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AVIAN INFLUENZA H5-SUBTYPE ANTIBODIES IN APPARENTLY HEALTHY LOCAL POULTRY IN LIVE BIRD MARKETS IN JIGAWA STATE, NIGERIA

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Abstract

Jigawa State with over 90% of its poultry being extensively managed local poultry has a vast wetland, the Hadejia Nguru wetland, which serves as a center for aggregation of migratory birds from Europe and Asia. The wetland provides a point of interaction between migratory wild birds, resident wild birds and local poultry which are of great significance in the ecology of *avian influenza*. *Avian influenza* outbreak in Jigawa State involved local poultry usually traded in live bird markets and constitutes the bulk of the poultry population in the State. The study surveyed for *avian influenza* antibodies in local poultry in live bird markets. Three hundred and ninety six sera were collected and tested by *haemagglutination inhibition* test. Overall *avian influenza* H5 subtype antibodies prevalence rate and mean titre were 12.1 % and $7.73 \pm 0.35 \log_2$ respectively. The prevalence rate and mean titre for chickens were 12.15% and $7.30 \pm 0.50 \log_2$ respectively. The figures were 16.39% and $9.90 \pm 0.10 \log_2$ and 9.92% and $6.92 \pm 0.58 \log_2$ for ducks and guinea fowls respectively. Taura live bird market had the highest prevalence of 39.1% with a mean titre of $9.9 \pm 0.11 \log_2$. Chickens, ducks and guinea fowls in live bird markets in Jigawa State were exposed to H5 subtype antigen and are likely to maintain *avian influenza* virus in the markets. There is a need for continuous active surveillance for *avian influenza* virus in live bird markets especially in States where there are high likelihoods of poultry-migratory wild bird interaction.

Key words: *Avian influenza*, Jigawa State, Live bird markets, Local poultry

ANTICORPS DE L'INFLUENZA AVIAIRE SOUS-TYPE-H5 CHEZ DES VOLAILLES LOCALES APPAREMMENT EN BONNE SANTE SUR LES MARCHES D'OISEAUX VIVANTS DANS L'ETAT DE JIGAWA AU NIGERIA

Résumé

L'État de Jigawa, où plus de 90% des volailles sont élevés en système extensif, dispose d'une vaste zone humide, le marécage Hadejia Nguru, qui sert de centre de regroupement des oiseaux migrateurs d'Europe et d'Asie. La zone humide constitue un point d'interaction entre les oiseaux sauvages migrateurs, les oiseaux sauvages sédentaires et les volailles locales, qui sont d'une grande importance dans l'écologie de la grippe aviaire. L'épidémie de grippe aviaire dans l'État de Jigawa a affecté les volailles locales habituellement vendues sur les marchés d'oiseaux vivants et qui constituent la majeure partie de la population de volailles dans cet État. La présente étude avait pour objet d'examiner les anticorps de la grippe aviaire chez la volaille locale sur les marchés d'oiseaux vivants. Trois cent quatre-vingt-seize sérums ont été prélevés et testés au moyen du test d'inhibition de l'hémagglutination. Le taux de prévalence global des anticorps de la grippe sous-type H5 et le titre moyen étaient respectivement de 12,1% et de $7,73 \pm 0,35 \log_2$. Le taux de prévalence et le titre moyen pour les poulets étaient respectivement de 12,15% et $7,30 \pm 0,50 \log_2$. Les chiffres étaient 16,39% et $9,90 \pm 0,10 \log_2$ et 9,92% et $6,92 \pm 0,58 \log_2$ respectivement pour les canards et les pintades. Le marché d'oiseaux vivants de Taura avait la plus forte prévalence de 39,1% avec un titre moyen de $9,9 \pm 0,11 \log_2$. Les poulets, canards et pintades vivants sur les marchés d'oiseaux vivants dans l'État de Jigawa ont été exposés à l'antigène du sous-type H5 et sont susceptibles de maintenir le virus de la grippe aviaire sur les marchés. Il est nécessaire de procéder à une surveillance active et permanente du virus de la grippe aviaire sur les marchés d'oiseaux vivants, en particulier dans les États où les probabilités d'interaction entre la volaille et les oiseaux migrateurs sauvages sont élevées.

Mots-clés : Influenza aviaire ; État de Jigawa ; Marché d'oiseaux vivants ; Volaille locale

Introduction

Local poultry (LP) which form more than 80% of the over 150 million poultry in Nigeria and 90 % of poultry in Jigawa State, represents a biodiverse rare gene pool and is a source of livelihood to villagers (Abdu *et al.*, 1992; Adene and Oguntade, 2006). It is also the main source of poultry meat and eggs which is at risk of being lost to highly pathogenic (HPAI) (Abdu *et al.*, 1992).

