2012). The two pathogens are however equally attracting attentions as agents of various infections as well as in transmission of drug resistance in and through food animals, poultry inclusive (Walker et al., 2002; Ogunleye et al., 2015).

In poultry for example, they have been associated with: mortalities due to septicaemia and respiratory signs (Bapat et al., 1985; Hussein et al., 2008; Hai-ping, 2009); embryonic death within the shell (Hebat – Allah and Mohamed, 2004); early yolk sac infection of young chicks within first week of life (Amare et al., 2013); and reproductive failures in chickens (Cabassi et al., 2014). All these pathologic conditions constitute a great economic loss to poultry production especially due to the associated mortalities (Hartl et al., 1997; Satish and Priti, 2015).

Apart from the roles of Proteus mirabilis and Pseudomonas aeruginosa in economic losses attributable to mortalities due to the various infections they cause in poultry, they have been documented to carry multi drug resistant traits in poultry from some parts of the world. For instance, five Pseudomonas aeruginosa isolated from a case of broilers omphalitis due to hatchery contamination in the mid -2000 at Alabama were resistant to sulfisoxazole, ceftiofur, penicillin, lincomycin, bacitracin, oxytetracycline, erythromycin, naladixic acid, and tetracycline, but all isolates were sensitive to gentamicin (Walker et al., 2002). There are also various reports indicating increase in the emergence of multi drug resistant poultry-origin P. mirabilis in countries like Korea and Croatia to mention a few (Kim et al., 2005; Tonkic et al., 2010, Aragon et al., 2008; Park et al., 2010). Ninety four percent (34/36) of the Proteus mirabilis isolates from 27/70(39%) chicken droppings from Bangladesh were multidrug resistant; and tetracycline had the highest resistance of 94 %(34/36) while the lowest resistance of 17% (6/36) was observed for ciprofloxacin (Nahar et al., 2014). In contrast however, the Proteus mirabilis

isolated from 3 (25%) of the 12 birds sampled from Ajmer region of Rajasthan, India showed a high level of resistance to tetracycline (100%), whereas all the isolates were susceptible to amikacin, ampicillin, amoxy clav, aztreonam, cefepime, cefezime, cefotaxime, cefopodoxime, ceftazidime, ceftriaxone, cefazolin, chloramphenicol, ciprofloxacin, cotrimoxazole, cefoxitin, gentamicin, imipenem, netilmicin and ofloxacin. All the tested species were also sensitive to gentamicin thus in terms of definition of multidrug resistance as resistance to 2 or more antibiotics, the isolates were 0% multidrug resistant (Dadheech et al., 2015).

From the foregoing, there is the possibilities of variations in the antibiotic susceptibilities pattern exhibited by these Gram negative bacteria from one region to the other, its surveillance in different countries and even region within each country is however imperative to track the resistance pattern in order to curtail possible antibiotic resistance spread through the organism within poultry industry.

In the current work, we examined 19 Proteus mirabilis and 35 Pseudomonas aeruginosa isolated from the cloaca swab of some commercial poultry from Nsukka, Enugu State, Nigeria (a major City from Eastern region of Nigeria where a lot of commercial poultry production activities takes place). The isolates were screened for their antibiotics susceptibilities to 10 commonly used antibiotics for food animals and humans in Nigeria. The fluoroquinolone resistant isolates were subsequently screened for point mutation of the quinolone resistant determining region by PCR assay.

Materials and Methods

Bacteria isolates:

The 54 Gram negative bacteria comprising of 19 Proteus mirabilis and 35 Pseudomonas aeruginosa studied were isolated from aseptically collected swabs from cloaca region of life birds from some commercial poultry houses in Nsukka, Enugu State, Nigeria. They were screened for their antibiotics susceptibilities. They were identified as Proteus mirabilis and Pseudomonas aeruginosa based on standard morphological and biochemical bacteriological procedures (Barrow and Felthams, 2004; Garcia and Isenberg, 2007). They were further confirmed as Proteus mirabilis and Pseudomonas aeruginosa with Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03 according to the manufacturers procedures and by 16S ribosomal RNA PCR identification procedure.

16S RNA Identification of the Proteus mirabilis

The 54 isolates were subjected to PCR 16S ribosomal RNA identification as previously described by Weisburg et al 1991 with some modification. Chromosomal DNA were produced from the isolates by heating the LB broth cultures at 99^0C for 15minutes. A 100µl of the boiled isolates were mixed with equal volume of PCR grade water, 1 µl of the mixture was used as DNA template in a 50 µl reaction. The DNA was amplified using QS PCR reagents(New England Bio labs) containing 1µM of fD2= 5'AGATTTGATCATGGCTCAG3' and rP1 = 5'ACGGCTACCTTGTTACGACTT3', 10 µl QS buffer, 1 µl dNTPs, 0.25 µl fD1, 0.25 µl rP1, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water, using the PCR protocol: initial denaturation step at 98^0C for 30 seconds, followed by 35 cycles of DNA denaturation at 98^0C for 10 seconds, primers annealing at 55^0C for 30seconds, primers extension at 72^0C for 1minutes 15seconds followed by final extension at 72^0C for 7minutes. The amplified products were resolved with precasted E- gel in an Electrophoresis unit (Life Technologies).

The amplified products were purified and sequenced at Iowa State University DNA sequencing facilities (Ames, IA, USA). The identities of the sequenced products were analysed by using BLASTN 2.2.31+ as described by Zhang et al., 2000.

Determination of Resistance to Kanamycin, ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, levofloxacin, ceftazidime, ceftriaxone, cefepime and amikacin

The 54 isolates were grown aerobically in breakpoint concentrations of 32µg/mL each for kanamycin, ceftazidime, ceftriaxone, amikacin, ampicillin, and cefepime; 64 µg/mL for streptomycin, 16 µg/mL for chloramphenicol, sulfamethoxazole at 1024µg/mL and 8µg/mL for levofloxacin (all from SIGMA- ALDRICH) according to standard method(CLSI, 2009)

Resistance was assigned if flocculent growth was observed after 16h of aerobic growth at 37^0C.

Analysis of the Quinolone resistant Determinant Region (QRDR) for the Levofloxacin resistant isolates

Subsequently, Levofloxacin MICs were determined for the 13/15(86.66%) Proteus mirabilis and 21/25 (84.00%) Pseudomonas aeruginosa isolates that were resistant at 8µg/mL breakpoint according to standard method (CLSI, 2009). The high fluoroquinolone resistant isolates were subsequently screened for point mutation through the amplification of the GyrA QRDR and DNA sequencing of the PCR product. It was carried out as previously described (Ogunleye, 2012).

A 560 base pair region of GyRA of the crude boiled DNA was amplified with a universal Forward and reverse oligonucleotide F=5´ATGAGCGACCTTGCGAGAAATA CACCG3´ and R=5´TTCCATCAGCGCC CTTCAATGCTGATGTCTTC3´ using QS polymerase reagent(New England Bio labs) in a 50µl containing 10 µl QS buffer, 1 µl dNTPs, 0.25 µl QRDR F, 0.25 µl QRDR R, 0.5 µl QS enzyme, 10 µl QS enhancer and 27 µl PCR water, using the PCR protocol: initial denaturation at 98^0C for 30seconds, and 35cycles of 98^0C for 10seconds, 55^0C for 30 seconds, 72^0C for 1minute 15seconds and final extension at 72^0C for 7minutes. The amplified products were resolved with precast E- gel in an electrophoresis unit (Life Technologies).

The amplified products were purified and sequenced at Iowa State University DNA sequencing facilities (Ames, IA, USA).

Results

Nineteen of the isolates were identified as Proteus mirabilis and 35 as Pseudomonas aeruginosa according to the conventional bacteriological analysis and the identities were confirmed with the 16s ribosomal RNA PCR analysis (Tables 1 and 2).

All the 19 Proteus mirabilis isolates were multidrug resistant. They all had 100% resistance to: ceftazidime, ceftriaxone, amikacin, sulfamethoxazole, chloramphenicol, and streptomycin. There were 94.74 %(18/19) resistance to cefepime; 89.47 % (17/19) to both kanamycin and ampicillin and 78.95% (15/19) to levofloxacin. The 13/15 (86.66%) isolates that were levofloxacin resistant at 8µg/mL breakpoint had high levels of resistance as indicated by a high level of their minimum inhibitory concentration ranges between 16 µg/mL to >128 µg/mL(Table 3). A total of 13.33% (2/15) of the high levofloxacin resistant isolates had point mutations. Isolate E36 had 2 point mutation at MIC 128µg/mL while isolate RO 44 displayed 3 point mutations at an MIC 16 µg/mL (table 3).

Likewise all the 35 Pseudomonas aeruginosa isolates were multidrug resistant. They had 100% resistance to five of the antibiotics tested including: cefepime, sulfamethoxazole, chloramphenicol, ampicillin and streptomycin. There were 97.14 %(34/35) resistant to three of the antibiotics namely: ceftazidime, ceftriazone and amikacin. The percentage resistance for kanamycin was 94.29 %(33/35) and 71.43 %(25/35) for levofloxacin. The 21/25(84%) isolates that were levofloxacin resistant at 8µg/mL breakpoint all had high minimum inhibitory concentration ranges between 16 µg/mL to 128 µg/mL. Of all the isolates screened for the presence of point mutation at the GyrA region of the quinolone resistant determining region, 5/21(23.81%) had between 4-26 point mutations as shown in Table 4.

Table 1: Proteus mirabilis from the Eastern part of Nigeria

isolate	16s RNA identity	Source	ceftaz	Ceftria	amik	cefep	Levo	Sulf	Chloram	Kan	amp	Strep
H24nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
E313	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
XII 11	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
F42nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	S	R
XII7nlf	Proteus mirabilis	Poultry E	R	R	R	R	S	R	R	R	R	R
FI3nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
F2	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
RO39	Proteus mirabilis	Poultry E	R	R	R	R	S	R	R	R	S	R
RO 23	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
RO 22	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
R O 4 4 nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
XI 4nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
R O 21nlf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
F17nlf	Proteus mirabilis	Poultry E	R	R	R	R	S	R	R	R	R	R
E39nlf	Proteus mirabilis	Poultry E	R	R	R	R	S	R	R	S	R	R
E316	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
E36	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	R	R	R
F40nlf	Proteus mirabilis	Poultry E	R	R	R	S	R	R	R	R	R	R
O117lf	Proteus mirabilis	Poultry E	R	R	R	R	R	R	R	S	R	R

Ceftaz=ceftazidime; ceftria= ceftriaxone; amik= amikacin; cefep= cefepime; levo=levofloxacin; sulf= sulfamethoxazole, kan= kanamycin; amp= ampicillin; strep= streptomycin; poultry E= poultry from the Eastern Nigeria.

Table2: Pseudomonas aeruginosa from the Eastern part of Nigeria.

Isolate	16s rna identity	Source	ceftaz	ceftria	Amik	cefep	levo	sulf	Chloram	kan	amp	Stress
E 319nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
F4nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
X I 19NLF	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
XI5nlf	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
D24nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
E11nlf	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
E312	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
E32nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
F51nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
E210	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO16	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
F30nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO 37	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
E31	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
KO 49	P.aeruginosa	poultry E	S	S	S	R	R	R	R	R	R	R
F12	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO28nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO17	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO 8	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
`F36nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
O17	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
E310	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
XIII7	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R

Isolate	16s rna identity	Source	ceftaz	ceftria	Amik	cefep	levo	sulf	Chloram	kan	amp	Stress
O12	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO 42	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
F44	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
F49	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
XII6nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	S	R	R
RO 50nlf	P.aeruginosa	poultry E	R	R	R	R	S	R	R	R	R	R
F45nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
F 16	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
XI10nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
XIII 1nlf	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R
RO48	P.aeruginosa	poultry E	R	R	R	R	R	R	R	S	R	R
XIII 8	P.aeruginosa	poultry E	R	R	R	R	R	R	R	R	R	R

P. aeruginosa= Pseudomonas aeruginosa; Ceftaz=ceftazidime; ceftria= ceftriaxone; amik= amikacin; cefep= cefepime; levo=levofloxacin; sulf= sulfamethoxazole, kan= kanamycin; amp= ampicillin; strep= streptomycin; poultry E= poultry from the Eastern Nigeria.

Table 3: Quinolone resistant determining region (QRDR) screening in Proteus mirabilis from poultry, Eastern, Nigeria.

Isolate	16s rRNA identity	No of point mutation	levo(8µg/mL	Levo MIC
F40nlf	P mirab	Nil	R	64µg/mL
E36	P mirab	2	R	128µg/mL
E 313	P mirab	Nil	R	32µg/ml
F42	P mirab	Nil	R	32µg/mL
RO 44	P mirab	3	R	16µg/mL
RO22	P mirab	Nil	R	32µg/ml
H24nlf	P mirab	Nil	R	64µg/mL
RO21nlf	Pmirab	Nil	R	128µg/mL
FI 3nlf	P mirab	Nil	R	32µg/ml
RO 23	P mirab	Nil	R	32µg/ml
XII 11	P mirab	Nil	R	>128µg/mL
F2	P mirab	Nil	R	64µg/mL
E38	P mirab	Nil	R	>128µg/mL

P mirab= Proteus mirabilis; Levo= levofloxacin

Table 4: Quinolone resistant determining region (QRDR) screening in Pseudomonas aeruginosa from poultry, Eastern, Nigeria

Isolate	16s rRNA id	No ofpoint mutation	levo(8µg/mL	Levo MIC
RO 28nlf	P aerug	Nil	R	16µg/mL
D24nlf	P aerug	Nil	R	64µg/mL
RO 16	P aerug	Nil	R	64µg/mL
E31	P aerug	Nil	R	128µg/mL
F45nlf	P aerug	8	R	128µg/mL
E312	P aerug	Nil	R	128µg/mL
O 17	P aerug	26	R	128µg/mL
E310	P aerug	Nil	R	32µg/mL
XII 9	P aerug	4	R	128µg/mL
F36nlf	P aerug	7	R	128µg/mL
E319	P aerug	Nil	R	64µg/mL
F1	P aerug	Nil	R	64µg/mL
E 32nlf	P aerug	Nil	R	16µg/mL
F12	P aerug	Nil	R	64µg/mL
KO49	P aerug	18	R	128µg/mL
F30 nlf	P aerug	Nil	R	128µg/mL
O12	P aerug	Nil	R	128µg/mL
E210	P aerug	Nil	R	64µg/mL
F16nlf	P aerug	Nil	R	64µg/mL
XIII 1	P aerug	Nil	R	128µg/mL
F4nlf	P aerug	Nil	R	16µg/mL

P aerug= Pseudomonas aeruginosa; Levo= Levofloxacin.

Discussion

The antibiotic resistance patterns observed for the Proteus mirabilis and Pseudomonas aeruginosa from cloaca healthy chickens from Nssuka, Enugu State, Nigeria, displayed a high level of resistance. The 78.95% - 100% resistance for Proteus mirabilis observed for the 10 antibiotics and the high fluroquinolone MICs ranges between 16µg/mL- >128µg/mL with observation of 2 and 3 point mutation from 2 of the isolates shows that the poultry from the studied area carries multidrug resistant pathogens that could constitute potential public health threats through antibiotic resistance transmission to the public through poultry. The observed high level of resistance in the bacteria isolates is consistent with similar reports from different parts of the world ((Kim et al., 2005; Tonkic et al., 2010, Aragon et al., 2008; Park et al., 2010). It is however unlike a higher level of susceptibilities reported for poultry origin Proteus mirabilis isolates from Ajmer region of Rajasthan, India where all the isolates were susceptible to amikacin, ampicillin, amoxy clav, aztreonam, cefepime, cefezime, cefotaxime, cefopodoxime, ceftazidime, ceftriaxone, cefazolin, chloramphenicol, ciprofloxacin, cotrimoxazole, cefoxitin, gentamicin, imipenem, netilmicin and ofloxacin. All the tested species were also sensitive to gentamicin thus in terms of definition of multidrug resistance as resistance to 2 or more antibiotics, the isolates were 0% multidrug resistant (Dadheech et al., 2015).

Likewise for the Pseudomonas aeruginosa isolates studied, the 71.43% - 100% resistance for the 10 antibiotics, high fluoroquinolone MICs ranges of between 16µg/mL- 128µg/mL and 5/21(23.81%) 4-26 point mutations at the GyrA of the quinolone resistant determining region also portrays presence of potential public health threat in terms of multidrug resistant pathogens that could be transmitted to the public through food chain. The observation also agrees with similar reports of poultry associated multi drug resistant Pseudomonas aeruginosa isolated from a case of broilers omphalitis due to hatchery contamination Alabama that conform to multi drug resistant definition based on their resistance to sulfisoxazole, ceftiofur, penicillin, lincomycin, bacitracin, oxytetracycline, erythromycin, nalidixic acid, and tetracycline (Walker et al., 2002).

The high level of antibiotic resistance so observed in this study can be attributed to high level of misuse as well as abuse of these antibiotics in the study area just as in some other parts of Nigeria (Unpublished data). The observation from this study lends a great support for the need for consistent and well organized public health education on the public health implications of antibiotic drug abuse in the poultry industries across all the different zones of Nigeria.

References:

Amare A, Amin AM, Shiferaw A, Nazir S, and Negussie H, 2013. Yolk Sac Infection (Omphalitis) in Kombolcha Poultry Farm, Ethiopia. AEJSR., 8 (1): 10-14.

Aragon LM, Mirelis B, Miro E, Mata C, Gomez L, Rivera A, Coll P, Navarro F, 2008. Increase in beta-lactam-resistant Proteus mirabilis strains due to CTX-M- and CMY-type as well as new VEB- and inhibitor-resistant TEM-type beta-lactamases. J. Antimicrob. Chemother., 61: 1029-1032.

Bapat DA, Krtlkarrri V B, Ninrje DV, 1985. Mortality in chicks due to P. aeruginosa. Indian J. of Animal Science., 55(7): 538 - 539.

Barrow GI, Felthan RKA,2004. Cowan and Steels identification of Medical bacteria 4th edition Cambridge University Press., 50–145.

Cabassi CS, Taddei S, Predari G, Galvani G, Ghidini F, Schiano E, Cavirani S, 2004. Bacteriologic finding in ostrich (Struthio camelus) eggs from farms with reproductive failures. Avian Dis., 48:716–722.

CLSI, 2009. Method of dilution antimicrobial susceptibility test for bacteria that grow aerobically: Approved Standard- 8thEdn. CLSI document M31-A3, 2009; 1-99. Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne, Pennsylvania, USA.

Dadheech T, Reena Vyas R, Rastogi V, 2015. Antibiotic resistance of aerobic bacterial isolates of Proteus mirabilis from sick layer chickens infected with septicaemia and salpingitis in Ajmer region of Rajasthan. WJPPS., 4(07): 2002-2011.

Garcia LS, Isenberg HD, 2007. Clinical Microbiology Procedures Handbook Vol. 1, Second edn. Update ASM Press American Society for Microbiology., 1752 N St., N.W. Washington, DC 20036-290.

Hai-ping HE, 2009. Isolation and identify of Pseudomonas aeruginosa in chicken dead-embryos. Chinese Qinghai J Anim Vet Sci., 3: 25-27.

Hartl A, Mollmann U, Schrinner E, Stelzner A, 1997. Pseudomonas aeruginosa infection in embryonated heh's eggs. An alternative in vivo model for the screening of antibacterial substances. Arzneimittelforschung., 47: 1061-1064.

Hebat – Allah, Mohamed AE, 2004. Some studies on Pseudomonas species in chicken embryos and broilers in ASSIUT Governorate. Ass. Univ. Bull. Environ. Res., 7(1): 23-30.

Hussein SA, Nassan AH, Sulaiman RR, 2008. Bacteriological and pathological study of yolk sac infection in broiler chicks in Sulaimani District. J Dohuk Univ., 1: 48-56.

Kassim Z, Aziz AA, Haque QM, Cheung HAS, 2003. Isolation of Proteus mirabilis from severe neonatal sepsis and central nervous system infection with extensive pneumocephalus. Eur J Pediat., 162(9):644–645.

Kim SH, Wei CI, An H, 2005. Molecular characterization of multidrug-resistant Proteus mirabilis isolates from retail meat products. J Food Prot., 68: 1408-1413.

Lysy J, Werczberger A, Globus M, Chowers I, 1985. Pneumatocele formation in a patient with Proteus mirabilis pneumonia. Postgrad Med J., 61:255–257.

Morrison AJ Jr, Wenzel RP, 1984. Epidemiology of infection due to Pseudomonas aeruginosa. Rev. Infect. Dis., 6(Suppl. 3):627–642.

Nahar A, Siddiquee M, Nahar S, Anwar KS, Islam S, 2014. Multidrug Resistant- Proteus mirabilis isolated from Chicken droppings in commercial poultry farms: bio-security concern and emerging public health threat in Bangladesh. J Biosafety Health Educ., 2: 120. doi:10.4172/2332-0893.1000120.

NNIS. 2004. National Nosocomial Infection Surveillance System. National Nosocomial Infection Surveillance (NNIS) System report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control., 32:470–485.

Ogunleye AO, 2012. Identification of GyrA mutations conferring fluoroquinolone resistance in Pseudomonas aeruginosa isolated from poultry in Ibadan, Oyo State, Nigeria. Afr J Microbiol Res, 6: 1573-1578.

Ogunleye AO, Adetosoye AI, Carlson SA, 2015. Rats as possible agents of antibiotic resistant Pseudomonas aeruginosa transmission through poultry in Ibadan, Oyo State, Nigeria. IRJM., 6(3): 037-041.

Park SD, Uh Y, Lee G, Lim K, Kim JB, Jeong SH, (2010) Prevalence and resistance patterns of extended-spectrum and AmpC beta-lactamase in Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Salmonella serovar Stanley in a Korean tertiary hospital. APMIS., 118: 801-808.

Satish S, Priti, M, 2015. Pseudomonas aeruginosa Infection in Broiler Chicks in Jabalpur. International J Ext Res., 6:37-39 http://www.journalijer.com e-Print ISSN: 2394-0301.

Tavichakorntrakool R, Prasongwaltana V, Sungkeeree S, Saisud P, Sribenjaloux P, Pimratana C, Bovornpadungkitti S, Sriboonlue P, Thongbookerd V. 2012. Extensive characterizations of bacteria isolated from catheterized urine and stone matrices in patients with nephrolithiasis. Nephrol Dial Transplant., 27(11): 4125-4130..

Tonkic M, Mohar B, Sisko-Kraljevic K, Mesko-Meglic K, Goic-Barisic I, Novak A, Kovacić A, Punda-P□lić V, 2010. High prevalence and molecular characterization of extended-spectrum beta-lactamase-producing Proteus mirabilis strains in Southern Croatia. J Med Microbiol., 59: 1185-1190.

Unger JD, Rose HD, Unger GF, 1973. Gram-negative pneumonia. Radiology. 107(2):283–91.

Walker SE, JE Sander, JL Cline and JS Helton, 2002. Characterization of Pseudomonas aeruginosa isolates associated with mortality in broiler chicks. Avian Dis, 46: 1045-1050.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ, 1991. 16S Ribosomal DNA Amplification for Phylogenetic Study. J Bact., 173(2): 697-703.

Zhang Z, Schwartz S, Wagner, Miller W, 2000. A greedy alogarithm for aligning DNA sequences.2000. J comput Biol., 7(1-2): 203-214.

MÉTHODOLOGIE POUR L'IMPLÉMENTATION DU RECENSEMENT NATIONAL DU CHEPTEL ANIMAL EN RÉPUBLIQUE DE DJIBOUTI

Matete G O[1], Idriss Ali Soultan[2], Omar Abdoulkader Mohamed[2], Farah Abdi Djama[3] Abdoulkader Ismail[3], et Fallou Guèye[3]

[1]Ruge Goshen Consulting : george.matete@gmail.com

[2]Direction de la Statistique et des Études Démographiques (DISED)

[3]Organisation des Nations unies pour l'alimentation et l'agriculture

Résumé

La Direction de la Statistique et des Études Démographiques (DISED) et la Direction de l'Élevage et des Services Vétérinaires (DESV) avec l'appui technique de la FAO à Djibouti (à travers le TCP/DJI/3501) ont développé d'une façon participative une méthodologie pour le recensement du cheptel animal en République de Djibouti. Cette méthodologie a été élaborée en prenant en compte les avis des leaders d'opinion clés et des experts techniques locaux ainsi que la documentation scientifique existante dans ce domaine.

La méthodologie propose l'utilisation du relevé aérien pour l'identification des emplacements des ménages éleveurs et l'estimation de leurs effectifs ainsi que de la taille et de la composition de leurs troupeaux en fournissant les coordonnées GPS. Le relevé aérien permettra également d'identifier et de fournir les coordonnées géographiques de tous les points d'eau. L'agent recenseur va interviewer le chef de ménage de chaque exploitation d'élevage identifiée. La technique « boule de neige » sera utilisée pour accélérer l'identification des ménages éleveurs.

Mots-clés: méthodologie, recensement du bétail Djibouti

METHODOLOGY FOR IMPLEMENTING THE NATIONAL LIVESTOCK CENSUS IN THE REPUBLIC OF DJIBOUTI

Abstract

The Department of Statistics and Demographic Studies (DISED) and the Directorate of Livestock and Veterinary Services (DESV) with the technical support of FAO in Djibouti (through TCP / DJI / 3501) developed a participatory methodology for livestock census in Djibouti. The methodology development took into account the views of key opinion leaders and local technical experts as well as the scientific literature in the field.

The methodology proposes the use of aerial survey to identify where pastoralist households are located and to estimate their numbers, as well as the size and composition of their herds by providing GPS coordinates. The aerial survey will also identify and provide geographical coordinates of all water points. The enumerators will interview heads of households for each identified livestock farm. The "snowball" technique will be used to accelerate identification of pastoralist households.

Keywords : methodology, livestock census, Djibouti

Introduction

La République de Djibouti (RdD), est l'un des plus petits pays d'Afrique, avec une superficie de 23 200 kilomètres carrés et une population de 939 298 habitants en 2014, selon les statistiques de la Direction de la statistique et des études démographiques (DISED).

L'absence d'informations quantitatives et qualitatives fiables limite le succès et l'efficacité des projets d'élevage. Les mouvements transfrontaliers des troupeaux sont courants pour les ménages nomades en RdD. Les troupeaux passent la saison des pluies à l'intérieur des frontières de la RdD, mais peuvent aussi se déplacer dans les zones de pâturage en Somaliland, en Érythrée ou en Éthiopie pendant la saison sèche, d'où la nécessité de faire un recensement du cheptel animal[1]. La DISED et la DESV, avec l'appui technique de la FAO à Djibouti (à travers le TCP/DJI/3501), ont développé d'une façon participative une méthodologie pour le recensement du cheptel animal en RdD.

L'objectif est de concevoir une méthodologie pour déterminer le nombre d'animaux d'élevage, la composition par espèce ainsi que la distribution spatiale, dans le pays. Les données seront utilisées pour estimer les paramètres de production et la prévision de leur évolution.

Matériel et méthode

Cette méthodologie a été élaborée en prenant en compte les avis des leaders d'opinion et des experts techniques locaux ainsi que la documentation scientifique existante dans ce domaine. Il s'agit d'une analyse exhaustive des méthodologies appliquées dans d'autres pays ou contextes et de les adapter au contexte djiboutien.

Le recensement du cheptel propose de diviser la RdD en cinq strates:

- Strate I: Elle se compose des villes principales et de leurs périphéries respectives (Djibouti-ville, Dikhil, Ali-Sabieh, Arta, Tadjourah et Obock).
- Strate II: Elle regroupe les terres se trouvant à moins de deux kilomètres d'une source d'eau importante ou des routes ou des marchés. Il s'agit notamment des villes rurales telles que As Eyla, Holl, Loyada, Yokobi, Randa, Balho, Dorra, Khor Angar et Moulhoule.
- Strate III: Elle regroupe les terres se trouvant entre deux et dix kilomètres d'une importante source d'eau ou de pâturages.
- Strate IV: Elle regroupe les centres d'intérêt spécial par exemple, les marchés de bétail, les centres de quarantaine, les camps de personnes déplacées et les installations militaires.
- Strate V: Elle regroupe toutes les autres terres qui ne sont très connues comme le désert et les montagnes.

La période pour réaliser le recensement du cheptel animal national de la RdD doit être comprise entre Octobre/Novembre ou Mars/Avril. Cela a été mentionné que c'était le meilleur moment pour faire le recensement parce qu'il correspond à la saison la plus favorable dans toute la sous-région et où on est susceptible de trouver les populations et le bétail sur place. Cette intervention peut être combinée avec une éventuelle campagne de vaccination des animaux contre les maladies animales majeures.

Le présent document comprend une liste des travaux préparatoires et les meilleures pratiques pour la conduite du recensement du bétail en utilisant deux approches intégrées, (i) un relevé aérien et (ii) un dénombrement avec des agents recenseurs d'une part pour les zones urbaines et d'autre part pour les zones rurales.

[1]*Par définition, le recensement du cheptel animal implique que les exploitations d'élevage sont énumérées sur la base individuelle, de groupe ou de la propriété institutionnelle au sein du territoire de la République de Djibouti pendant une période de référence bien définie.*

Résultats et discussion

Méthodologie du recensement du cheptel animal
Travaux préparatoires

Les travaux préparatoires nécessaires à la réalisation d'un recensement national du cheptel animal (RNCA) sont les suivants :

i. de définir le cadre légal du RNCA;
ii. de préparer le budget et de mobiliser le fonds nécessaires pour l'exécution du RNCA;
iii. d'arrêter un calendrier d'exécution du recensement qui met en exergue les différentes activités et décliné en trois étapes : (a) pré-dénombrement, (b) dénombrement et (c) post-recensement;
iv. de mettre en place l'organisation administrative;
v. de concevoir un plan de communication ad hoc: le développement de la stratégie de communication, la consultation des usagers, la publicité du recensement et la promotion des produits du recensement ;
vi. de mettre en place un mécanisme pour assurer la bonne exécution des activités et de la qualité des données ;
vii. de préparer les zones de dénombrements (ZD) et la liste des ménages;
viii. de préparer des outils statistiques, développement de programme de tabulation et la conception de base de données ;
ix. de préparer le questionnaire ;
x. de faire des enquêtes pilotes du RNCA ;
xi. de préparer le manuel de formation des agents recenseurs ;
xii. de préparer le plan de tabulation du rapport ;
xiii. de planifier la diffusion des résultats du RNCA ;
xiv. de préparer le recrutement du personnel;
xv. de mettre en place un mécanisme permettant d'éviter tous les biais sur les données.

Des recommandations détaillées sont disponibles (Division de statistique des Nations Unies, 2008)

En outre, pour une exécution efficace et réussie du RNCA, nous devrons tenir compte des meilleures pratiques qui ont réussi dans d'autres pays, notamment ceux de la sous-région (p.ex. Somalie, Ethiopie et Soudan du Sud) :

- l'engagement au plus haut niveau des leaders politiques, traditionnels et religieux ;
- la mise en place d'une structure de pilotage du RNCA ;
- le développement d'une stratégie de communication pour le RNCA ;
- l'élaboration d'une matrice d'analyse des risques ;
- l'élaboration du questionnaire (papier ou numérique PDA) ;
- la mobilisation des ressources et des moyens logistiques ;
- la conduite d'un recensement pilote ;
- la conduite de l'enquête post-recensement ;
- Suivi et évaluation.

Le questionnaire comprend :

- Information sur le ménage
- Information sur le bétail
- Information sur la production

Méthodologie du recensement aérien

Le recensement aérien est effectué en utilisant la technique dite « Vol de reconnaissance systématique » (SRF) décrite par Norton-Griffiths (1978). Les zones de dénombrement du bétail à l'échelle du pays pour des emplacements mesurant 10 km x 10 km sont créées selon une procédure qui garantit que chaque partie de la zone de dénombrement est uniformément contrôlée. (Figure 1). Les avions volent à une altitude moyenne de 106 mètres au-dessus du sol, avec des ajustements sur un intervalle de 30 mètres. La vitesse sera de 180 km/h pour prendre des photographies aériennes (des observations) directes et obliques d'animaux d'élevage et estimer les troupeaux d'animaux d'élevage réparties le long d'une route déterminée (Buckland et al., 1993; Brochers et al., 2002; Cassey et McArdle, 1999).

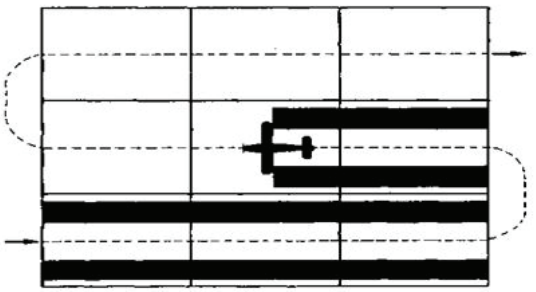


Figure 1: Vol d'aéronefs en lignes parallèles sur les zones de pâturages en saison sèche.

L'enquête aérienne va suivre des lignes de vols systématiques, pour couvrir 60% de la zone totale. Après avoir pris connaissance des zones de survols existantes et des procédures pertinentes pour les demandes de soutien et d'autorisation de survol par les autorités compétentes du Gouvernement de la RdD. Chaque ligne de vol va utiliser de manière systématique une grille de dimensions fixes. La photographie est à une résolution de 15 cm. L'imagerie verticale a été recueillie à l'aide d'une lentille 60 mm. L'analyse des données pour l'enquête aérienne fournit des estimations de la population d'animaux d'élevage calculées en utilisant la méthode 2 de Jolly des Unités de Taille Inégales (Jolly 1969). La première étape consiste à calculer la densité moyenne pour l'ensemble de la zone à partir de l'équation D = Σ yi / Σ ai, où yi est le total des animaux comptés dans la grille / la case i de la grille, la zone de la case ai de la grille, i l'échantillon numéro (1, 2, 3, ai, ..., n) et n le nombre total de case de la grille comptées. La population totale est donc P = DA où A est la zone de recensement de la surface totale et la densité moyenne D par unité de surface.

Les résultats sont présentés comme la taille estimée de la population, la densité (ind / km2) + leur erreur standard (SE) et la taille moyenne de groupe + SE. Lorsque la variance de la population est estimé à : Var (Y)= {N (N-n) $\sum$y2+D2$\sum$a2-2D$\sum$ay}/n (n-1) où N est le total des cases possibles de la grille dans la zone d'étude et n le nombre de cases de la grille échantillonnés. L'erreur standard de Y est SE (Y) = $\sqrt{}$ Var (Y). La précision de Y peut être exprimée par le coefficient de variation (CV), qui est calculé par CV (Y) = SE (Y) / Y, qui peut être commodément exprimée en pourcentage. Alternativement, la précision peut être indiquée par le calcul des limites de confiance pour Y à un degré de probabilité donné. Le 95% de CL (Y) = ± t × SE (Y), où «T» = n-1 degrés de liberté. Les valeurs de t de Student sont obtenues à partir des tableaux.