The local poultry industry which is highly developed in Jigawa State is very important to the rural economy through provision of income, supplementary food and employment to the rural populace would be at risk of being lost from poultry diseases especially *avian influenza*.

Jigawa State with over 90 % of its poultry extensively managed local poultry has a vast wetland, the Hadejia Nguru wetland. This wetland serves as a center of aggregation of migratory' birds from Europe and Asia. The wetland provides a point of interaction between local poultry, resident wild birds and migratory wild birds significant in *avian influenza* ecology (Willians *et al.*, 2008).

As local poultry are usually taken to LBMs for sales and slaughter at home, LBMs are important points of interaction between local poultry from different villages (Aye, 2010). When *avian influenza* virus is established in a LBM, it can spread to other markets and farms through contaminated equipment, bird, people and vehicles (Henning *et al.*, 2012).

When antibodies to *avian influenza* were detected in apparently healthy local chickens in Jigawa during the 2006 HPAI outbreak in Nigeria it raised fears that these chickens may act as reservoirs thereby maintaining and spreading *avian influenza* virus to commercial poultry (Wakawa, 2009).

Local poultry are traded in LBMs in Jigawa State and the markets serve as mixing points for poultry from different households and villages hence their *avian influenza* (AI) status is a reflection of the AI status within the State. This study assessed the prevalence of *avian influenza* antibodies among local poultry

in Jigawa State.

Materials and Methods

Study area

study was carried out in Jigawa State which lies between latitudes 11°N and 13°N and longitudes 8°E and 10° 35E covering a land area of about 22,410 km². Jigawa State is bordered to the West by Kano State, to the East by Bauchi and Yobe States and to the North by Kastina State and Niger Republic. It has an average annual rainfall of 300-1000 mm and temperature varying between 15-35°C. The climate is tropical continental and the vegetation Sudan Savannah (JARD, 1994).

Jigawa State has twenty seven local government areas with a huge wetland, the Hadejia Nguru wetland, which covers an area of approximately 16 km² in Jigawa and Yobe states (Barbier *et al.*, 1991).

Sampling frame

Multistage random sampling techniques was employed in order to represent all the three senatorial districts, daily, every other day and weekly live bird markets (LBMs), wetlands LBMs, and border markets as shown in Table 1. Based on the aforementioned sampling technique, eight LBMs namely Babura, Dutse, Gujungu, Jahun, Kafin Hausa, Maigatari, Ringim and Taura LBMs in seven local government areas were selected as study LBMs and blood samples were collected between August, 2008 and April, 2009.

Data collection

A structured questionnaire was administered to consenting live bird traders prior to sample collection. The questionnaire gather information on poultry source and vaccination history.

Sample collection and processing

Local poultry were selected without replacement and their species, age, sex and any abnormal condition noted prior to collection of blood sample.

Two milliliter of blood collected

through brachial vein of poultry using 21 G sterile hypodermic needles and 2 ml syringes carefully observing asepsis were allowed to clot at room temperature and sera obtained were stored at -200 C until used for serology.

Detection of avian influenza antibodies by HI test

An alpha haemagglutination inhibition (HI) test was performed on all poultry sera using standard procedures recommended by OIE (OIE, 2009). The test antigen used was an inactivated H5 subtype-antigen while the positive serum was an H5N2 serum both prepared by Istituto Zooprofilattico OIE/FAO Laboratory for AI and NDV delle Venezie.

Data analysis

Data generated were analysed by descriptive statistics using Statistical Package for Social Sciences (SPSS) version 17 program. The frequency, mean, standard error of mean and chi square values of cross tabulations were calculated. Values of $p \leq 0.05$ were considered significant.

Results

Three hundred and ninety six local poultry in eight LBMs in Jigawa State were sampled comprising 17.4% (69) from Babura, 6.3% (25) Dutse, 27% (107), 4.3% (17) Jahun, 1.8% (7) Kafin Hausa, 33.8% (134) Maigatari, 3.5% (14) Ringim and 5.8% (23) from Taura LBM. The overall prevalence of avian influenza H5 subtype antibodies was 12.1 % with a mean titre of $7.73 \pm 0.35 \log_2$. The prevalence of avian influenza H5 antibodies in guinea fowls, chickens and ducks were 9.92% (12/121), 12.15% (26/214) and 16.39% (10/61) respectively. Based on age, the prevalence rates in adult and young poultry were 11.9% (39/327) and 23.1% (3/13) respectively, with growers having a prevalence

rate of 10.7% (6/56). The avian influenza H5 subtype antibodies prevalence rate based on sex were 7.96% (9/113) and 13.78% (39/283) for male and female respectively.

All poultry sampled in Kafin Hausa and Ringim LBMs did not have antibodies to H5 subtype avian influenza antibodies. Amongst the LBMs with AI H5 subtype sero-positive poultry, Babura LBM had the lowest prevalence rate of 5.8% (4/65) and the highest being Tura LBM with 39.0% (9/23). However, poultry in Jahun LBM had the highest mean antibody titre of $10.0 \pm 0.11 \log_2$ and Babura LBM had the lowest mean titre of 6.00 ± 1.08 among the sero-positive LBMs. All the poultry in Jahun and Taura had H5 antibodies titre of $\geq 7 \log_2$ (%) ($p = 0.01$) (Table 2).

The mean avian influenza H5 antibodies titre in guinea fowls was $6.92 \pm 0.58 \log_2$, $7.30 \pm 0.50 \log_2$ in chickens and $9.90 \pm 0.1 \log_2$ in ducks. The mean titre for adults and young poultry were $7.79 \pm 0.38 \log_2$ and $7.67 \pm 1.20 \log_2$ respectively while the grower titre was $7.43 \pm 1.21 \log_2$. Male poultry had a mean titre of $7.33 \pm 0.88 \log_2$ with $7.82 \pm 0.38 \log_2$ in females.

The antibody titre among local poultry varied with 46.9 % having H5 subtype antibody titre of $10.0 \log_2$ and 63.3% (31) had antibody titre of $\geq 7 \log_2$. All ducks sampled had AI H5 antibody titre of $\geq 7 \log_2$, 59.3% (16) of chickens and 41.7% (5) of guinea fowls had titre of $\geq 7 \log_2$ ($p = 0.015$) (Table 3).

Only ducks were traded in Taura, Ringim, Kafin Hausa and Jahun LBMs while chickens and guinea fowls were traded in Dutse, Maigatari, Gujungu and Babura LBMs. The prevalence rate of H5 subtype antibody of chickens in Babura LBM was 6.97 % (Table 4).

Discussion

The study confirms the exposure of local poultry in Jigawa State to avian influenza

Table 1: Selection of live bird markets to participate in the study.

Senatorial District	Weekly LBMs	Every other day LBMs	Daily LBMs	Wet lands LBMs
A	2	1	0	2
B	1	0	1	0
C	1	0	0	0
Total	4	1	1	2

Table 2: Distribution of avian influenza H5 antibodies in local poultry in live bird markets in Jigawa State.

Live bird market	No. sampled	No. positive (prevalence) (%)	Mean titre \pm SE (Log_2)	Poultry with titre $\geq 7 \log_2$ (%) ($p = 0.01$)
Babura	69	4 (5.8)	6.00 \pm 1.08	25.0
Dutse	25	9 (36.0)	9.10 \pm 0.64	90.0
Gujungu	107	10 (9.3)	6.30 \pm 0.68	30.0
Jahun	17	1 (5.9)	10 \pm 0.00	100
Kafin Hausa	7	0 (0.0)	0.0 \pm 0.0	0.0
Maigatari	134	15 (11.2)	6.8 \pm 0.60	53.3
Ringim	14	0 (0.0)	0.0 \pm 0.0	0.0
Taura	23	9 (39.1)	9.9 \pm 0.11	100
Total	396	48 (12.1)	8.04 \pm 0.39	63.3

Table 3: Distribution of avian influenza H5 subtype anybodies in chickens, ducks and guinea fowls in live bird markets in Jigawa State.

Species	No. of sample	Prevalence (%)	Mean titre \pm SE (\log_2)	Titre $\geq 7 \log_2$ (%)
Chicken	214	26 (12.2)	7.73 \pm 0.35	59.3
Duck	61	10 (16.4)	9.90 \pm 0.10	100
Guinea fowl	121	12 (9.9)	6.92 \pm 0.58	41.7
Total	396	48 (12.1)	8.04 \pm 0.39	63.3

Table 4: Distribution of avian Influenza H5 sero-prevalence by species of poultry and location of live bird markets in Jigawa State.