L'enquête aérienne :

i. Identifie avec précision l'emplacement et un nombre approximatif de ménages d'éleveurs, la taille du troupeau, les animaux d'élevage et le nombre d'animaux, et l'emplacement et le type de toits et des sources d'eau à sec de la saison grâce à un système de positionnement géographique; fournir une indication photographique des exploitations d'élevage. En outre, les imageries pourraient être analysées pour la faune, le type de couverture du sol, la zone agricole et le type d'utilisation des terres.
ii. Aide à la cartographie en fournissant des systèmes d'information géographique (SIG) pour les cartes coordonnées de l'imagerie satellite et le dénombrement basé sur les photos numériques et base de données géographique numérique des établissements de référence couvrant l'ensemble du pays. Ceux-ci seront utilisés pour décrire la situation physique, mais aussi des données sociales, économiques et culturelles qui sont interconnectés. Concevoir des secteurs de dénombrement / des Strates basées sur des grappes d'habitations et d'avoir systématiquement les coordonnées géographiques (latitude et longitude) de tous les logements de chaque strate. Cette conception capte chaque ménage résidant dans le cercle sélectionné / state au moment de l'entrevue, y compris ceux qui n'ont pas de logement permanent ou qui sont temporairement absents de leur logement. Cette conception élimine aussi la sous-représentation résultant de populations mobiles.
iii. Donne des effectifs indicatifs de l'élevage dans leur domaine vital. L'enquête aérienne

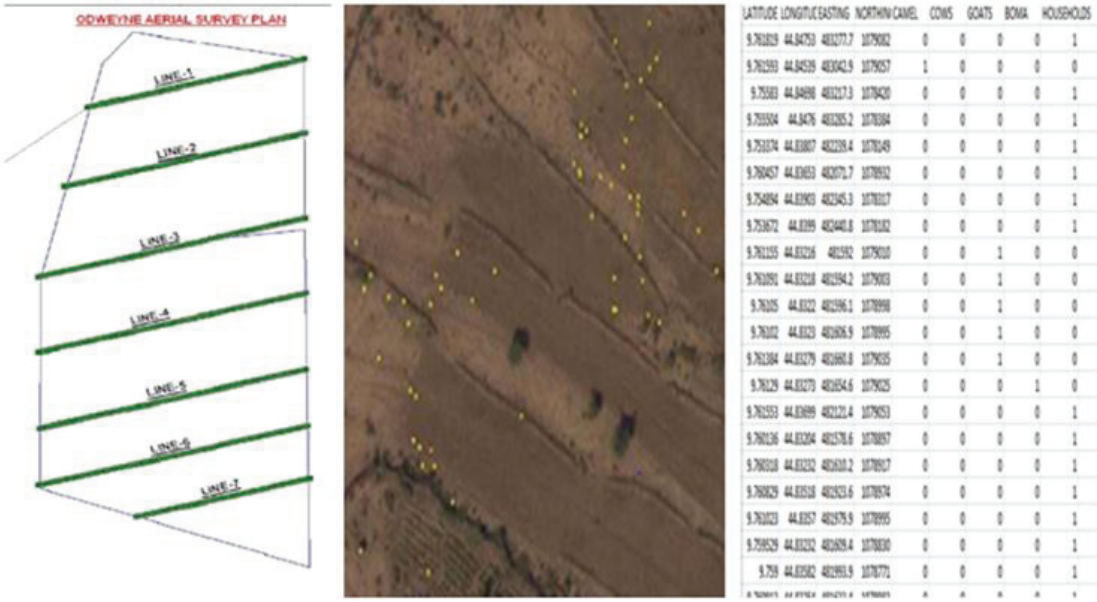


Figure 2: Enquête aérienne en Somaliland.

est proposée comme une procédure rentable pour obtenir des estimations non biaisées d'animaux, les tendances des troupeaux dans les grandes régions et leurs emplacements avec un intervalle de confiance de 90% (IC équivalent à un niveau de α = 0,10 de signification) de ± 25% ou moins) dans un court laps de temps. Un exemple du travail accompli en Somaliland est fourni ci-dessous (Figure 2).

Recensement des animaux par interview

L'agent recenseur va interviewer le chef de ménage de chaque exploitation d'élevage identifiée. Les données recueillies comprendront le jeune animal gardé à la maison; les données sur la productivité (naissances, mortalités, achats, ventes, données sur la santé animale, la production laitière); les dossiers d'élevage des femelles adultes; les mouvements des troupeaux - et les transhumances des animaux, les points d'eau; les infrastructures pour les animaux, par exemple les points d'eau, les contraintes et les opportunités perçues; le type de travail et leur utilisation, les principaux lieux de regroupement des animaux et les pâturages les plus courants à chaque saison. La technique « boule de neige » sera utilisée pour accélérer l'identification des ménages d'éleveurs.

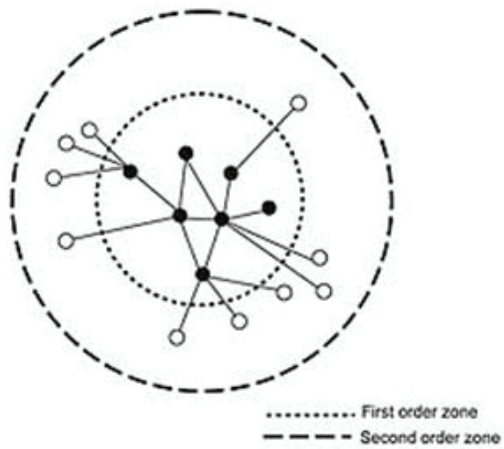


Figure 3: "Boule de neige pour l'identification de troupeaux d'animaux.

Méthodologie de recensement du cheptel animal sédentaire

La méthode de facto pour compter les troupeaux d'animaux va être mise en œuvre en utilisant du personnel préalablement formé (recenseurs par interview). L'approche comprend une opération complexe de collecte active de données et d'informations sur les animaux, la participation des ménages et des institutions spécialisées de l'élevage (abattoirs, quarantaine, fermes) accompagnée par la compilation, l'évaluation, l'analyse et la diffusion des données démographiques,

économiques et sociales concernant les animaux d'élevage en RdD en utilisant « Principles and Recommendations for Population and Housing Censuses, Revision » 2 (Nations Unies, Division de la statistique, 2008). L'unité de dénombrement sera le ménage individuel ou des campements de 758 zones de dénombrement fournis par la Direction de la statistique (voir Tableau 1).

Les données et informations de chaque ménage d'éleveurs seront collectées avec des questionnaires par un agent de recensement dans un secteur de dénombrement spécifié. Cela se fera dans un délai de cinq (5) jours pour répondre aux exigences de l'universalité et de la simultanéité.

Les exigences logistiques et budgétaires pour le RNCA sont estimées dans le Tableau 2 et à l'Annexe 1.

Méthodologie de recensement du cheptel animal nomade

La façon la plus appropriée pour localiser les cheptels animaux nomades et transhumants et/ou leurs propriétaires dépend des circonstances nationales et locales, et peut varier dans différentes parties de la République de Djibouti (RdD). Pour des raisons logistiques, les enquêtes de terrain doivent utiliser des cadres de recensement ciblés sur les sites où les animaux se rassemblent, comme les points d'eau, les points de vaccination, les cuves et les courses de pulvérisation, mais peuvent également inclure les campements temporaires saisonniers, les routes et les marchés des animaux, comme abordé dans les sections suivantes. Il y a deux problèmes ici: le premier est de localiser les animaux, c.-à-d. quels points de dénombrement doivent être choisis (points d'eau; cuves d'immersion, etc.). La seconde est, une fois que les points de dénombrement ont été choisis, comment collecter les données sur le nombre d'animaux vivants (questionnaires ou des observations directes).

L'utilisation de méthodes de collecte de données conventionnelles dans les recensements du cheptel animal en milieu rural conduit à une exclusion totale ou partielle des communautés pastorales. L'objectif du recensement est la prise en compte des ménages de pasteurs dans la collecte des données dans les zones rurales (Schelling, 2013). La méthodologie proposée combine l'utilisation du système de positionnement géographique (GPS) pour localiser les troupeaux et de la technique d'échantillonnage en boule de neige pour l'identification rapide et l'introduction rapide aux répondants »dans les établissements et groupes géographiques (Himelein et al., 2014).

Table 1: Répartition des zones de dénombrement en République de Djibouti.

Strates	ZD Urbaine	ZD Rurale	Nombre de ZD
Arrondissement 1	74	0	74
Arrondissement 2	119	0	119
Arrondissement 3	38	0	38
Arrondissement 4	156	0	156
Arrondissement 5	141	0	141
Région d'Ali-Sabieh	28	22	50
Région de Dikhil	22	42	64
Région de Tadjourah	12	45	57
Région d'Obock	8	15	23
Région d'Arta	10	26	36
Total	**608**	**150**	**758**

Tableau 2: Ressources humaines nécessaires pour la réalisation du recensement national du cheptel animal en République de Djibouti.

	Nombre de dénombreurs	Superviseur du dénombrement	Coordinateurs	Véhicules
Djibouti-ville	40	4	1	2
Région d'Ali-Sabieh	157	16	4	5
Région de Dikhil	197	20	5	6
Région de Tadjourah	330	33	9	9
Région d'Obock	347	35	9	10
Région d'Arta	87	9	3	3
Total	**1156**	**117**	**31**	**35**

Dans les zones rurales, un point GPS sera identifié au centre du campement. Tous les animaux dans les ZD / campement éligible se trouvant à l'intérieur d'un cercle tracé autour des points sélectionnés sont interrogés d'une manière conforme à la culture en intégrant les sous-unités de la famille élargie (Figure 4).

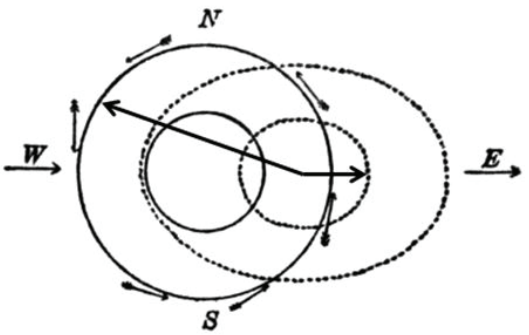


Figure 4: Recensement en cercles concentriques dans les states II et III.

Les superviseurs et agents recenseurs devront contacter les anciens du village et d'autres personnes bien informées pour recueillir des informations sur le nombre de maisons, la population et les caractéristiques socio-économiques du village principal et quatre grands villages satellites. Pendant ce temps, l'un des enquêteurs fait le tour des villages et fait un décompte physique du nombre de têtes d'animaux dans ces villages. Les principaux villages et les villages satellites sont ensuite reportés sur une carte en utilisant le système de positionnement géographique (GPS), qui a fourni les coordonnées (longitudes et latitudes) pour ces villages.

L'enquête de terrain sera utilisée pour administrer un questionnaire spécifique aux chefs des ménages. Les données collectées vont inclure les jeunes animaux élevés dans le ménage; les données sur la productivité (naissances, mortalités, sorties, ventes, données sur la santé animale, la production de lait); les registres des animaux femelles adultes; les mouvements des troupeaux et les zones de transhumances, les points d'eau; les infrastructures d'élevage, par exemple, points d'eau, contraintes et opportunités perçues; type de main d'œuvre et son utilisation, les principaux sites de rassemblements d'animaux et les pâturages les plus courants de chaque saison.

Le comptage sur le terrain sera nécessaire pour obtenir une ventilation fiable par âge et par sexe. Mais certaines méthodes de comptage sur le terrain seront mieux indiquées que d'autres pour cela. Les jeunes animaux ne peuvent pas tous être vaccinés, être amenés aux réservoirs d'eau ou même être amenés aux points d'eau. Les mâles peuvent être tenus à l'écart de certaines interventions, car ils peuvent être difficiles à manipuler ou il y a des craintes par rapport à leur fertilité pouvant être endommagée. Ainsi, alors que le comptage aux points d'eau, aux réservoirs d'eau et aux points de vaccination pourra être utile, il pourra produire des résultats partiels et biaisés.

L'enquête sur le terrain est la meilleure façon d'obtenir des détails sur les structures de l'âge et du sexe et même la possibilité de différencier entre les ovins (moutons) et les caprins (chèvres). Différencier les moutons des chèvres n'est pas toujours facile à distance, avec la position de la queue pouvant peut-être constituer le meilleur indicateur.

Pour obtenir plus de détails sur les systèmes de production, tels que la façon dont les animaux sont nourris et abreuvés, les niveaux de consommation des ménages et à quels âges les animaux sont vendus et pour quelles raisons, il est nécessaire de rencontrer et de parler avec les producteurs; il n'y a pas d'autres moyens d'obtenir ces informations.

En cas de grands troupeaux, il peut être préférable de prendre des photos numériques ou la couverture vidéo pour le comptage ultérieur ou utiliser le décompte à la main, ou un compteur qui peut être cliqué une fois pour chaque animal compté et fournir un total croissant de tous les animaux comptés. Un décompte à la main peut être obtenu avec des compteurs simples ou multiples, de sorte que différents types ou espèces peuvent être comptés en même temps.

Analyse statistique

Les données des questionnaires, le nombre d'animaux présentés aux vétérinaires ou le contrôle/traitement, et les totaux estimés de l'enquête aérienne devraient tous être analysés par un seul groupe pour la cohérence de l'approche et la présentation. L'équipe du projet devrait travailler avec les statisticiens afin de leur expliquer les informations spécifiquement recherchées et de décider de la manière de la présentation. Ce travail préparatoire doit être entrepris avant que les données ne soient présentées aux statisticiens, de sorte que l'analyse et la présentation des résultats peuvent se produire aussi rapidement que possible et que le travail dans le reste du pays puisse être entamé sans délai.

Point clés

1. La RdD, a une population pastorale très dispersée sur une grande surface. Procéder à un recensement au sein des communautés pastorales exige une planification et l'affectation de budget adéquat. L'inaccessibilité, le caractère mobile des habitants, un niveau élevé de taux d'analphabétisme, et l'immensité de la zone reste les grands défis dans la conduite du recensement national du cheptel animal (RNCA). Les leadeurs coutumiers et religieux sont essentiels pour mobiliser la population pastorale et améliorer les réponses des interviewés.
2. Afin d'éviter des omissions ou des doublons, les cartes des zones de dénombrement (ZD) seront préparées comme ceux de la population urbaine sédentaire. Ces cartes de ZD seront rendues possibles par l'utilisation de l'imagerie par satellite. Les limites des ZD sont définies par des structures permanentes comme des collines ou des rues, etc.
3. Le nombre de ménages dans chaque ZD sera compris entre150 et 200. La taille d'une ZD pastorale peut déroger à cette fonction de la densité de la population.
4. Des efforts doivent être faits pour limiter la distance entre les points de visite parce que la plupart des recenseurs seront à pied une fois qu'ils seront dans les limites de leur ZD de travail. L'accès à chaque ZD se fera avec des minibus ou avec des 4x4. Une équipe plus grande sera utilisée dans les campements pastoraux pour s'assurer que l'enquête est menée aussi vite que possible.
5. Les enseignants pourront être recrutés pour ces exercices. Les superviseurs doivent faire partie du personnel de la DISED ou de la DESV pour résoudre les problèmes techniques. Pour la mise en œuvre de l'enquête, chaque équipe de recenseur sera muni d'un dispositif GPS dans lequel les coordonnées de la ZD spécifique est pré chargé. Les équipes d'agents recenseurs seront conduits aussi près que possible de la ZD et se déplacent ensuite le reste du chemin à pied, si nécessaire. La collecte de données en temps réel et la synchronisation se fera au moyen de questionnaires électroniques

dans les tablettes avec menu déroulant.

6. L'imagerie satellite: l'imagerie par satellite sera demandée à l'USAID et l'ensemble de données spatiales décrivant la couverture terrestre, l'utilisation des terres, et d'autres caractéristiques géographiques sera utilisé comme entrée pour délimiter des strates mutuellement exclusives. La méthodologie propose d'utiliser une résolution de 5 mètres de SPOT 5 et imageries Quickbird résolution 60 cm pour la délimitation des zones de dénombrement (ZD) et des limites dans les zones pastorales.

Remerciements

Nous tenons à remercier le commissaire au plan chargé de la statistique M. Amareh Ali Said, pour avoir facilité à travers la Direction de la Statistique et des Études Démographiques (DISED), l'élaboration de la présente méthodologie. Nos remerciements vont également à l'endroit de M. Mohamed Ahmed Awaleh, ministre de l'Agriculture, de l'Eau, de la Pêche, de l'Élevage et des Ressources Halieutiques de la République de Djibouti, pour l'intérêt qu'il a porté à ce projet de recensement national du cheptel animal (RNCA) en République de Djibouti.

Nous remercions Mme Emmanuelle Guerne-Bleich, représentante de l'Organisation des nations-unies pour l'alimentation et l'agriculture (FAO) auprès de la République de Djibouti et de l'IGAD (Autorité Intergouvernementale pour le Développement) pour sa confiance et son engagement.

Ce travail a été financé par la FAO dans le cadre du projet TCP/DJI/3501 "Méthodologie pour un recensement national du cheptel animal.

Bibliographie

Borchers D L, Buckland S T and Zucchini W (2002). Estimating animal abundance. Springer, London, England.

Buckland S T, Anderson D R, Burnham K P and Laake J L (1993). Distance sampling: Estimating abundance of biological populations. Chapman and Hall, London, England.

Cochran W G (1977). Sampling techniques. John Wiley and Sons, New York, New York, USA.

Himelein K, Eckman S and Siobhan Murray S (2014). Sampling Nomads: A New Technique for Remote, Hard-to-Reach, and Mobile Populations. Journal of Official Statistics, 30 (2): 191-213, http://dx.doi.org/10.2478/jos-2014-0013

Jolly G M (1969). "Sampling methods for aerial census of wildlife populations." East African Agriculture and Forestry Journal 34: 46-49.

Norton-Griffiths M (1978). Counting Animals. Handbook No.1 in a series of handbooks on techniques currently used in African wildlife ecology (Ed: J. Grimsdell). African Wildlife Foundation, Nairobi.

Schelling E (2013). Guidance note on sampling methodologies to enhance the inclusion of mobile and semimobile pastoralists. UNICEF, Nairobi.

United Nations Statistics Division (2008). Principles and recommendations for population and housing censuses, Revision 2. Statistical Papers Series M, 67 (2). UNSD, New York.