Live bird market	Guinea fowl prevalence (%)	Chicken prevalence (%)	Duck Prevalence (%)
Babura	3.8	6.97	NT
Dutse	0	36.0	NT
Gujungu	24.14	3.85	NT
Jahun	NT	NT	5.88
Kafin Hausa	NT	NT	0.0
Maigatari	6.25	15.71	NT
Ringim	NT	NT	0.0
Taura	NT	NT	39.1

NT= Not Tested

H5 subtype antigen. Since these poultry had not been vaccinated, it is likely that the antibodies were developed from exposure with a field virus possibly following contact with wild waterfowl which are natural reservoirs of avian influenza virus. In fact avian influenza virus has been isolated from healthy waterfowls in Nigeria (EFSA, 2006; Gaidet et al., 2008). The high antibody titre ($\geq 7 \log_2$) to avian influenza in chickens and ducks indicates circulation of a field avian influenza virus (Boven et al., 2008).

The sero-conversion of local poultry sampled might imply that these birds are survivors from the 2006-08 avian influenza outbreak or were exposed to low pathogenic avian influenza virus. But the high antibody titre indicates a recent exposure to avian influenza virus which might be circulating within the local poultry population in Jigawa State (Durosinlorin et al. 2009). The circulation of a low pathogenic avian influenza field virus with the risk of mutating to a highly pathogenic form after circulating

in susceptible poultry poses a threat to local poultry biodiversity and livelihood of local poultry farmers in Jigawa State (Harder and Werner, 2006). The high antibody prevalence rate in ducks confirms that ducks are likely to maintain *avian influenza* virus within a population (Hanson, 2005). Similar to previous studies, the prevalence for H5 antibodies in young poultry was higher than in adults confirming reports that young poultry are more susceptible to AIV than adults hence the higher sero-conversion (Kasemsuwan *et al.*, 2009).

Since all poultry sampled at Taura LBM were ducks and the LBM had the highest prevalence with all the ducks having antibody titer $\geq 7 \log 2$, ducks purchased from this LBM are likely to spread the *avian influenza* virus to household poultry if and when new additions are purchased from the LBM.

In Jigawa State, most local poultry farmers use poultry manure to fertilize crop farms usually located next to their houses. The poultry manure is usually purchased from commercial farms in Kano State that likely have been involved in the H5N1 outbreaks in 2006 and 2007. Thus the exposure of local poultry to AIVs might be as a result of contact with poultry litter used as manure (Assam *et al.*, 2011). The constant interaction of local poultry kept on free range with wild birds increases their risk of exposure to *avian influenza* virus. Similarly, most commercial poultry farm attendants keep local poultry and are in constant contact with commercial poultry there by exposing their local poultry to *avian influenza* virus.

Jigawa state having wetlands which attracts migratory wild birds from Europe increases the risk of introduction of *avian influenza* virus to local poultry which are most likely to interact especially as there were reports of high mortality in local poultry prior to the first reported case of *avian influenza* in Nigeria. The proximity of poultry farms to rivers, ponds and lakes increases the risk of contact with migratory birds which are reservoirs of *avian influenza* virus (EFSA, 2006).

Apparently healthy local chickens, ducks and guinea fowls traded in the LBM in Jigawa State have been exposed to *avian influenza* virus with high antibody prevalence in ducks, young and female poultry. Ducks presented for

sale at Taura LBM had the highest prevalence for *avian influenza* H5 antibodies posing a serious threat in the control of *avian influenza* in Nigeria and West Africa. This study highlights the need for continuous active surveillance for *avian influenza* virus in LBMs especially in States with high likelihood of poultry-migratory bird interaction.

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EFFECT OF CONCENTRATE SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF SMALLHOLDER DAIRY CATTLE IN RUNGWE DISTRICT, TANZANIA.

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Abstract

A study was conducted in Rungwe district in Tanzania, to assess the effect of concentrate supplementation on reproductive performance of smallholder dairy cattle. Cattle used were crossbreds, mainly between Friesian (*Bos taurus*) and indigenous Tanzania Short Horn Zebu (*Bos indicus*). All animals were managed under farmers conditions with zero-grazing mainly on natural pasture grasses and cultivated fodder comprising of Napier grass (*Pennisetum purpureum*), *Desmodium* spp. and Guatemala grass (*Tripsacum laxum*). The animals were allotted into supplemented (S) and control (C) groups. In addition to the basal diet, the S group received a supplement, which was formulated to contain 13.4 ME (MJ/kg), 138.1 g/kg CP, 2.3 g/kg Ca, 1.3 g/kg P and 19 mg/kg Cu. The S group had higher plasma P (1.65 vs. 1.52 ± 0.03 mmol/L) ($P < 0.05$), Cu (7.85 vs. 6.28 ± 0.31 μ mol/L) ($P < 0.001$), and PUN (4.11 vs. 3.32 ± 0.07 mmol/L) compared with the C group. Postpartum ovarian cyclicity resumed before 90 days in greater proportion in the S than the C group although the association between the groups and days to initiation of cyclicity (before or after 90 days postpartum) was not statistically significant ($P > 0.05$). Postpartum first visual oestrus was observed earlier in a larger proportion of the S compared with the C group and the association between the groups and days to postpartum first visual oestrus was significant ($P < 0.001$). The S group had significantly ($P < 0.05$) fewer days (103.2 ± 11.9) from parturition to first visual oestrus (DPO) than the C group (146.8 ± 15.3 days). It was concluded that there was a need for concentrate supplementation to improve the reproductive performance of dairy cattle in Rungwe district. Further studies using several supplement levels were recommended in order to come up with optimum supplement level for desired dairy cattle reproductive performance.

Key words: Concentrate supplement, reproductive performance, small holder dairy cattle.

EFFET DE LA SUPPLEMENTATION EN CONCENTRES SUR LA PERFORMANCE DE LA FONCTION REPRODUCTIVE DES VACHES LAITIÈRES DES PETITES EXPLOITATIONS DU DISTRICT DE RUNGWE EN TANZANIE.

Résumé

Une étude a été réalisée dans le District de Rungwe en Tanzanie dans le but d'évaluer l'effet de la supplémentation en concentrés sur les performances de la fonction reproductive des vaches laitières des petits exploitants. Les vaches utilisées dans l'étude étaient de race croisée, principalement entre frisonnes (*Bos taurus*) et zébus à courtes cornes indigènes de Tanzanie (*Bos indicus*). Tous les animaux ont été élevés dans les conditions de ferme, principalement en stabulation permanente, nourris principalement à l'herbe de pâturages naturels et aux fourrages cultivés comprenant l'herbe à éléphant (*Pennisetum purpureum*), *Desmodium* spp et l'herbe du Guatemala (*Tripsacum laxum*). Les animaux ont été répartis en groupe recevant un supplément (Groupe S) et en groupe témoin (Groupe C). Outre le régime de base, le groupe S a reçu un supplément dont la formule contenait 13,4 ME (MJ/kg), 138,1 g/kg CP, 2,3 g/kg Ca, 1,3 g/kg P et 19 mg/kg Cu. Le Groupe S avait des valeurs plus élevées de plasma P ($1,65$ contre $1,52 \pm 0,03$ mmol/L) ($P < 0,05$), Cu ($7,85$ contre $6,28 \pm 0,31$ μ mol/L) ($P < 0,001$) et PUN ($4,11$ contre $3,32 \pm 0,07$ mmol/L) par rapport au Groupe C témoin. Le cycle ovarien post-partum a repris avant 90 jours chez une très grande proportion du Groupe S par rapport au groupe C, bien que l'association entre les groupes et les jours avant le début du cycle (avant ou après 90 jours post-partum) n'était pas statistiquement significative ($P > 0,05$). Les signes visuels du premier œstrus post-partum ont été observés plus tôt chez une plus

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grande proportion du groupe S par rapport au groupe C, et l'association entre les groupes et les jours précédant l'apparition des premiers signes visuels de l'oestrus post-partum était significative ($P < 0,001$). Le groupe S avait un nombre de jours significativement ($P < 0,05$) moins élevé ($103,2 \pm 11,9$) entre la mise-bas et l'oestrus visuel (DPO) par rapport au Groupe C ($146,8 \pm 15,3$ jours). Il a été conclu qu'une supplémentation en concentrés était nécessaire pour améliorer la performance de la fonction reproductive des vaches laitières du District de Rungwe. D'autres études utilisant plusieurs niveaux de suppléments ont été recommandées en vue de trouver le niveau de complément optimal pour la performance souhaitée des vaches laitières.

Mots-clés : Supplément concentré ; Performance de la fonction reproductive ; Vaches laitières des petites exploitations

Introduction

Rungwe district is located in the southern highlands zone (SHZ) of Tanzania. Over 80% of the population in the district is engaged in agriculture. In addition to local zebu cattle, smallholder farmers in the area keep crossbred dairy cattle. Introduction of the improved dairy cattle breeds took place mostly as a result of activities of private and public projects (Mwakyembe, 1996a). However, the increase in dairy cattle population has been lower than projected in the area where long calving interval (CI) has been suspected to be a contributing factor (Mwakyembe, 1996b).