PREVALENCE OF FISH DISEASES IN FISH FARMS IN KATSINA STATE, NIGERIA

Dauda A B[1] and Ibrahim H I[2]
[1]Department of Fisheries and Aquacultural Technology, Federal University Dutsin-Ma PMB 5001, Dutsin-Ma, Katsina State, Nigeria
tdabak@gmail.com, +2348062085120
[2]Department of Agricultural Economics and Extension, Federal University Dutsin-Ma, PMB 5001, Dutsin-Ma, Katsina State, Nigeria

Abstract

Prevalence of fish disease in fish farms in Nigeria was investigated using data from structured questionnaire obtained during farm visits, and analysed using descriptive statistics and multiple linear regression. Findings from the study revealed that majority of the farm manager's (45.7) have less than 3 years of experience in fish farming. Most of the farms (60%) have operated for less than 5 years. Few of the fish farms (8.5%) have more than 21 ponds/tanks. Diseases outbreak has been experienced in about 54.3% of the fish farms among which 31.6% experience it often. Bacteria diseases were reported to be the dominant diseases in the state and the associated economic loss was valued between ☐50,000 and ☐1,000,000. Majority of the farmers (89.5%) were able to control the diseases with 84.2% of them controlling the diseases with antibiotics. The regression result had coefficient of determination 0.624 with f-ratio 7.746 (p=0.000), Manager's knowledge, size of the farm and number of ponds have influence on estimated financial loss to diseases. Prevalence of fish diseases and its associated economic loss in fish farms in the state is on the high side, therefore the needs to improve on management of diseases to save the industry.

Key words: antibiotics; aquaculture; bacteria; disease outbreak; economic loss.

LA PREVALENCE DES MALADIES DU POISSON DANS LES FERMES PISCICOLES DE L'ETAT DE KATSINA AU NIGERIA

Résumé

La prévalence des maladies du poisson dans les fermes piscicoles du Nigeria a été étudiée en utilisant les données d'un questionnaire structuré obtenues lors des visites aux fermes, et analysées à l'aide de statistiques descriptives et de la méthode de la régression linéaire multiple. Les résultats de l'étude ont révélé que la majorité des exploitants (45,7) avait moins de 3 ans d'expérience en élevage de poissons. La plupart des exploitations agricoles (60%) fonctionnaient depuis moins de 5 ans. Peu de fermes piscicoles (8,5%) avaient plus de 21 étangs / réservoirs. L'apparition des maladies a été étudiée dans environ 54,3% des fermes piscicoles, elle était fréquente dans 31,6% des fermes. L'étude a révélé que les maladies bactériennes étaient dominantes dans cet État, et la perte économique associée a été évaluée entre ☐50.000 et ☐1.000.000. La majorité des agriculteurs (89,5%) était en mesure de contrôler les maladies ; 84,2% d'entre eux traitaient les maladies avec des antibiotiques. Le résultat de la méthode de la régression linéaire avait un coefficient de détermination de 0,624 avec f-ratio de7,746 (p = 0,000). La connaissance des gestionnaires, la taille de la ferme et le nombre d'étangs ont une influence sur la perte financière estimée associée aux maladies. La prévalence des maladies du poisson et la perte économique qui en résulte dans les fermes piscicoles de l'État de Katsina sont élevées. Par conséquent, il est nécessaire d'améliorer la prise en charge des maladies pour sauver ce secteur.

Mots-clés : antibiotiques ; aquaculture ; bactéries ; foyer de maladie ; perte économique.

Introduction

Aquaculture is the farming of aquatic organisms by intervention of man in the rearing process to enhance production and private ownership of the stock being cultivated, compared to fishing, this activity allows a selective increase in the production of species used for human consumption, industry or sport fishing . It has been the engine driving growth in total fish production since mid 1990s, as capture production has leveled off (Dauda et al., 2014). There had been tremendous growth in global aquaculture in the last fifty years, from total production of less than a million to 63.6 million tons in 2011 and a corresponding value of US$126.77 billion, the current status also represent over 30 percent growth between 2006 and 2011 from 47.3 million tons in 2006 to 63.6 million tons in 2011 (FAO, 2012). Though the history of aquaculture has been long and can be dated back to 475 BC in china, it became important in the late 1940's, because the method of aquaculture is being used to restock the waters as a complement to natural spawning (Cruz et al., 2012). The history of aquaculture in Nigeria can be dated back to 1950's and since then the production has been witnessing gradual growth. The aquaculture production in Nigeria increased greatly from 40,000 metric tons in 2006 contributing 6% of domestic fish production (Adeogun et al., 2007) to 200,535 metric tons in 2011 contributing about 24% of domestic production (FAO, 2012). As stated by Cressey (2009), aquaculture business is now very lucrative and intensive culture system that is characterized with high stocking density with expectation of rapid growth and high survival rate is now well practiced.

According to Ajani et al. (2011), intensive culture systems expose cultured fish to a lot of stress and make them susceptible to disease attacks. Disease is now regarded as a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries (Bondad-Reantaso et al.,2005; Dauda et al.,2015). According to Cruz et al. (2012) outbreaks of viral, bacterial, and fungal infections have caused devastating economic losses worldwide, China reported disease-associated losses of $750 million in 1993, while India reported $210 million losses from 1995 to 1996. Though there is problem of poor farm record keeping in Nigeria and the exact monetary loss to diseases in aquaculture may be difficult to ascertain. Ajani et al (2011) stated that, Fish diseases in most farms in Nigeria has led to a substantial monetary loss to fish farmers as the production costs will be on increase due to disease outbreak while the investment loss will occur in dead fish, cost of treatment and reduction in growth.

Aquaculture development in Katsina State, Nigeria is still at it infancy though there is concerted effort to sustain the growth, however there is need to resolve some major problems hampering the development of aquaculture in the state (Dauda et al., 2015). Disease has been identified as a major constraint to aquaculture development in the state and therefore there is need for detailed understanding of the prevalence and incidence of fish disease in the state in other to put in place necessary measures to combat the menace. This study therefore examines the prevalence of fish diseases as well as associated economy loss to diseases in fish farms in Katsina State, Nigeria.

Material and methods

Study area

The research work was done in Katsina state, Nigeria. The state is in the North-western part of the country, with a land mass of 24,192 km² and estimated population of 6,483,429 million (NPC, 2006). The state has three agro-ecological zones namely, Sahel savannah in the north-eastern part of the state, Sudan savannah in the central and Northern guinea savannah in the southern part of the state. The Sahel zone is characterized with generally sandy soil of

low fertility and rainfall of less than 500mm per annum, the Sudan savannah has mostly sandy soil and rainfall in the zone is between 500 and 900mm per annum and duration of rainy season of 90-130 days, while for the Northern guinea savannah the rainy period ranged between 130 and 190 days and the rainfall is between 1000mm and 1400mm per annum (Adekunle et al., 2005)

Data collection and analysis

Data was collected with the use of structured questionnaire during farm visits to all the known farms in the state, as obtained in the list of fish farms in the state provided by Katsina State Agricultural and Rural Development Agency (KTARDA). Data were collected on the demographic characteristics of the farms and socio-economic characteristics of the farm managers as well as on prevalence, management and associated economic loss to fish diseases in the fish farms. Data collected were analysed using descriptive statistics and multiple linear regression. The multiple linear regression analysis was used to assess the relationship between estimated financial loss to diseases and demographic characteristics. The implicit models of the regression are:

$Y = f(X1, X2, X3, X4, X5, X6, X7, e)$,

Y = Estimated financial loss to diseases

X^1 = Manager's Educational status

X^2 = Manager's years of experience in fish farming

X^3 = Manager's Knowledge of fish farming

X^4 = Age of the farm in years

X^5 = Size of the farm in hectares

X^6 = Number of ponds/tanks

e = Error term

Results

Demographic characteristics of the fish farms and farm managers

The important demographic characteristics of the fish farms visited and that of the managers are reported in Table 1. Majority of the farm managers (40%) have secondary school certificate and below, while 25.7% of them are graduate, only 8.6% are fisheries and aquaculture graduate. Some 20% of the manager had undergone formal training/ certificate programme in fish farming but 48.6% constituting the majority were only trained on the job without any formal certification. Majority of the managers (42.8%) have 1 to 3 years experience in fish farming and Larger percentage, constituting 60% belonged to fish farmer's association. Majority of the fish farms (60%) in the state are between age of 1-5 years, while the dominant size of the farms (57.1%) is between 0.01 and 0.50 hectares with only 2.9% having land mass above 3.10 hectares but below 8.00 hectares. The number of ponds also largely varied with a total of 60% constituting the majority of the farms having between 1 and 5 holding facilities.

Prevalence and management of fish diseases in fish farms in Katsina State

The response on prevalence of fish diseases, management and economic loss due to the diseases are presented in Table 2, Disease outbreak has been experienced by 54.3% of the farms in the state at one point or other, among the farms that experienced diseases, majority (52.6%) had not experienced diseases outbreak often but 31.6% reported that they had experienced it often. Dominant majority (89.5%) of the farms that had experienced disease outbreak were able to identify the cause of the diseases and list of major diseases and symptoms observed and mentioned by the farmers are broken skull, fin and tail rot, swollen gut, itch, lesions and haemorrhage. Among the farms visited, 57.9% reported bacteria diseases (Fin and tail rot) as the main disease experienced. Majority of the farms, (68.4%) had 1 to 3 ponds affected, with estimated economic loss varying from less than ☐50, 000 to over ☐1,000,000 and majority of the farms constituting, 42.1% of those who have experienced disease outbreak had estimated lost between ☐51, 000 to

Table 1: Demographic characteristics of the fish farms and farm managers

Variable	Response	Frequency	Percentage
Manager's Educational status			
	Postgraduate	5	14.3
	Graduate	4	11.4
	Diploma/NCE	12	34.3
	Secondary school	9	25.7
	Primary school	2	5.7
	Quranic education	2	5.7
	Non-formal education	1	2.9
	Total	**35**	**100.0**
Manager's Knowledge of fish farming			
	Fisheries graduate	3	8.6
	Agriculture/related courses graduate	6	17.1
	Training/certificate programme	7	20.0
	Trained on the job	17	48.6
	Others	2	5.7
	Total	**35**	**100.0**
Manager's years of experience in fish farming			
	Less than a year	1	2.9
	1-3	15	42.8
	4-6	8	22.9
	7-9	4	11.4
	10 and above	7	20.0
	Total	**35**	**100.0**
Membership of fish farmer's association			
	Yes	21	60.0
	No	14	40.0
	Total	**35**	**100.0**
Age of the farm in years			
	Less than 1 year	1	2.9
	1-5	21	60.0
	6-10	6	17.1
	11-15	5	14.3
	16-20	2	5.7
	Total	**35**	**100.0**
Size of the farm in hectares			
	0.01-0.50	20	57.1
	0.60-1.00	6	17.1

Variable	Response	Frequency	Percentage
	1.10-3.00	8	22.9
	3.10- 8.00	1	2.9
	Total	**35**	**100.0**
Number of ponds/tanks			
	1-5	21	60.0
	6-10	5	14.3
	11-15	5	14.3
	16-20	1	2.9
	21 and above	3	8.5
	Total	**35**	**100.0**

Table 2: Prevalence and management of fish diseases in fish farms

Variable	Response	Frequency	Percentage
Disease outbreak			
	Yes	19	54.3
	No	16	45.7
	Total	**35**	**100.0**
Times experienced disease outbreak			
	Once	3	15.8
	Not-often	10	52.6
	Often	6	31.6
	Total	**19**	**100.0**
Disease identified			
	Yes	17	89.5
	No	2	10.5
	Total	**19**	**100.0**
Disease type			
	Unidentified	2	10.5
	Fungi	1	5.3
	Bacteria	11	57.9
	Nutritional	1	5.3
	Others	4	21.0
	Total	**19**	**100.0**
No of ponds/tanks affected			
	1-3	13	68.4
	4-6	2	10.5
	7-9	1	5.3
	10 and above	3	15.8
	Total	**19**	**100.0**

Variable	Response	Frequency	Percentage
Financial loss due to diseases (□)			
	< 50000	2	10.5
	51000-100000	8	42.1
	101000-500000	7	36.8
	501,000-1000000	1	5.3
	>1000000	1	5.3
	Total	**19**	**100.0**
Diseases controlled			
	Yes	17	89.5
	No	2	10.5
	Total	19	100.0
Diseases treated by	Self	6	31.6
	Consultant	13	68.4
	Total	**19**	**100.0**
Disease treated with			
	Antibiotic	16	84.2
	Salt	1	5.3
	Others	2	10.5
	Total	**19**	**100.0**

Table 3: Relationships between demographic characteristics and prevalence, management and estimated loss to fish diseases

Variables	Regression Coefficient	T-ratio	Pvalue
Manager's Educational status (X1)	-9377.208	-0.337	0.739
Manager's Years of experience (X2)	17721.210	1.452	0.158
Manager's Knowledge of fish farming (X3)	83741.866	2.041*	0.051
Age of the fish farm (years) (X4)	-8624.002	-0.906	0.373
Size of the fish farm (Hectares) (X5)	-124390.702	-2.888***	0.007
Number of ponds/tanks (X6)	43794.124	5.957***	0.000
R2 %	62.4%		
F-Ratio	7.746***		0.000

□100, 000 to diseases. Larger percentage of 89.5% was able to control diseases on their farms, majority (68.4%) employed the services of consultant for disease treatment and the treatment was done using antibiotics by 84.2% of the respondents.

Relationships between demographic characteristics of the farm and farm managers and estimated economic loss to diseases in fish farms

The result of regression analysis ran to elicit the relationship between demographic characteristics and economic loss to diseases is presented in Table 3., The coefficient of determination (R2) is 0.624 with f-ratio 7.746 which is significant at 1% level (P = 0.000). This implies that 62.4% of the variation in the estimated financial loss is determined by the explanatory variables.

Among the demographic characteristics, size of the farm in hectares,

number of ponds/tanks affected and manager's knowledge of fish farming have significant influence on the estimated financial loss. Size of the farm have t-ratio -2.888 which is significant at 1% (P=0.007), number of ponds/tanks affected have t-ratio, 5.957 which is significant at 1%level (P=0.000) and Manager's knowledge of fish farming have t-ratio 2.041 which is significant at 10% level (P=0.051).

Discussion

Though majority of the farm manager's have post secondary school educational but only a few of them are graduate, this can be regarded as a problem because considering the needs for technology adaptation and innovation in the dynamic world of fish farming, the ones with lower level of education may not be able to catch-up very fast with advancement in fish farming practices. This agrees with the report of Abdsalam et al. (2010) who stated that the level of education of a farmer is an important factor that determines his/her ability to understand policies or programmes that affect farming. Moreover, among the graduate, only a minute percentage have educational background that has direct link with fish farming, this potent more danger as most of the farm will rely more on external advice and information and there may even be difficult in differentiating between quack and expert in terms of advice, this result is in tandem with that of Dauda et al. (2015) who noted inadequate technical expertise in the aquaculture industry of the region. The fish farming industries in the state is handled with people who are just learning with majority having less than 5 years on the job, so it may be difficult in handling unexpected emergency among which may result into disease outbreak on the farm. Participation of majority of the fish farm managers in fish farmer association is a good thing and if the discussion in the meetings is robust it will be a good opportunity of bridging the gap between the old and the new fish farmers and as well assist those with lesser educational qualification.

Disease outbreak has been experienced in larger numbers of the fish farms in the state and majority of the farms have experienced disease outbreak more than once though less than half of the farms experienced diseases often, the disease outbreak in fish farms in Katsina State Nigeria can be regarded as a major problem and this is in line with findings of Bondad-Reantaso et al. (2005) and Dauda et al. (2015). Ability to identify the type of disease in most of the farm is a good one, the report of the findings indicated that bacteria diseases are the most prominent one among the different types of disease reported, this can be justified by the ubiquitous characteristics of bacteria and availability of many of the opportunistic pathogenic bacteria in culture water especially when the quality of the water is bad (Danba et al., 2014). The bad water quality encourages diseases in two ways, one it will be carrying a lot of opportunistic disease causing organism and two, it will stress the fish and make it susceptible to attack from the pathogens. As stated by Akinwole and Iyiola (2012), the ultimate success or failure of an aquaculture operation is determined by water quality of the culture environment. Though the study revealed that majority of the farms have between 1-3 ponds affected, but over 15.8% having 10 ponds and above affected represent a lot of economic loss especially in a state where fish farming is relatively at its infancy and small scale farmers are still dominating the industries. Despite the higher number of small scale fish farms in the study area, a good number of the farms still loses above □50,000 to diseases per annum while some loses over a million naira per annum, this can be classified as huge economical loss. If it is not properly checked, the loss may grow to the level of loss in fish farms in China and India as reported by Cruz et al. (2012). Majority of the farms reported that they were able to control the disease and this is putting a hope for future development of aquaculture in the state, but larger number of the farms could not control the disease on their own but have to employ the services of a consultant. That is another economic loss to diseases and representing overall increment in the cost of fish production (Ajani et al., 2011), and therefore reduces the expected profit,

if this is continuous and not well managed, it may render aquaculture business non-lucrative as opposed to the opinion of Cressey (2009). The treatment of the disease using antibiotics, though solving the problem on the temporary basis but it should not be encouraged because it poses more future danger. This is in line with the report of WHO (1999) who documented existence of various antibiotic resistant strain of human pathogen such as motile Aeromonas spp., Edwarsiella. tarda, Escherichia coli, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae and observation of Kim et al. (2004) and Sorum (2006) who mentioned that aquatic bacteria can also develop resistance genes as a consequence of exposure to antimicrobial agents. The possible occurrence of antimicrobial residues in aquaculture products may also pose threat to human health (WHO, 2006).

The coefficient of determination of the regression is well above 50% and significant, this indicated that the explanatory variables have influence on the estimated financial loss to fish diseases and therefore the postulated effect of individual parameters can be relied upon. This is in line with the findings of Onyeneke and Nwaiwu (2012). Manager's knowledge of fish farming have significant effect on the estimated financial loss to diseases, this can be associated with larger number of the manager's with inadequate knowledge of fish farming and therefore the high prevalence of fish disease which finally resulted in economic loss. This finding agrees with the report of Abdulsalam et al. (2010) who stated that knowledge of a farmer is an important factor that determines his/her ability to understand policies or programmes that affect farming. Majority of the fish farms in the state operates on a small portion of land, and consequentially the need for intensification which may lead to fast degradation of water quality and subsequently stress the fish and expose them to diseases, therefore lead to high economic loss (Bondad-Reantaso, 2005). Number of ponds/tanks in the farms also have significant effect on the estimated financial loss, this may be connected with the fact that the higher the number of ponds/tanks in the farms, the higher the tendency of diseases prevalent (when there is outbreak) and the higher the possible financial loss to diseases

Conclusion

The findings of the research established that prevalence of fish diseases and its associated economic loss in fish farms in the state is on the high side and therefore there is need to improve on management of diseases to save the industry from collapsing. Size of the farms, number of ponds/tanks and manager's knowledge of fish farming are important factors affecting economic loss to fish diseases. The industry therefore require intervention of government and extension agents to assist with man power development, and to encourage the farm owners to increase their farm size in order to reduce high level of intensification that if not well managed will continue to encourage prevalence of fish diseases. Training and re-training of the farm owners and farm managers on best practices for management of fish farms is also recommended.

References

Abdulsalam Z, Yaro A M, Alobo A J, 2010. Assessment of level of awareness on climate change in some selected local government area of Jigawa State, Nigeria. Proceedings of the 11th Annual National Conference of National Association of Agriculutural Economics, November 30 – December 3, 2010, FUT Minna, Niger State, Nigeria.

Adekunle A A, Olowu T A, Ladele A A, 2005. Bridging the Communication Gap between Scientists and Farmers in Katsina State of Nigeria. Published by International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 26pp.

Adeogun O A, Ogunbadejo H K, Ayinla O A, Oresegun A, 2007. Urban Aquaculture: Producer perceptions and practices in Lagos State Nigeria. Middle-East J. Sci. Res. 2(1): 21 – 27

Ajani E K, Akinwole A O, Ayodele I A, 2011. Fundamentals of Fish Farming in Nigeria. Walecrown publishers Ibadan. 158pp.

Akinwole A O, Adeola I O, 2012. Interrelationship

of Temperature, pH, Dissolved Oxygen and Nitrogenous waste in Fish culture systems. NJRED, 6: 38-42.

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Tan Z, Shariff M, 2005. Disease and health management in Asian aquaculture. Vet. Parasitol, 132: 249–272

Cressey D, 2009. Aquaculture: future fish. Nature, vol. 458, no. 7237, pp. 398–400.

Cruz M T, Ibanez A L, Hermosillo O A M, Saad H C R, 2012. Use of Probiotics in Aquaculture. International Scholarly Research Network ISRN Microbiology, Volume 2012 (2012), Article ID 916845, 13 pages

Danba E P, Bichi A H, Ishaku S, Ahmad M K, Buba U, Bingari M S, Barau BW, Fidelis U F, 2014. Occurrence of Pathogenic Bacteria associated with Clarias gariepinus in Selected Fish Farms of Kumbotso Local Governement Area of Kano State, Nigeria. Bayero Journal of Pure and Applied Sciences, 7(2): 145 – 149

Dauda A.B., A. Dasuki and A.H.Bichi, 2015. Analysis of Constraints to Aquaculture Development in Sudano-Sahelian Region of Nigeria. Tropical and Subtropical Agroecosystems, 18 (2): 189 -193. http://www.veterinaria.uady.mx/ojs/index.php/TSA/article/view/2074

Dauda A B, Oladele A H, Elaigwu A M, 2014. Analysis of Availability and Consumption of Cultured Fish in some Local Government Areas (LGAs) of Katsina State. Production Agriculture and Technology (PAT); 10 (2): 110-118, www.patnsukjournal.net/currentissue

FAO, 2012. The State of the World Fisheries. Food and Agriculture Organization, Rome, Italy. 230pp.

Kim S, Nonaka L, Suzuki S, 2004. Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. FEMS Microbiol. Lett. 237, 147– 156.

NPC, 2006. Population and housing census: Enumerators manual. National Population Commission (NPC), Federal Republic of Nigeria. pp 1-16.

Onyeneke R U, Nwaiwu J, 2012. Micro level analysis of beef and fish in Imo State, Nigeria. Agricultural Research and Reviews; 1(1)1 – 8. http://www.wudpeckerresearchjournals.org/ARR

Sørum H, 2006. Antimicrobial drug resistance in fish pathogens. In: Aarestrup, F.M. (Ed.), Antimicrobial Resistance in Bacteria of Animal origin. ASM Press, Washington DC, pp. 213– 238.

World Health Organization, 2006. Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance: Seoul, Republic of Korea, 13– 16 June 2006.

World Health Organization, 1999. Joint FAO/NACA/WHO Study Group on food safety issues associated with products from aquaculture. WHO Technical Report Series, No 883

HEALTH PROPHYLAXIS IN GOAT BREEDING IN SOUTHERN AND CENTRAL CÔTE D'IVOIRE

Emanfo A S A[1]*, Esmel M E[2], Komoin-Oka A M A C[3], Koné M W[1]
[1]Laboratoire de Biologie et de Cytologie Animale, UFR des Sciences de la Nature, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.
[2]Agence Nationale d'appui au Développement rural, BP v 183 Abidjan, Côte d'Ivoire,
[3]Laboratoire Central Vétérinaire de Bingerville (LCVB), BP 206 Bingerville, Côte d'Ivoire.

Summary

A survey on the health prophylaxis in goat farming was conducted, in five regions of southern and central Côte d'Ivoire, within 45 goat farms. It aimed to appreciate the methods of breeders in animal management, identify common diseases, describe their symptoms and take inventory of products used to treat them. The results revealed that 93.3 % of farmers have not received any training on farming techniques against 6.7 % of breeders coming from a farming school. The feed is ensured mainly by urban fodder (48.9 %) and natural pastures (51.1%); none breeder has a modern pasture. Most farmers (77.8 %) have never practiced the disinfection of their farms and livestock equipment. Evoking semiotics, 75.6 % of farmers have found cases of death without symptoms. The observed frequency of diarrhea was 88.9 %, including 13.3 % of bloody diarrhea. Furthermore, 62.2 % of farmers claimed to have seen worms in feces. Slimming frequent symptom is estimated by 15.6 % of livestock farmers and signs of loss of appetite in 13.3 % of them. Only 6.7 % of farmers used treatments, once per quarter, of their animals; while 75.6 % did so occasionally. Veterinary drugs used are: Levamisole (6.7 %), Albendazole (71.2%), Ivermectin (13.3 %) and oxytetracycline (8.9 %). Incorrect use of these products by some breeders may cause the resistance of gastrointestinal parasites.

Keywords: Investigation, health prophylaxis, dwarf goats, Ivory Coast

LA PROPHYLAXIE SANITAIRE EN ÉLEVAGE CAPRIN AU SUD ET AU CENTRE DE LA CÔTE D'IVOIRE

Resumé

Une enquête sur la prophylaxie sanitaire en élevage caprin a été réalisée dans cinq régions du sud et du centre de la Côte d'Ivoire, au sein de 45 installations caprines. Elle visait à apprécier les méthodes des éleveurs dans la gestion des animaux, identifier les pathologies courantes, décrire leurs symptômes et faire l'inventaire des produits utilisés pour les traiter. Les résultats révèlent que 93,3 % des éleveurs n'ont reçu aucune formation sur les techniques d'élevage contre 6,7 % d'éleveurs issus d'une école d'élevage. L'alimentation est assurée essentiellement par des fourrages urbains (48,9 %) et des pâturages naturels (51,1 %) ; aucun éleveur ne dispose d'un pâturage moderne. La plupart des éleveurs (77,8 %) n'ont jamais pratiqué la désinfection de leurs fermes et du matériel d'élevage. Evoquant la sémiologie, 75,6 % des éleveurs ont constaté des cas de mortalité sans symptômes. La fréquence des diarrhées observées est de 88,9 %, dont 13,3 % de diarrhées sanguinolentes. Par ailleurs, 62,2 % des éleveurs prétendent avoir observé des vers dans les fèces. Les signes d'amaigrissement sont estimés fréquents par 15,6 % des éleveurs, et les signes d'inappétence par 13,3 % d'entre eux. Seulement 6,7 % des éleveurs réalisent des traitements une fois par trimestre de leurs animaux pendant que 75,6 % le font à l'occasion. Les produits vétérinaires utilisés sont : Lévamisole (6,7 %), Albendazole (71,2 %), Ivermectine (13,3 %) et Oxytétracycline (8,9 %). La mauvaise utilisation de ces produits, par certains éleveurs, pourrait provoquer la résistance des parasites gastro-intestinaux.

Mots-clés : Enquête, prophylaxie sanitaire, caprin nain, Côte d'Ivoire

*corresponding author email: emanfoalex@gmail.com

Introduction

En Côte d'Ivoire, malgré des conditions climatiques favorables à l'élevage, la production nationale de proteine animale couvre moins de la moitié (46,35 %) de la consommation (FAO, 2004 ; Mahieu, 2014).

Pour combler ce déficit et relever le défi de la sécurité alimentaire, le dévéloppement et l'intensification de l'élevage des caprins constituent l'une des priorités de l'Etat ivoirien (MIPARH, 2007). Les petits ruminants représentaient 60 % des animaux de boucherie, d'après le rapport 2001 du service d'inspection de la direction départementale du ministère de l'agriculture et des ressources animales de Korhogo, et la consommation de cette viande est cencée augmenter de 30% jusqu'en 2030, selon les prévision (FAO, 2015). En Côte d'Ivoire, l'approvisonnement des abattoirs reste tributaire des pays sahéliens (Komoin-Oka, 1999 ; Achi et al., 2003 ; CIRAD, 2013). Malgé les efforts dans l'installation et l'encadrement des éleveurs, la production reste insuffisante dans le Sud du pays pour plusieurs raisons dont les principales sont:

- Le faible attachement des populations du sud de la Côte d'Ivoire à l'élevage.
- La fréquente apparition de pathologies, surtout parasitaires.
- l'absence d'une stratégie de gestion et de prévention parasitaire, pour les caprins (CNRA, 2015)

Bien qu'un programme systématique de déparasitage interne soit en vigueur, avec des médicaments subventionnés, la moitié des mortalités des jeunes ruminants est encore imputable aux parasites gastro-intestinaux (Achi et al., 2003 ; Kaplan et al.,2004). La pérennité des parasites dans les installations est surtout liée à un encadrement peu adéquat et une situation sanitaire médiocre des animaux (Belem et al., 2000 ; Mahieu, 2014 ; CNRA, 2015). Une enquête épidémiologique réalisée par Achi en 2003 sur les petits ruminants dans le nord, a permis d'identifier Trichostrongylus colubriformis (96 %), Haemonchus spp (82 %), et Oesophagostomum columbianum (54 %), comme étant les parasites majoritaires. Dans la zone forestière sud, Komoin-Oka a réalisé en 1999 la même recherche sur les ovins de race Djallonké. Les espèces prédominantes étaient Trichostrongylus colubriformis (89,7 %) et Haemonchus contortus (84,1%). Néanmoins, dans la zone sud, aucune base de données n'est disponible sur les parasites gastro-intestinaux du caprin, ni sur les facteurs favorisant la prolifération de ces parasites dans les élevages. La présente étude est une enquête qui vise à établir un diagnostic des conditions d'élevage du caprin nain dans les zones sud et centre de la Côte d'Ivoire.

Matériel et méthodes

Période et zones et d'étude

Cette étude s'est déroulée durant la période de Janvier à Juillet 2015. Le sud et le centre de la Côte d'Ivoire ont constitué les zones d'étude notamment les régions du N'Zi (Dimbokro), du Moronou (Bongouanou), de la Mé (Adzopé) de l'Agneby-tiassa (Tiassalé, Azaguié), et le district autonome d'Abidjan (figure 1). La répartition des fermes en fonction des localités est résumée dans le tableau 2.

Echantillonnage

La population cible comprenait tous les éleveurs de caprin connues dans cinq localités du sud et du centre de la Côte d'Ivoire. L'identification des exploitations a été fondée sur des données provenant d'un recensement des éleveurs de caprin réalisé par l'Agence Nationale d'Appui au Développement Rural (ANADER) en 2013. Par ailleurs, des informations recueillies auprès de certains éleveurs ont été utilisés pour identifier ceux qui n'étaient inscrits.

La sélection des fermes enquêtées était basée sur les critères suivants :

- la disponibilité d'un parc de nuit (enclos) pour faciliter la capture puis l'observation des animaux.
- la volonté de l'éleveur de collaborer.

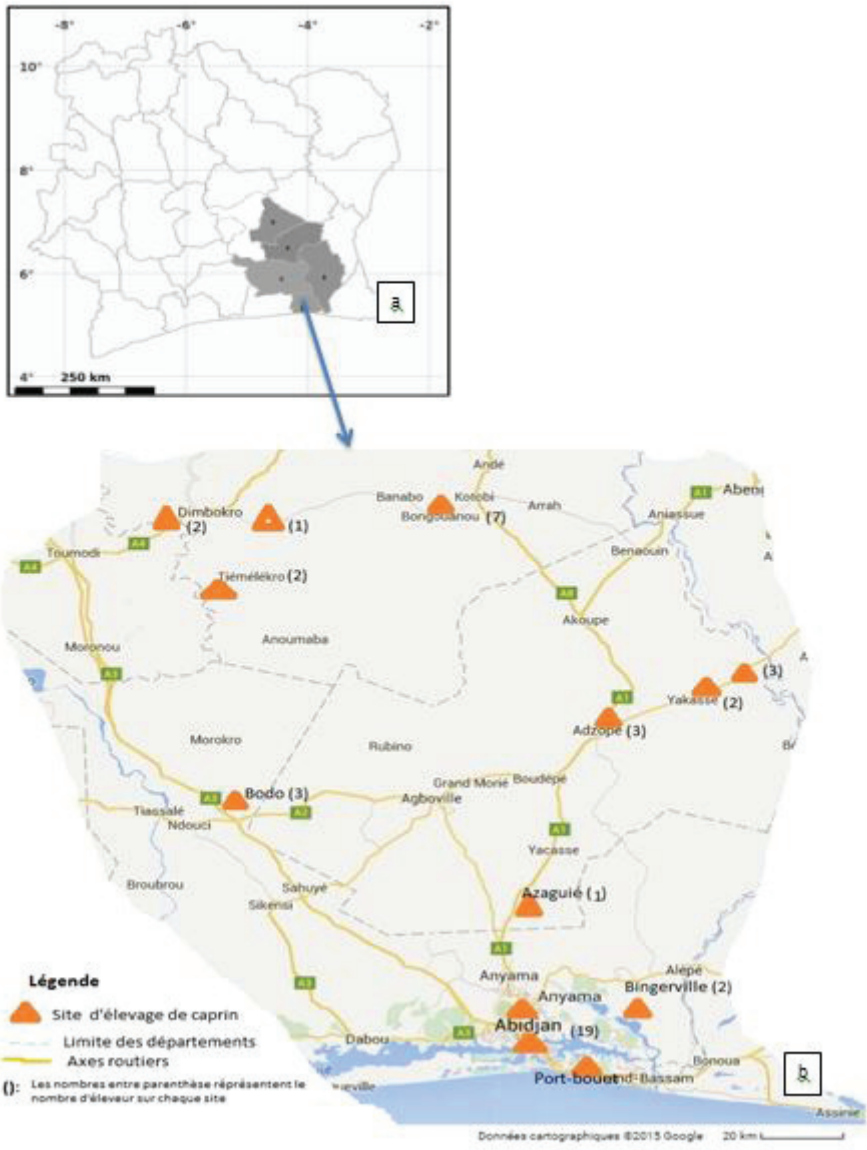


Figure 1 : (a) Présentation de la zone d'étude ; (b) localisation des élevages caprins. (Creative, 2014 ; Google, 2015 ; CNTIG, 2011). Modifié avec le logiciel Paint.NET de Microsoft.)

Au total, 45 éleveurs ont été retenus sur les 51 identifiés. L'unité statistique de l'enquête était un éleveur remplissant les conditions ci-dessus définies.

Une partie de l'enquête a été réalisée auprès de huit vétérinaires, neuf techniciens d'élevage et quatre firmes pharmaceutiques en vue d'inventorier les pathologies diagnostiquées dans les fermes caprines et identifier les différentes molécules antiparasitaires conseillées contre les parasites gastro-intestinaux affectant les caprins. Les agents de santé animale ont été sélectionnés pour la conduite de l'enquête sur la base d'un seul critère : ils intervenaient dans la zone d'étude sur des fermes caprines. Parallèlement, des enquêtes ont été menées auprès des éleveurs et des marchands de caprins.

Enquête sur le terrain

Sur ces sites, les interviews à partir d'un questionnaire préétabli couplées à des observations ont été orientées autour de huit axes : l'identification de l'éleveur et de sa ferme, la conduite de l'élevage et de l'hygiène, les symptômes de pathologies, les modes de traitements et le circuit d'approvisionnement et de distribution des produits d'élevage. Les observations ont consistés à l'appréciation visuelle de l'état sanitaire des animaux (diarrhées, amaigrissements, larmoiements, gales), l'hygiène des installations et la photographie des notices ou des boites vides des médicaments utilisés. L'identification des éleveurs et l'organisation des rencontres ont été possibles grâce à la collaboration de l'ANADER. Le tableau 1 présente les axes autour desquels était orientée l'enquête, ainsi que les variables considérées pour caractériser chaque paramètre.

Analyses statistiques

Les données de l'enquête ont subi une analyse descriptive à l'aide du logiciel SPSS. Le test de Chi2 a permis de comparer les proportions obtenues. Aussi, une analyse des correspondances multiples a-t-elle été réalisée sur dix variables (figure 3) pour différencier les types d'élevage.

Résultats

Identification de la zone d'étude

La localisation géographique des installations est présentée dans le tableau 2. Les nombres entre parenthèse représentent les effectifs des installations dans les différentes zones. Au total cinq régions, sept villes, sept quartiers et neuf villages ont été prospectés.

Identification de l'éleveur

Les paramètres d'identification révèlent que 93,3% des éleveurs sont de sexe masculin, d'âge variant entre 21 et 68 ans. Le tableau 3 présente la distribution des âges des éleveurs. Il fait ressortir que la moyenne d'âge est de 42,2 ± 12 ans (mode = 31 ; aplatissement = -0,516).