Most of available information on reproductive performance (RP) of dairy cattle in SHZ is similar to other parts of Tanzania (Table 1). In most cases the information indicates suboptimal RP in terms of age at first calving (AFC), interval from calving to conception (CCI) and CI. This is in contrast to what is expected in a well-managed dairy herd, where heifers would typically show first oestrus from 7 to 18 months of age and 65 to 70 % of the cows conceive on first service with an average of 1.3 to 1.7 services per conception (Roberts, 1971). Generally, recommended AFC in a well-managed dairy herd is 24 months (Quigley *et al.*, 1996) and less than 10% of cows are expected to have reproductive problems and CI should be between 360 and 390 days (Roberts, 1971). In order to attain this CI, cows must be pregnant within 85 - 115 days after calving.

Variations in RP result largely from influence of environmental (80%) compared with genetic (20%) factors (Lotthammer, 1989; Mwatawala *et al.* 2003). Possible environmental factors that can contribute to the suboptimal RP include poor nutrition exacerbated by seasonal

variations in pasture quality and quantity, low oestrus detection rates, reproductive diseases and disorders and functional abnormalities. It is assumed that the suspected suboptimal RP in Rungwe district is related to improvements in dairy herd genetics brought about by use of *B. taurus* x *B. indicus* crossbreds which do not respond well in local environmental conditions. In addition, Gimbi, *et al.* (2002) found out that steaming up of animals was not normally done and mineral supplementation was not regular in Rungwe. Suboptimal RP of cows related to improvements in milk yield and increased nutrient demands placed on the improved cattle has been reported in the United States (Washburn *et al.*, 2002), Australia (Macmillan *et al.*, 1996), Ireland (Roche, 2000) and the United Kingdom (Royal *et al.*, 2000).

Nutritional interventions using various levels of locally available feed resources to supplement smallholder dairy cattle has been shown to improve production and reproductive performance and proved to be economically viable in a number of African countries (Guerouali, 1999; Boodoo *et al.*, 1999).

In Tanzania, several supplementation trials have been reported in smallholder crossbred dairy cattle using variable supplemental ingredients. However, most studies were short term and aimed more at evaluation of effects of energy, protein and mineral supplementation in milk production and composition than reproductive performance (Urassa, 1999; Msangi *et al.*, 2004; Mlay *et al.*, 2005).

Since smallholder dairying is an important avenue for rural development in developing countries through its contribution to increases in livestock and farm production, income generation from sales of milk and

dairy product, provision of jobs and transfer of money from urban to peri-urban and rural areas (Mdoe et al. 2000; Kristensen et al. 2004), and sub optimal RP is evident from the reviewed studies, the present on-farm study was carried out to investigate the effect of supplementation on RP of smallholder dairy cattle in Rungwe district.

Null hypothesis: Supplementation with concentrates will not improve reproductive performance of smallholder dairy cattle in Rungwe district

Alternative hypothesis: Supplementation with concentrates will improve reproductive performance of smallholder dairy cattle in Rungwe district

Materials and Methods

Experimental design and sampling

A longitudinal study with multistage, purposive sampling was set where three divisions and within each division, three wards were selected. Two villages and 4 farmers per village were selected from each of the selected wards. A sample of 72 cows and heifers (about 7 months pregnant) were purposefully selected from the farmers and alternately allocated as obtained into control and supplemented treatment groups.

Experimental animals, their management and feeding

Animals used were crossbreds, mainly between Friesian (*Bos taurus*) and indigenous Tanzania Short Horn Zebu (*Bos indicus*) cattle. All animals were managed under farmers conditions where they were zero-grazed mainly on natural pasture grasses and cultivated fodder comprising Napier grass (*Pennisetum purpureum*), *Desmodium* spp. and Guatemala grass (*Tripsacum laxum*), which always constituted less than half of the basal diet. Banana pseudostems and leaves were increasingly used during the dry season due to shortage of natural and cultivated pastures. Maize stover, bean and groundnut straw, and sweet potato vines were also used when available. Home made supplementary feeding, comprising hominy meal, sunflower seed cake and commercial minerals was provided

mostly to lactating cows. When available the concentrate was fed twice a day during milking. Health management involved check-ups and treatments by local veterinary field officers.

The supplemented group was given a concentrate in addition to the basal diet. Literature from Rungwe district (Table 2) and tables of nutrient requirements for dairy cattle (ARC, 1990) were used to estimate the amount of metabolisable energy (ME), crude protein (CP), calcium (Ca) and phosphorus (P) available in the pasture grasses. Estimated nutrient balance at dry matter intake (DMI) of 2.5% of cattle body weight (Mussa, 1998) in Rungwe district were computed and shown in Table 3. The specifications for nutrients requirements used were for pregnant and lactating animals weighing 320.8 kg and producing 8.4 litres of milk per day.

The supplement was made to cover the deficits to enable the animals to approximately meet their daily requirements for ME, CP, Ca and P (ARC, 1990) assuming the ME and CP contents of ingredients to be 13.8 MJ/kg and 116 g/kg for hominy meal (HM) and 12.6 MJ/kg and 219 g/kg for sunflower seed cake (SSC), respectively (Bwire and Wiktorsson, 1996).

The supplement was formulated to contain 13.4 ME (MJ/kg), 138.1 g/kg CP, 2.3 g/kg Ca, 1.3 g/kg P and 19 mg/kg Cu using hominy meal (76%), sunflower seed cake (23%) and commercial mineral supplement (1%), which was Super Maclick® (Coopers Kenya Ltd). The supplement was fed at a rate of 7 g/kg Bwt/day for 2 months before calving and 10 g/kg Bwt/day after calving during the rainy season and 8 g/kg Bwt/day for 2 months before calving and 11 g/kg Bwt/day after calving during the dry season.

Mean daily dietary nutrient balances before and after calving were estimated using predicted DMI and tabulated dietary nutrient requirements (ARC, 1990) for an 8 to 9 months pregnant and lactating animal weighing 287.1 kg and producing 12.0, 11.9, and 10.9 litres of milk per day during the first, second and third months post-calving, respectively.

Sample collection and measurement of variables

Forty basal feed samples were randomly collected from feeding troughs in the three

Table 1: Reproductive performance parameters in different types of cattle and farms in Tanzania

Reproductive parameters			Cattle/farm type	Place	Source
¹ AFC (months)	² CCI (days)	³ CI (days)			
		490-503	Smallholder (B.Taurus x B. indicus)	Kagera	Rugambwa et al. (1994)
		453	Smallholder (B.Taurus x B. indicus)	Northeast Tanzania	Msanga et al. (1999)
		477 (335-860)	Smallholder	Highland areas (Mt. Meru)	Kanuya et al. (2000)
30.2±0.8		426±18	Large farm (Friesian, Ayrshire and Jersey)	Morogoro	Mujuni et al. (1990)
		433±8	Smallholder (B.Taurus)	Mbeya	Mchau, (1991)
		443±10	Smallholder (B.Taurus x B. indicus)	Mbeya	Mchau, (1991)
33-39		375-489	Large farms	Iringa & Mbeya	Kifaro, (1995)
36.7		484.9	Smallholder	Iringa & Mbeya	Balikowa, (1997)
	237±14	517±22		Arumeru	Kanuya et al. (1998)
32.8		404±13 (RS)	(Ayrshire)	Iringa	Kanuya and Greve, (2000)
		466±20 (DS)			
		480	Smallholder (B.Taurus x B. indicus)	Iringa & Mbeya	Mwakalile et al. (2002)

¹AFC = Age at first calving

²CCI = Interval from calving to conception

³CI = Calving interval

wards (20 during rainy season and 20 during dry season). Milk samples were collected once every 5 days from 14 to 90 days post calving for determination of progesterone concentration. Milk samples were also collected when heat signs were observed on days different from the days scheduled for sampling. Blood samples were collected monthly starting about 60 days prepartum to 90 days postpartum. All samples were analysed at the Department of Animal Science (DASP) laboratory of Sokoine University of Agriculture, in Morogoro Tanzania.

Feed, blood and milk sample analyses

The feed samples were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), Ca, P and Cu and in vitro organic matter digestibility (IVOMD). Proximate analysis was done according to procedures of AOAC (1990). Progesterone concentrations in milk were determined using self-coating

milk progesterone radioimmunoassay (RIA) kit (FAO/IAEA Programme, 1999 Bench protocol, version – ScRIA 3.1). Plasma Ca, P and Cu levels were determined by spectrophotometric techniques (Kessler and Wolfman, 1964; Paynter, 1987) while urea in blood plasma was determined using a NED Dye method kit (Span Diagnostics Ltd, Surat, India)

Derived reproductive performance parameters

Following calving, farmers were involved in oestrus detection and hand-mating the animals. The date of conception was obtained retrospectively after confirmation of pregnancy by rectal palpation at about 60 days post mating. Reproductive performance parameters were derived as follows:

- Days from parturition to resumption of ovarian cyclic activity (DPOA) were determined by RIA of milk progesterone. Increase in progesterone concentration after calving above 1 ng/ml or more for 2

consecutive sampling days was considered an indication that ovulation had taken place (Terqui *et al.*, 1982).