Concernant leur nationalité, 51,1 % d'entre eux sont des non-nationaux notamment des nigériens (24,4 %), des burkinabés (20,0 %) et des maliens (6,7 %). Par ailleurs, seulement 6,7 % des éleveurs sont issus d'une école d'élevage ; les autres (93,3 %) n'ont reçu aucune formation sur les techniques d'élevage (44,4 %), ou s'appuient sur des connaissances empiriques traditionnelles (48,9 %) dans la gestion de leurs fermes. L'analyse des niveaux d'étude montre que 62,2 % des éleveurs sont analphabètes, 11,1 % ont arrêté les études au primaire, 15,6 % au secondaire et 11,1 % au supérieur. En moyenne, 2,1 (min = 1 ; max =4) personnes assurent la conduite des animaux dans chaque installation étudiée. Les groupes ethniques rencontrés au cours de notre enquête sont présentés dans le tableau 4.

Identification de la ferme

Les élevages urbains et péri urbains représentent 55,6 % des installations enquêtées. Les périodes d'installation des différentes unités caprines sont d'une répartition régulière depuis les quatorze dernières années (2001-2015). Le pourcentage d'élevage mixte est de 80,0 % contre 20,0 % d'élevage mono spécifique. Les animaux élevés en association sont les ovins (71,1 % des éleveurs), les bovins (37,8 %), la volaille (60,0% %) et les chiens (22,2 %).

Conduite de l'alimentation

L'étude des méthodes de conduite de l'alimentation montre que 51,1 % des éleveurs associent à la fois des graminées et des légumineuses tandis que 28,9 % d'entre eux ne distribuent que des graminées et 20,0 % que des légumineuses. Les espèces de fourrage utilisées sont : Panicum maximum, Brachiaria ruziziensis, Panicum maximum, Brachiaria ruziziensis, Pennisetum purpureum, Ficus exasperata, Ficus benjamina et Moringa oleifera. Quant aux compléments alimentaires, 97,7 % des éleveurs les utilisent ; il s'agit notamment des sous-produits agricoles (73,3 %), agro-industriels (24,4 %).

Tableau 1 : Paramètres d'élevage et variables les caractérisant

Paramètres étudiés	**Variables considérées**
Identification de la zone d'étude	Régions ; villes ; quartiers ou villages
Identification de l'éleveur	Age, activité principale, pays d'origine, langue, genre, niveau d'étude, formation au métier d'éleveur et nombre de personnes embauchées.
Identification de la ferme,	Année de création, mode d'élevage et type d'élevage.
Conduite de l'alimentation	Espèces fourragères distribuées, type de compléments distribués et apport en eau.
Conduite de l'hygiène,	Origine du fourrage et son conditionnement, source de l'eau de boisson, fréquence et mode de nettoyage, fréquence et mode de désinfection et désinfectants utilisés
Symptômes de pathologies	Présence de vers dans les fèces, pertes d'appétit, amaigrissements, diarrhées et mortalités.
Mode de traitements	Suivi vétérinaire, déparasitage interne, fréquence de déparasitage interne, produits vétérinaires utilisés, plantes médicinales utilisées et méthodes de traitement.
Circuit d'approvisionnement et de distribution des produits.	Origine des animaux et marchés d'écoulement des produits.

Tableau 2 : Situation géographique des éleveurs enquêtés

Régions	**Villes**	**Quartier / Village**	**Nombre d'éleveur (45)**
		Abobo	5
	Abidjan (19)	Attécoubé	5
Lagunes (21)		Port-Bouët	5
		Adjamé	4
	Bingerville	Agriculture	2
		Agou	3
Mé (8)	Adzopé	Abongoua	3
		Yapokoi	2
N'Zi (5)	Dimbokro	Ediakro	3
		Koffi Ahoussoukro	2
		Tiemelekro	2
		Kangandi	1
Moronou (7)	Bongouanou	Assahara	2
		Assié koumassi	2
Agneby-tiassa (4)	Azaguié	Azaguié corridor	1
	Tiassalé	Bodo 2	3

Tableau 3 : Distribution des âges des éleveurs

N	Valide Manquante	45 0
Moyenne		42,24
Erreur std. de la moyenne		1,820
Médiane		42,00
Mode		31
Ecart-type		12,207
Aplatissement		-,516
Erreur std. d'aplatissement		,695
Intervalle		47
Minimum		21
Maximum		68
Centiles	25	31,00
	50	42,00
	75	52,00

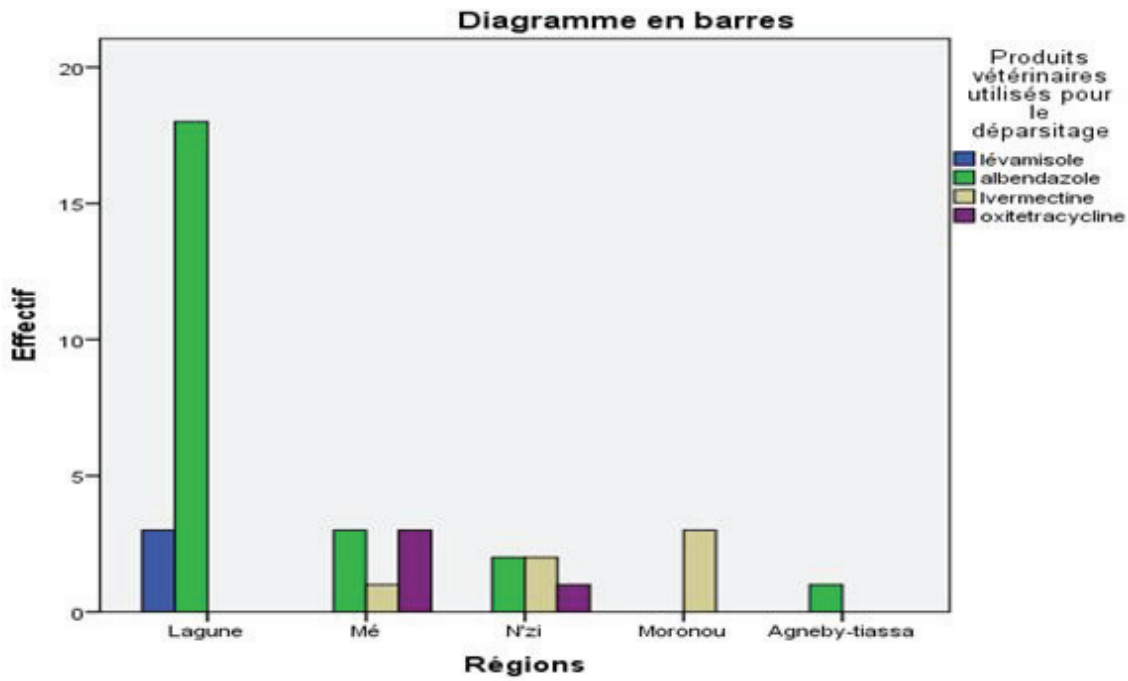


Figure 2 : Utilisation des produits vétérinaires en fonction des zones d'étude

Tableau 4 : Groupes ethniques rencontrés au cours de l'enquête

		Effectifs	Pourcentage	Pourcentage valide	Pourcentage cumulé
Valide	Baoulé	4	8,9	8,9	8,9
	Agni	8	17,8	17,8	26,7
	Hausa	11	24,4	24,4	51,1
	Dioula	13	28,9	28,9	80,0
	Moré	9	20,0	20,0	100,0
	Total	**45**	**100,0**	**100,0**	

Conduite de l'hygiène dans les élevages

Les résultats sur l'étude des conditions d'hygiène présentent une forte utilisation des fourrages urbains (48,9 %) et des pâtures naturelles (51,1 %). Aucun éleveur ne disposait d'un pâturage moderne. Seuls 8 % des éleveurs séchaient le fourrage pendant 24 heures avant sa distribution. Quant à l'origine de l'eau de boisson, les résultats stipulent que 37,8 % des éleveurs utilisent de l'eau de la Société de Distribution d'Eau de Côte d'Ivoire (SODECI). Mais, la majorité des éleveurs (51,1 %), installés en zone rurale, utilise l'eau des puits. Par contre, 4,4 % d'entre eux utilisent l'eau des marigots.

L'analyse des fréquences de nettoyage dans les installations montre que certains éleveurs (33,3 %) nettoient les enclos au moins une fois par jour. Mais seulement 8,9 % d'entre eux associent balayage et lavage. Le tableau 5 révèle que 77,8 % des éleveurs n'ont jamais pratiqué de désinfection.

Symptômes de pathologies

Selon les éleveurs :

Evoquant la sémiologie, 75,6 % des éleveurs dénoncent des cas de mortalité sans symptômes. En zone urbaine, les animaux adultes sont les plus touchés ; tandis qu'en zone rurale ce sont les plus jeunes. La fréquence des diarrhées observées est de 88,9 %, dont 13,3 % de de type sanguinolent. Mais, 57 % des éleveurs estiment que les mortalités à la suite des diarrhées sont rares. Par ailleurs, 62,2 % des éleveurs prétendent avoir observé des vers dans les fèces. Quant aux signes d'amaigrissement et d'inappétence respectivement 15,6 % et 13,3 % des éleveurs estiment qu'ils sont fréquents.

Selon les agents de santé animale :

Tous les agents consultés ont affirmé que les maladies parasitaires internes et externes étaient les plus diagnostiquées dans les élevages caprins. Les méthodes de diagnostic de ces pathologies étaient l'observation à l'œil nu de l'animal (gales, anneaux de Ténia, poils piqués, amaigrissement et œdèmes sous la gorge) et rarement (11,8 %) les analyses coprologiques.

Modes de traitements

Considérant les méthodes de traitement, 64,4 % des éleveurs ont une fois consulté des vétérinaires. Par contre, 17,8 % ne l'on jamais fait, bien qu'ils réalisent le déparasitage interne de leurs animaux. Quelques éleveurs (6,7 %) le réalisaient une fois par trimestre pendant que 75,6 % le faisaient à l'occasion. Les produits vétérinaires utilisés sont : Lévamisole (6,7 %), Albendazole (71,2 %) Ivermectine (13,3 %) et Oxytétracycline (8,9 %).

Leur utilisation en fonction des zones est illustrée par la figure 1. Les doses de produits utilisées sont établies selon la posologie préconisée pour les ovins, chez les éleveurs suivis par des agents de santé animale (64,4 %). Les éleveurs qui ne sont pas suivis (17,8 %) s'appuient sur la notice lorsqu'elle est disponible. Dans le cas contraire, la dose est administrée de manière aléatoire. Par ailleurs, aucun éleveur n'utilise des plantes anthelminthiques ni toute autre plante pour soigner les caprins. Le tableau 6 met en évidence les éleveurs qui traitent leurs animaux sans l'avis d'un agent de santé animale.

Tableau 5 : Rythmes de désinfection dans les fermes

	Effectifs	Pourcentages
Existence d'un programme de désinfection	8	17,8
Pas de programme de désinfection	37	82,2
Total	**45**	**100,0**

Fréquence de désinfection

	Effectifs	Pourcentages
Une fois par mois	8	17,8
Moins d'une fois par trimestre	2	4,4
Jamais	35	77,8
Total	**45**	**100,0**

Tableau 6 : Tableau croisé Suivi vétérinaire * Déparasitage interne

		Déparasitage interne		Total
		Oui	Non	
Suivi vétérinaire	animaux suivis par un vétérinaire	29a	0b	29
	animaux non suivis	8a	8b	16
	Total	**37**	**8**	**45**

Chaque lettre en indice indique un sous-ensemble de Déparasitage interne catégories dont les proportions de colonne ne diffèrent pas de manière significative les unes des autres au niveau ,05.

Approvisionnement en reproducteurs et distribution des produits d'élevage.

Toutes les installations caprines (19 installations) situées dans le district d'Abidjan s'approvisionnent à l'abattoir de Port-Bouët. Par ailleurs, la majorité des éleveurs en milieu rural (53,3 %) achètent les reproducteurs non loin de leurs localités. Seuls deux éleveurs ont constitué leurs cheptels à partir de caprins achetés au nord de la Côte d'Ivoire. Concernant la vente, seule la viande est commercialisée. Les acquéreurs principaux sont les restaurants et les marchés de bétail. L'autoconsommation n'est enregistrée que dans 4,4 % des installations.

Les tableaux 7 et 8 présentent respectivement les lieux d'approvisionnement et les marchés de distribution des produits d'élevage.

Différentiation des types d'élevage

L'analyse des correspondances multiples réalisée sur les variables a permis de distinguer trois types d'éleveurs représentés par les figures 3 et 4 :

1er type : (dimension 1) on distingue, les éleveurs de la ville d'Abidjan caractérisés par une formation traditionnelle, l'utilisation de fourrage urbain et un rythme de nettoyage de deux fois par jour. Ils sont installés dans les marchés de bétail et ont pour activité principale l'élevage et la vente d'animaux (éleveur-vendeurs). Ce groupe représente 42,2 % des éleveurs interrogés.

2ème types : (dimension 2), Le deuxième type d'éleveur est issu d'une école d'élevage ; il représente 6,6% des éleveurs interrogés. Ils ont un niveau d'étude universitaire et sont souvent des étudiants ou des fonctionnaires, l'élevage de caprins étant pour eux une activité secondaire, ou temporaire (expérimentation, loisir). Ils pratiquent un élevage urbain ou périurbain autour de deux villes Azaguié et Bingerville. Ces villes abritent plusieurs écoles de formation en élevages.

3ème type : (dimension 1 et 2) Le troisième type d'éleveur (51,2 %) regroupe ceux installés en milieu rural dans les départements de

Tableau 7 : Lieux d'approvisionnement

	Effectifs	Pourcentage
Abattoir	19	42,2
Nord du pays	2	4,4
Sud	11	24,4
Centre	13	28,9
Total	**45**	**100,0**

Tableau 8 : Marchés de distribution des produits d'élevage.

	Effectifs	Pourcentage
particulier et restaurant	43	95,6
autres	2	4,4
Total	**45**	**100,0**

Dimbokro, Tiassalé, Adzopé, Bongouanou. Ils sont caractérisés par une absence de formation ou ayant une formation traditionnelle, ils sont, en général, analphabètes ou de niveau primaire et pratiquent l'agriculture industrielle (café, cacao, hévéa, etc.). Ils utilisent les pâtures naturelles. Les animaux sont parqués dans des enclos pour éviter la destruction des cultures. Aussi, l'entretien de ces enclos est-il négligé (rythme de nettoyage de moins d'une fois par jour).

Des variables comme la perte d'appétit, les symptômes d'amaigrissement et les mortalités sans symptôme ne sont associées à aucune catégorie dans le graphique.

Discussion

Le nombre des éleveurs consulté est réduit (45) du fait de la petite taille de la population cible dans la zone d'étude. D'abord, les peuples de la zone sud ivoirienne sont connus pour leur faible attachement à l'élevage, au profit des cultures vivrières et industrielles (FAO, 2004 ; Mahieu, 2014). Ensuite, la quasi-totalité des caprins élevés dans cette zone est en divagation. Ceci dénote la faible attention accordée, par l'éleveur, sur les animaux. Par conséquent, des informations statistiques fiables ne pouvaient être recueillies auprès de ces derniers.

L'élevage des caprins dans la zone d'étude est réalisé par des adultes dont la majorité à moins de 32 ans, presque tous, de sexe masculin (93,3 %). Le faible niveau de formation des éleveurs expliquerait le fait que la quasi-totalité ignore les techniques modernes d'élevage. Les nationaux sont fortement impliqués dans l'élevage des caprins (48,9 %). Mais ce secteur reste encore dominé par les non nationaux (51,1 %) notamment en zone urbaine.

Plusieurs installations caprines étaient localisées en zone urbaine et péri-urbaine du fait de la facilité d'écoulement des produits d'élevage. L'absence totale de pâturage moderne, les bâtiments non conformes aux normes zootechniques et l'association avec d'autres animaux dénotent le manque de professionnalisme des éleveurs. En effet, la présence de pâturage moderne permet d'assurer en quantité et en qualité la ration fourragère des ruminants. Le respect des normes zootechniques dans la construction des bâtiments permet d'assurer un confort pour les animaux qui permettra d'optimiser leur production (OIE, 1980 ; Najai et al., 2004 ; Cabaret et Nicourt, 2009). Par ailleurs, l'association de plusieurs animaux dans une même ferme favorise une prolifération des parasites ayant plusieurs hôtes. Elle peut également favoriser une adaptation, par mutation, des parasites à un nouvel hôte avec

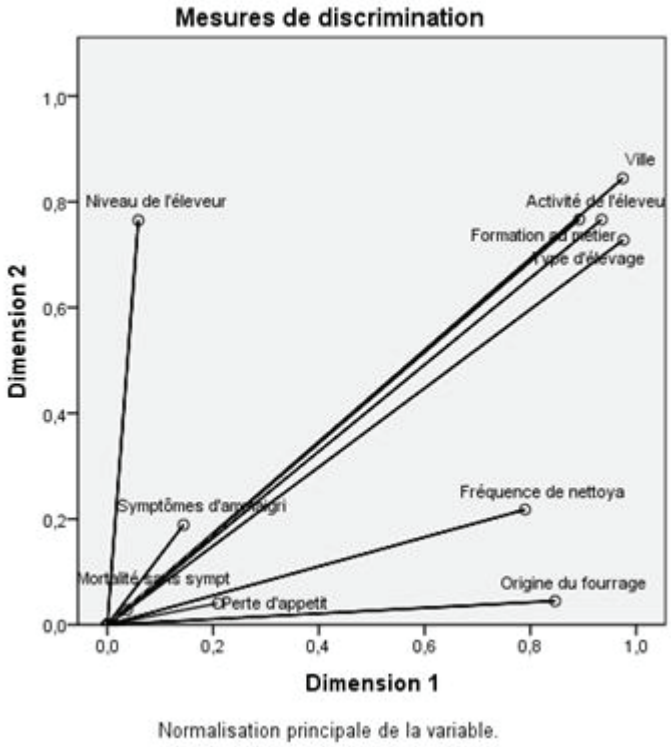


Figure 4 : Mesure de discrimination des variables

lequel ils sont permanemment en contact (Ba et Geerts, 1998 ; Bairden et al., 1995 ; Inderbitzen,1981).

En ce qui concerne la conduite de l'alimentation, les rations proposées par 97,7 % des éleveurs comporte du fourrage et du concentré. Mais, les rapports quantitatifs (fourrage /concentré) sont ignorés. La ration alimentaire des ruminants serait d'environ 80 % de fourrage et de 20 % de concentré (céréales et sous-produits agro-industriel). Une ration de plus de 30 % de concentré pourrait entrainer des météorisations. La ration fourragère du caprin serait d'environ 20 % de graminées et de 80 % de légumineuses (Bourbouze et Guessous, 1979 ; Cesar et Zoumana, 1999). Dans les installations étudiées la méconnaissance des valeurs nutritives des aliments et l'inexistence de formule alimentaire rendent aléatoire l'équilibre de la ration alimentaire. Certains éleveurs renforcent la ration lorsqu'il observe des symptômes d'amaigrissement.

Quant à la conduite de l'hygiène, le nettoyage réalisé dans toutes les installations est une avancée notable vers la réduction des charges parasitaires. Mais, l'inexistence d'un programme de désinfection dans 82,2 % des fermes favorise le maintien des espèces parasitaires dans ces installations. Ceci pourrait favoriser leur résistance aux anthelminthiques. Par ailleurs, la distribution d'eau propre (SODECI, puits protégés) est très améliorée (51,1 %). En revanche la négligence du nettoyage des abreuvoirs prédispose les animaux aux contaminations parasitaires.

Le fourrage prélevé en zone urbaine n'est pas recommandé pour nourrir des animaux d'élevage du fait de leur forte charge microbienne, surtout parasitaire. L'utilisation donc du fourrage urbain par 48,9 % des éleveurs constitue une dangereuse exposition des animaux aux infestations (Barger, 1999 ; Belem et al., 2000 ; MIPARH, 2007). Néanmoins, un séchage du fourrage, pendant 24 h pourrait permettre de réduire considérablement sa charge parasitaire (Emanfo et al., 2013).

Les symptômes d'amaigrissements et de diarrhées observés dans des exploitations

pourraient être causés par un effet combiné de la mauvaise conduite de l'alimentation et de l'hygiène déplorable (Abdelmadjit, 1978). Les symptômes des parasitoses gastro-intestinales sont souvent difficiles à percevoir sans un examen complémentaire (examen coprologique, méthode FAMACHA, etc.). L'inaccessibilité et la méconnaissance de ces méthodes chez la quasi-totalité des éleveurs pourraient expliquer l'absence de symptôme constaté par ceux-ci (Cabaret et Nicourt, 2009).

Le traitement des pathologies, en général, et des parasitoses, en particulier, doit être réalisé avec le plus grand soin pour éviter un surdosage qui pourrait être fatale aux animaux ou d'un sous-dosage à l'origine d'une éventuelle résistance des parasites (Najai et al., 2004). La consultation des vétérinaires en vue d'un diagnostic permet d'éviter ces éventualités. L'utilisation de déparasitant par 17,8 % des éleveurs, sans un diagnostic préalable, représente un risque de développement de résistance par les parasites. Par ailleurs, la rareté des médicaments spécifiques aux caprins pousse les éleveurs et certains agents de santé animale à se référer aux doses destinées aux ovins ou à la volaille (anticoccidien). Or, les caprins ont un métabolisme plus rapide que les ovins, ils éliminent plus vite certains déparasitants (benzimidazolés, ivermectine, lévamisole) (Inderbitzen, 1981 Burke et al., 2007 ; Okombe et Pongombo, 2013). En conséquence, les posologies figurant sur les produits et définies pour les ovins ne sont pas adaptées aux caprins, qui nécessitent des doses thérapeutiques supérieures pour obtenir une efficacité comparable à celle obtenue chez les ovins (Reinemeyer, 1993 ; Kouable Bi et Tre Bi, 2014).

Les différences observées entre les éleveurs résident uniquement dans leur localisation, leur formation et leur rythme de nettoyage des enclos. Les résultats sur l'aspect sanitaire semblent mitigés. En effet, des variables comme la perte d'appétit, les symptômes d'amaigrissement et les mortalités sans symptôme qui sont des indicateurs d'infestation parasitaire, ne sont associées à aucune catégorie dans le graphique de l'ACM. Ceci démontre que tous les éleveurs de caprin, dans ces trois types d'élevage, ne maitrisent pas encore les paramètres sanitaires.

Conclusion

Le système d'élevage des caprins dans la zone d'étude reste traditionnel, dans sa totalité, avec une faible installation des éleveurs issus des écoles de formation. La prophylaxie sanitaire est médiocre, dans l'ensemble et est caractérisée par une insuffisance d'entretien des installations et alimentation pauvre et précaire. La molécule antiparasitaire la plus utilisée est l'Albendazole. La méconnaissance des posologies, et la mauvaise utilisation des déparasitants dans ces zones pourraient engendrer la résistance des parasites gastro-intestinaux. Aussi la réalisation de test de résistance permettra d'évaluer l'ampleur de ce phénomène. Enfin, la régularité des symptômes d'infestations parasitaires (amaigrissement, inappétence, etc.) nécessite qu'on approfondisse les connaissances sur les parasites en cause.

Remerciements

Nous remercions l'Agence Nationale d'Appui au Développement Rural (ANADER) pour les ses effort consentis dans identification des éleveurs, et la facilitation de notre rencontre avec eux.

Références bibliographiques

Abdelmadjit M. S. : Contribution à l'étude des coccidioses des petits ruminants en élevage traditionnel tchadien, Thèse de Doctorat vétérinaire, Ecole inter-états des sciences et médecine vétérinaires de Dakar, 1978, 135 p.

Achi Y.L., Zinsstag J., Yéo N., Dea V. et Dorchies P.H. : Épidémiologie des helminthoses des moutons et des chèvres dans la région des savanes du Nord de la Côte d'Ivoire. Revue Méd. Vét., 2003, 154, 3, 179-188.

Ba H. Geerts S. : La résistance aux benzimidazoles des nématodes gastrointestinaux des petits ruminants en Gambie et au Sénégal. Rev. Elev. Méd. vét. Pays trop., 1998, 51(3) : 207-210.

Bairden K., Armour J., Duncan J. : A 4-year study on the effectiveness of alternate grazing of cattle and sheep in the control of bovine parasitic gastro enteritis. Vet. Parasitol., 1995, 119-132.

Barger I.A. : The role of epidemiological knowledge and grazing management for helminth control in small ruminants. In: Int. J. Parasitol., 1999, 29, p. 41-47.

Belem A.M.G., Nikiema Z.L., Sawadogo L. et Dorchies Ph. : Parasites gastro-intestinaux des moutons et risques d'infestation parasitaire des pâturages en saison pluvieuse dans la région centrale du Burkina Faso. Revue Méd. Vet., 2000, 151, 437-442.

Bourbouze A., Guessous F. : La chèvre et l'utilisation des ressources dans les milieux difficiles. Rev. Elev. Méd. vét. Pays trop., 1979, 32 (2) : 191-198

Burke J. M., Kaplan R. M., Miller J. E., Terrill T. H., Getz W. R., Mobini S., Valencia E., Williams M. J., Williamson L. H., Vatta A. F.: Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. Vet. Parasitol., 2007, 147 : 89-95.

Cabaret J. et Nicourt C. : Les problèmes sanitaires en élevage biologique : réalités, conceptions et pratiques Inra Productions Animales, 2009, 22 (3), 235-244.

Cesar J., Zoumana C. : Les régimes alimentaires des bovins, ovins et caprins dans les savanes de Côted'Ivoire, et leurs effets sur la végétation. Fourrages, 1999, 159 : 237-252.

CIRAD (Centre de coopération internationale en recherche agronomique pour le développement) : Etude relative à la formation du programme d'actions détaillé de développement de la filière lait en zone UEMOA, Annexe 3 : rapport d'activité, 2013, 26 p.

CNRA Centre National de Recherche Agronomique) : Programme Productions d'élevage filière petits ruminants, fiche technique, Côte d'Ivoire, 2015, 1p.

CNTIG (Comité National de Télédétection et d'Information Géographique), : Carte administrative, nouveau decoupage administrative, 2011, 1p.

Creative C., Ivory Coast regions, https:// creativecommons.org/, 2014.

Emanfo A. S. A. ; Komoin-Oka C. ; Karamako Y. ; Fantodji A., : Parasitic contamination of fodder consumed by the grasscutter breeding in urban and suburban areas of Abidjan (Cote d'Ivoire). European Journal of Scientific Research, 2013, 116 (2): 221-229

FAO (Food and agriculture organization): Evaluation des récoltes et des disponibilités alimentaires en Côte d'Ivoire, rapport spécial, édition FAO/PAM, Côte d'Ivoire, 2004, 34 p.

FAO (Food and agriculture organization) : Les petits ruminants, ressources importantes de la Côte d'Ivoire. Bulletin d'information FAO Côte d'Ivoire n°11, 2015, 12 p.

Google M., 2015 : Cartographiques ©2015 Google 10 km https://www.google.com/maps/

Inderbitzen F., Eckert J., Hofmann HR.: Parasitological effect of alternate grazing of cattle and sheep. In "Epidemiology and control of nematodiasis in cattle" Nansen P., Jorgensen R.J. & Soulsby E.J.L. Eds pp, 1981, 249-258.

Kaplan R. M., Burke J. M., Terrill T. H., Miller J. E., Getz W. R., Mobini S., Valencia E., Williams M. J., Williamson L. H., Larsen M., Vatta A. F. : Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol, 2004, 123: 105-120.

Komoin-Oka C., Zinsstag J., Pandey V.S., Fofana F. et N'depo A. : Epidémiologie des parasites des ovins de la zone sud forestière de la Côte d'Ivoire. Rev. Elev. Méd. Vét. Pays trop., 1999, 52, 39-46.

Kouable Bi B. N et Tre Bi T. C.O. : Evolution des exportations du bétail malien suite aux récentes crises. Rapport d'activité, 2014, 49 p.

Mahieu M., : Gestion du parasitisme gastro-intestinal des petits ruminants en zone tropicale humide, Thèse de Doctorat, Université de Lorraine, France, 2014, 178p.

MIPARH (Ministère de la Production Animales et des Ressources Halieutiques), : Production et consommation nationales. Direction de la planification et des programmes (DDP), Abidjan, Côte d'Ivoire, 2007., 4 p.

Najai S., Jaouad M., Khaldi G., Ben Hammouda M. et Djemali M. : Conduite traditionnelle des petits ruminants dans les régions arides tunisiennes : Savoir-faire du berger et exploitation durable des ressources pastorales, CIHEAM, 2004, 249- 253.

OIE (Office international des épizooties) : Importance des petits ruminants dans les programmes de production des pays d'Afrique. Bull. Off. int. Epiz., Rabat, Maroc, 1980, 92 (11-12), 1211-1225.

Okombe E.V. et Pongombo S.E.W. : Suspicion de la résistance aux benzimidazoles chez les strongles gastro-intestinaux du caprin à Lubumbashi, R.D. Congo, Int. J. Biol. Chem. Sci., 2013, 7(6): 2426-2433.

Reinemeyer C., Pringle J.: Evaluation of the efficacy and safety of Morantel tartrate in domestic goats. Vet. Hum. Toxicol., 1993, 35 : 57-61.

BIOECOLOGIE DES TABANIDÉS DANS LA ZONE PÉRIPHÉRIQUE DU DISTRICT DE BAMAKO AU MALI

Traore D*, Bass B et Kone F
Laboratoire Central Vétérinaire, BP 2295, Bamako, Mali

Résumé

Une prospection entomologique portant sur un total de 162 larves récoltés dans le cours d'eau de Farakoba, sur la route de Koulikoro, situé à une dizaine de kilomètres à l'Est de Bamako a été menée au Mali. Au total 165 larves de tabanidés en provenance des différents gîtes ont été collectées de juillet à septembre 2012 et mises en élevage au Laboratoire Central Vétérinaire (LCV) de Bamako. L'objectif de cette étude était d'apporter notre contribution à la connaissance actuelle de la survie larvaire de ces vecteurs dans ce cours d'eau. Les prélèvements de larves effectués dans dix sites géo référencés ont permis de confirmer la présence des espèces suivantes : Tabanus gratus, Tabanus biguttatus, Chrysops longicornis, Chrysops distinctipennis et Haematopota decora). Une nouvelle espèce Chrysops gresicolis a été identifiée. Des investigations plus approfondies sur l'ensemble du territoire permettront de déterminer la distribution, la fréquence et l'abondance de cette espèce au Mali.

Mots clés : larves, tabanidés, Chrysops gresicolis, Mali

TABANID BIOECOLOGY IN THE PERIPHERY OF BAMAKO DISTRICT, MALI
BIOECOLOGIE DES TABANIDES DANS LA ZONE PERIPHERIQUE DU DISTRICT DE BAMAKO AU MALI

Abstract

An entomological survey involving a total of 162 larvae collected in the water stream of Farakoba, on the road of Koulikoro, located about ten kilometers east of Bamako in Mali was conducted. In total 165 tabanids larvae from different deposits were collected from July to September 2012 and put in breeding at the Central Veterinary Laboratory (CVL). The objective of this study was to make our contribution to the current knowledge of the larval survival of these vectors in this stream. Samples of larvae performed in ten georeferenced locations have confirmed the presence of the following species: Tabanus gratus, Tabanus biguttatus, Chrysops longicornis, Chrysops distinctipennis and Haematopota decora). A new species Chrysops griseicolis was identified. Further investigations on the entire territory shall determine the distribution, frequency and abundance of this species in Mali.

Keys word: larvae, tabanids, Chrysops griseicolis, Mali

Corresponding author email : traore_daman@yahoo.fr

Introduction

Les tabanidés communément appelés taons sont un groupe important de diptères hématophages dont la taille varie entre 25 à 30mm. Elles comprennent 3.500 espèces décrites dont approximativement 900 en Afrique. L'essentiel des travaux sur ces insectes a été effectué par Oldroyd (1952, 1954,1957).

Concernant le Mali peu de travaux ont été effectués et ceux-ci ne concernent que le stade adulte et peu d'études ont été effectuées sur les larves. C'est pour cette raison que nous avons jugé utile d'apporter notre contribution à la connaissance de la vie larvaire de ces vecteurs. Les tabanidés ont depuis longtemps été reconnus comme insectes nuisibles à l'homme et aux animaux. Quand certaines espèces sont abondantes comme dans certaines régions côtières, les activités extérieures de l'homme peuvent être sérieusement perturbées.

Les tabanidés peuvent jouer un rôle important dans le transmission de la trypanosomiase provoquant une anémie, une perte de poids et une décroissante correspondante en production de lait et viande.

Les femelles des tabanidés déposent leurs œufs un à un en masse contenant de 50 à 700 œufs. En fonction de l'espèce les masses d'œufs peuvent être composées d'une à plusieurs rangées et varient en couleur, forme et dimension généralement de 5 à 25 mm ou plus de long et de 2mm de large (Goodwin, 1972).

Peu de succès ont été réalisés dans le domaine du contrôle des tabanidés. Les applications d'insecticides dans des zones restreintes ont été peu satisfaisantes, celles dans les zones plues étendues n'ont été que partiellement efficaces, alors qu'elles constituent un danger de plus en plus grand pour les organismes non cibles.

Dans certains Etats des expériences ont été faites sur les zones très étendues ; elles ont montré qu'un contrôle à court terme est possible avec des produits chimiques qui semblent ne pas avoir de sérieux effets sur les organismes non cibles (Goodwin, 1982).

Il faut noter que les insecticides ont donné plus de succès dans le contrôle des larves que dans celui des adultes, mais ils ont causé plus de dommage aux autres organismes.

Les répulsifs standards utilisés par l'homme et efficaces contre les moustiques ne sont pas efficaces contre les tabanidés. Leur utilisation pourrait même au contraire attirer les mouches à cause de la brillance qu'elle donne à la peau (Goodwin, 1982).

L'utilisation d'ennemis naturels comme moyens de contrôle est peu prometteuse bien qu'on pourrait s'attendre à une réduction de la population en libérant en masse dans la nature des œufs de parasite (Goodwin et al, 1982).

Les manipulations du niveau d'eau sont à l'origine de la destruction de certaines espèces de tabanidés. Cependant une telle manipulation n'est très souvent pas possible si le drainage réduit les lieux de reproduction de beaucoup d'espèces, il pourrait accroître des habitats de larves d'autres espèces (Goodwin, 1982).

L'utilisation de piège demeure le moyen de contrôle le plus promettant du moins dans les zones peu étendues. C'est ainsi que des pièges canopy sont utilisés dans les zones côtières de plusieurs Etats pour réduire la population de tabanidés sur les plages. Ils sont aussi utilisés avec succès dans des vergers d'orangers et même près des piscines ou les tabanidés attaquent les baigneurs.

Materiel et Methodes

Site :

La zone choisie pour la présente étude est un tronçon de la rivière Farakoba situé à l'entrée Est de Bamako sur la route de Koulikoro. Ce tronçon long de1Km est situé dans la partie sud de la rivière et s'étend du pont près du Laboratoire Central Vétérinaire jusqu'au fleuve Niger. En dépit de son caractère temporaire, le marigot présente un certain écoulement dans son lit assez tard en saison sèche.

Ce site a été choisi parce que les larves vivent en milieu humide et même aquatique d'une part, d'autre part les formations végétales

(arbustives et herbacées nous procurent divers types d'habitats de larves). La rivière constitue également un abreuvoir pour les animaux domestiques et même sauvages ; ceux-ci constituent une source de nourriture pour ces mouches .Ainsi cette activité témoigne de la présence de larves d'insectes sur les bords humides qui constituent un lieu de ponte favorable des tabanidés

Coordonnées des sites

Site 1 : N 1266776° W 00791630°
Site 2 : N 1266917° W 00791641°
Site 3 : N 1266893° W 00791330°
Site 4 : N 1266883° W 00791331°
Site 5 : N 1266869° W 00791333°
Site 6 : N 1266850° W 00791327°
Site 7 : N 1266338° W 00791324°
Site 8 : N 1266338° W 00791325°
Site 9 : N 1266808° W 00791327°
Site 10 : N 1266795° W 00791326°

Caractéristiques morphologiques du site

Le marigot a creusé son lit dans des grès très anciens qui appartiennent à la série de Sotuba. Ces grès sont surmontés d'une puissante cuirasse ferrugineuse épaisse de plusieurs mètres.

Une coupe transversale du marigot présente de la berge au lit mineur, les formations pédologiques suivantes (Figure2). Au sommet de la berge, la cuirasse ferrugineuse plus ou moins démantelée en surface et transformée en gravillon .Cette partie est presque en dehors du lit majeur. Dans le lit majeur (frange atteinte au moment des hautes eaux) on remarque un alluvionnement selon le gradient ci- après : sable plus ou moins grossiers ; sable fins plus limons ; limon ; argile. Le fond du lit est occupé par un soubassement de grès anciens très durs.

Végétation

Sur les deux rives du marigot on remarque une savane arbustive ou dominent dans les conditions normales le karité (Vitellaria paradoxa) et le néré (Parkia biglobasa). Parmi les autres espèces de cette savane on peut retenir : Combretum ghasalense, C. glutinosum, C. micrathum, Terminalia avicenoides, Pterocarpus lucens, Bombax costatum.

La strate herbacée comprend une autre espèce : Andropogon gayanus, Cymbopogon giganteus, Pennisetum pedicellatum, Dactyloctnium aegyptuin.

Cette végétation de savane est dans un état de dégradation avancée sous l'action de la culture et de la coupe. Le long du marigot a certainement existé une formation de forêt galerie ainsi que le témoigne les éléments suivants : Berlinia hendolotiana, Daniellia oliveri , Macrosphyra longistyla, Detarium senegalense,Canthium cornelia, Dictyandra arboresca, Raphia sudanica, Ficus capensis, Ficus iteophylla, Securinega virosa, Mucuna pruriens, Cassia obovata, Parkia Biglobosa, Bombax costatum, Khaya senegalensis, Acacia macrostachya, Crateva religiosa, Phyllantus dicoidens (flore illustrée du Sénégal par Berhaut (1954, 1967).

On peut également remarquer dans cette végétation la présence d'une grande plantation de manguiers (Manguifera indica.) En plus de ces types de végétation l'on peut noter également la présence d'une flore aquatique composée de plantes suivantes : Numphéa lotus, N. micrantha, N. maculata, Eicornia natans, Ceratophyllum demersum, Utricularia gibba varoxeleta, Jussiea repens var diffusa, Jussiea erecta, Panicum repens, Panicum subalbidium, Brachiara stigmatisata (Berhaut 1954, 1967).

Climat

Notre zone d'étude se trouve dans le district de Bamako. Elle occupe la frange la plus méridionale du Sahel africain correspondant à la zone soudanienne. Elle bénéficie de ce fait d'un climat tropical assez humide avec un total des précipitations annuelles de 878 millimètres mais avec une saison sèche et une saison des pluies bien marquées. Le mois le plus sec ne reçoit en effet pas la moindre goutte de pluie (précipitations égales à 0 mm en décembre) tandis que le mois le plus pluvieux est bien arrosé (précipitations égales à 234 mm en août). Les pluies régulières estivales permettent le développement d'une savane arborée ainsi que la culture de plantes telles que le sorgho,

le maïs. On note deux saisons : la saison sèche qui s'étend de septembre à mai et la saison des pluies de juin à octobre. La pluviométrie varie de 800 à 1000 mm.

Matériel :

Seaux, pelles, pinces flexibles, tamis, bassines, flacons de récupération.

Méthodes

Prélèvement et étalage des échantillons :

Ce travail, réalisé entre les mois de juillet et septembre 2012 au Laboratoire Central Vétérinaire de Bamako au Mali a consisté à prélever une partie du substrat susceptible de renfermer les larves. Avec des seaux les échantillons des sites étaient quotidiennement apportés au laboratoire et étalés sur des tamis de manière qu'ils soient en contact avec la lumière. Ainsi chaque semaine du lundi au vendredi les échantillons des sites étaient prélevés.

Les tamis dont le cadre est en bois sont de forme rectangulaire, ils ont une longueur de 3 mètres sur 0, m 60 de large et d'une profondeur de 20 cm, son fond est constitué d'un grillage double de mailles de 15 mm. Les tamis sont posés sur des briques jouant ainsi le rôle de support.

Les échantillons qui représentaient une partie du substrat où vivent les larves sont composés de : boues prélevées sur les berges où au fond de l'eau des plantes entières (surtout les herbacées flottantes ou vivant fixées au fond de l'eau ou sur la berge) détritus de toutes sortes pouvant représenter un milieu favorable au développement des larves. Sur chaque tamis sont disposés 4 échantillons différents, en dessous desquels sont posées 4 bassines de réception contenant de l'eau. Ces bassines jouent un rôle important car l'assèchement de l'échantillon oblige les larves à s'enfoncer de plus en plus profondément dans le substrat et finissent par être recueillies dans celles - ci.

Analyse des échantillons

Identification des constituants de l'échantillon :

Elle a eu lieu dans le laboratoire, et a consisté à préciser les constituants vivants et minéraux des échantillons.

Dénombrement des larves :

Il a consisté à déterminer les larves de tabanidés des autres espèces de larves d'abord (Exemple larve de coléoptère, tipulidé) ensuite la séparation des différentes espèces de tabanidés et à déterminer leur nombre. Toutes les larves sont manipulées avec des pinces flexibles, car elles sont fragiles. Après la séparation, les larves de tabanidés sont soumises à un élevage dans des flacons.

Elevage des larves :

Les larves issues du dénombrement quotidien étaient d'abord examinées à la loupe binoculaire pour détermination et séparées en deux lots.

Le premier lot résultant du dénombrement et qui est composé de larves mortes est soit jeté soit conservé dans l'alcool à 80° pour identification ultérieure.

Le deuxième lot composé de larves vivantes était destiné à l'élevage. Ces larves sont placées individuellement dans des flacons en verre pour les grosses larves et les petits flacons en plastique pour les petites larves contenant du sable propre mouillé exempt de matière organique. Ce sable est maintenu humide durant toute la durée de la vie larvaire.

Pour la nourriture, les larves recevaient une provision hebdomadaire de foie frais, et étaient contrôlées tous les 2 jours pour connaître exactement les dates de mue, de pupaison et d'émergence. Après une pupaison la dernière exivue larvaire est conservée dans l'alcool car elle se détériore rapidement dans les flacons.

La gaine pupale est recueillie après émergence de l'adulte et gardée dans l'alcool. La gaine pupale et l'exivue larvaire jouent un rôle important dans l'identification des espèces.

Au total 165 larves de tabanidés en provenance des différents gîtes ont été mises en élevage de juillet en septembre. Cet élevage des larves nous a permis de produire 2 lots :

- un premier lot dont nous avons pu déterminer l'espèce à partir du stade larvaire, grâce aux travaux de Goodwin

(1978, 1979) qui renferme 162 larves identifiées ;

- un deuxième lot dont nous n'avons pas pu déterminer l'espèce à partir de l'état larvaire, car jusqu'ici ces larves n'ont pas été décrites dans une publication. Ce lot renfermait 3 larves, dont une qui était à l'état de pupe, une morte, et la troisième qui continuait son cycle larvaire.

Resultats

Résultats obtenus : pourcentages par rapport au total des échantillons collectés

Tabanus gratus……=72 larves sur 162 soit 44%

Tabanus biguttatus…..= 43 larves sur162 soit 25%

Chrysops longicornis...= 14 larves sur162 soit 8%

Chrysops distinctipennis =10 larves sur162 soit 6%

Haematopota…… = 7 larves sur 162 soit 4%

Au cours de cette étude 3 genres (Tabanus, Chrysops et Haematopota) et 5 espèces (Tabanus gratus, Tabanus biguttatus, Chrysops longicornis, Chrysops distinctipennis et Haematopota decora) ont été identifés. L'éclosion d'un lot de larves a révélé la présence d'une nouvelle espèce du genre chrysops, Chrysops griseicolis (diptera tabanidae) dans le cours d'eau de Farakoba au Mali. La même espèce avait été rapportée dans les régions éthiopiennes (Oldroyd (1952, 1954, 1957).

Discussion

Les études précédentes menées par Goodwin et al. (1979) ont montré que les femelles des tabanidés déposent leurs œufs un à un en masse contenant de 50 à 700 œufs. En fonction de l'espèce les masses d'œufs peuvent être composées d'une à plusieurs rangées et varient en couleur, forme et dimension généralement (5 à 25 mm ou plus de long) et (2mm de large).

En fonction de l'espèce les masses d'œufs pouvaient être composées d'une à plusieurs rangées et varient en couleur, forme et dimension généralement de 5 à 25 mm ou plus de long et de 2mm de large.

Certaines femelles ont montré une préférence pour certain site d'ovo position, mais en général les œufs sont pondus sur la végétation ou d'autres supports au-dessus de l'eau ou du sol humide.

La plupart des tabanidés pour lesquelles les informations sont disponibles se reproduisent dans des habitats aquatiques ou semi aquatiques bien que les troncs d'arbres et les rochers soient utilisés. Cependant comme les recherches continuent la liste des espèces se reproduisant en habitat terrestre s'accroît.

Les œufs constituaient un amas blanchâtre quand ils viennent d'être pondus ; ensuite la masse s'assombrit rapidement en différentes nuances qui vont du brun au noir suivant les espèces. Mais pour la plupart des espèces la forme et la couleur des œufs sont inconnues.

Les œufs éclosent généralement 5 à 8 jours après la ponte et les larves s'enfoncent dans l'eau ou le sol. Le développement larvaire peut prendre un an ou plus, mais chez certaines espèces le stade larvaire ne dure que 6 semaines (Goodwin et al. 1976).

Au moment de la fin de la vie larvaire, les larves qui sont aquatiques migraient vers les parties les plus sèches pour faire leur pupaison, de même que celles qui étaient dans des habitats terrestres migraient vers la surface du sol. La période pupale variait de 5 à 10 jours (plupart des chrysops), 1 à 3 semaines pour la plupart des gros tabanus.

Au cours de nos travaux l'émergence des adultes nous a permis d'identifier 3 genres et 5 espèces.

Une étude réalisée en Côte D'Ivoire par Acapovi (2005) confirme la présence de ces deux genres (Tabanus et Chrysops).

DIA et al (2005) ont également rapporté les mêmes espèces au Centre International de Recherche-Développement en élevage dans la zone subhumide (CIRDES) à Bobo Dioulasso au Burkina Faso.

Conclusion

Cette étude nous a permis de confirmer la presence de genres de tabanidés dont les genres Tabanus, Haematopota et Chrysops, Une nouvelle espèce, Chrysops gresicolis est rapportée. Les prospections doivent être élargies à d'autres zones du pays pour recueillir les données relatives à la présence, à la fréquence, à l'abondance des espèces de tabanidés et d'élucider leur rôle dans la transmission mécanique de la trypanosomose au Mali.

Remerciements

Les auteurs remercient sincèrement le Directeur du Laboratoire Central Vétérinaire pour avoir accepté de conduire cette étude au sien de son institution et pour avoir facilité le déroulement des travaux. Ils remercient les techniciens de l'unité de l'entomologie pour leur accompagnement et leur appui technique.

References.

Acapovi, G. L. (2005). Identification et bioécologie des tabanidés vecteurs mécaniques potentiels de la trypanosomose bovine dans les régions de savanes en Côte-d'Ivoire: Odienné et Korhogo Abidjan : Université de Cocody, 2005.- 137 p. Thèse : Entomologie vétérinaire.

Berhaut, J.(1954).FloreillustréeduSénégal. Tome I. Ed. Vol…A compléter

Berhaut, j.(1967).FloreillustréeduSénégal. TomeII. Ed.Vol Acompléter

Dia, M., Desquesnes, S., Hamadou1, J., Bouyer1, W.Yoni 1, A.S. Gouro (2005). Etude comparée de deux modèles de piège Tetra (petit et grand). Revue du CIRDES (2005)

Goodwin, J.T. (1972): immature stages of some eastern Nearctic Tabanidae (Diptera). Introduction and the genus Chrysops Meigen. The Journal of the Georgia Entomological Society, 7, 98-109.

Goodwin, J .T et Murdoch, W.P. (1974). A study of immature Neotropicaltabanidae. Diptera.. Annals of the Entomological Society 67, 85 -133

Goodwin, J.T. (1982). The Tabanidae (Diptera) of Mali. Miscellaneous Publications of the Entomological Society of America, 13, 1-141.

Goodwin, J.T. (1985). Notes on the seasonal and spatial distribution of Tabanidae (Diptera) at Foret. Myia, 3, 415-431.

Oldroyd, H. M.D. (1952).The horse flies of theEthiopianregion, Ed Vol: I Acomplléter

Oldroyd, H. M.D. (1954). The horse flies of the Ethiopian region. Ed. Vol 2… A compléter

Oldroyd, H. M.D. (1957).The horse flies of the Ethiopian region.Ed.Vol 3…Acompléter……..

Director of Publication
Prof. Ahmed Elsawalhy

Editor in Chief
Dr. Simplice Nouala

Editors
Dr. Edward Musiwa Nengomasha
Prof. James Wabacha
Dr. Mohamed Batu Duramany Seisay
Dr. N'Guetta Austin Bosso

Reviewers
Prof. Abdu Ayuba Paul
Prof. Abdullahi Alhaji Magaji
Dr. Adama Sow
Prof. Adel Abdel Azeem Mahmood Fayed
Dr. Amadou Traore
Prof. Ayayi Justin Ayih-Akakpo
Prof. Bassirou Bonfoh
Dr. Benedicta O. Mbu Oben
Prof. Benjamin Obukowho Emikpe
Dr. Bockline Omedo Bebe
Dr. Cyprien F. Biaou
Prof. Etienne Pamo Tedonkeng
Dr. Gilbert Komlan AKODA
Dr. Henri Kabore
Dr. Jacques Somda
Dr. James Okwee-Acai
Dr. Jean Marcel Mandeng
Dr. Jean Claude Fotsa
Prof. John David Kabasa
Prof. John Osita Arinze Okoye
Dr. Joseph Simbaya
Dr. Komlan AKODA
Dr. Langelihle Simela
Prof. Malek Zrelli
Dr. Norber Mbahin
Prof. Osama Rajab Mohamed Elwaer
Dr. Patrick Irungu
Dr. Samuel Wakhusama
Dr. Sarah Ossiya
Prof. Serge Niangoran Bakou
Dr. Tadele Tolosa Fulasa
Prof. Tarnagda Zekiba
Prof. Timothy Uzochukwu Obi
Dr. Unesu Ushewokunze-Obatolu
Dr. William Olaho Mukani

AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

- Veterinary microbiology, epidemiology
- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
- Emerging and re-emerging issues in animal resources
- Biosecurity
- Animal resources trade and value chain
- Socio economics and economics of animal resources development

Language

The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

Manuscripts Submission

Authors are invited to submit electronically their manuscripts via attachment only at bahpa@au-ibar.org in a secured PDF and word format. Manuscript can be sent by post in case of unavailability of internet services (authors should be aware that in this case it will take longer time to be published).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance.

To be considered for publication in the BAHPA, any given manuscript must satisfy the following criteria:

- Originality. BAHPA does not accept manuscripts that have already been published elsewhere. However, studies that replicate results that are already in the literature may be considered for publication, as the independent confirmation of results can often be valuable, as can the presentation of a new dataset.
- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and hold readers' attention.
- Usefulness. Manuscripts submitted must help researchers, trainers, educators and policy makers in all regions of Africa improve their effectiveness.
- Rigorous methodology. Manuscripts submitted must be based on valid and reliable information, documentation or sound concepts, empirically, logically and theoretically supported.
- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
- Because the guidelines are updated as appropriate, authors should check them again before they submit their articles. Manuscripts submitted for publication will be considered for acceptance on the understanding that they present original work which has not been published or submitted for publication elsewhere and that they are subject to peer review.

Types of contribution

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

Short Communications: are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

Review Articles: should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief..

Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes and special calls: The editor will, from time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/ conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

Submission Guidelines
Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, , title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted.
8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. . Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by '*et al.,*'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.,*. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.,* 2001)

The use of reference managing software is encouraged
The authors should be cited in a chronological order by year and then by a or b; in the reference list they should be listed alphabetically.

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

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

Illustrations
Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

Abbreviations, Symbols and Nomenclature
All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

Ethical guidelines
BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

Revising your article
When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

You should go through the referees' comments and for each comment mention whether you followed their suggestion or whether you disagree and wish to respond to the comment. If a referee has misunderstood a point, it is not necessarily their fault and may have been caused by ambiguity or lack of clarity in your article which needs to be corrected. Some authors copy out each of the referees' comments in turn and include their response immediately after. In other cases responses can be made referring back to the reports. Finally, please make sure that you send your revised article to us and not simply the original version again. This is a common mistake, especially when authors send in their work electronically. Electronic revised articles should contain all text and graphics files needed to generate the revised version, and not just those files that have changed.

By observing these guidelines you will be assisting the referees, who give up their time to review manuscripts. If you prepare your article carefully, this can save valuable time during the publication process.

Appeal of Decision
Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office with a detailed explanation for why they find reasons to appeal the decision within 14 days.

Proofs
One set of proofs will be sent to the author to be checked for printer's errors and should be returned within three days.

Offprints
25 offprints of each article will be supplied free of charge. Additional offprints may be ordered and paid for at the proof stage. Each extra offprint costs US $5.00.

Subscriptions
The annual subscription fee, including postage (surface mail) and handling is USD 100.00. Air mail charges are available upon request.

Back volumes
Back issues are also obtainable upon request at similar charges.

Desktop Publisher
Mr. Fahim Franz Kremeier