- Oestrus detection rate was calculated as the proportion of visible heats recorded by farmers expressed as a percent of those cows registered as being in oestrus by use of milk progesterone assay.
- Days from parturition to first visual oestrus (DPO) were calculated from farmers' records. The results were arbitrarily classified into 3 categories according to how soon the first visual oestrus was observed after calving. The DPO categories of < 90 days, 90 – 150 days and >150 days were used.

Statistical analyses

Data were analysed using SPSS 12.0.1 for windows (Copyright® SPSS Inc, 1989-2003). Descriptive analyses were carried out on natural pasture nutritional data. The procedure of general linear model, multifactorial between subjects analysis of variance was employed with treatment as a fixed factor while plasma Ca, P, Cu and Urea Nitrogen and their initial observations were included as dependent variables.

Contingency tables for percentages of animals that showed postpartum cyclicity before and after 90 days, and animals in different DPO categories for treatment factors were produced using the procedure of descriptive statistics. Pearson's chi-square test and Cramer's V test were used to determine the relationship between different categories in the contingency tables and to quantify the strength of relationships, respectively.

Results

Dry matter, metabolisable energy, crude protein and minerals in natural pastures

Mean values for DM, ME and CP, P, Ca and Cu in native pastures are shown in Table 4.

Daily dietary nutrient balance for supplemented animals

Mean daily dietary nutrients' balances before and after calving are shown in Table 5. ME and CP balances were positive for two months pre-calving and negative for two months post-calving. Calcium balance was negative from two months to one month before calving after which it turned positive up to two months post-calving. Negative balance was observed for P and Cu throughout the duration of experimental period.

Plasma mineral and urea nitrogen concentration for supplemented and control animals

There was insignificant difference in mean Ca values between the S and C treatment groups (Table 6). The mean plasma P as well as Cu concentrations for the supplemented group were significantly ($P < 0.05$) higher than the values for the control group. The mean plasma urea nitrogen (PUN) concentration of the supplemented group was significantly ($P < 0.001$) higher than the control group (Table 6).

Oestrus detection rate and reproductive performance parameters

Oestrus detection rate was 68%. The percentages of cows showing postpartum resumption of ovarian cyclicity before and

Table 2: Nutritive value of natural pastures in Rungwe district

	Wet season	Dry season	Mean
Dry matter (DM) (%)	13.62	39.19	25.11
Metabolisable energy (ME) (MJ/kgDM)	8.17	7.74	7.95
Crude protein (CP) (%)	8.42	12.51	10.46
Calcium (Ca) (%)	0.31	0.34	0.33
Phosphorus (P) (%)	0.22	0.19	0.21

Source: Mussa, (1998).

Table 3: Estimated nutrient balance per day for cows fed on pastures in Rungwe district

Two months before calving to calving					
Source	DMI (kg)	ME (MJ)	CP (g)	Ca (g)	P (g)
Pasture	8.55	68.0	894.3	28.3	18.0
Requirements		52.9	396.5	33.0	20.0
Balance		+15.1	+497.8	-4.7	-2.0
Calving to two months after calving					
Pasture	8.55	68.0	894.3	28.3	18.0
Requirements		104.0	1083.3	32.4	21.0
Balance		-36	-189.0	-4.1	-3.0

Table 4: Mean chemical composition of natural pastures fed to dairy cattle in Rungwe district

Component %	Mean \pm SEM	Minimum	Maximum
DM	29.4 \pm 2.2	14.9	42.7
ME (MJ/kgDM)	5.7 \pm 0.2	3.3	8.1
CP	9.3 \pm 0.5	6.4	14.0
CF	31.4 \pm 1.2	22.5	46.8
EE	1.0 \pm 0.1	0.5	1.7
Ash	13.6 \pm 1.0	8.9	27.5
Ca	0.6 \pm 0.1	0.1	1.3
P	0.2 \pm 0.02	0.1	0.4
Cu (mgCu/kgDM)	0.27 \pm 0.35	0.10	0.53

Table 5: Mean daily dietary nutrient balance for two months before and after calving for supplemented animals

Overall nutrient balance precalving (two months before calving to calving)						
Source	DMI (kg)	ME (MJ)	CP (g)	Ca (g)	P (g)	Cu (mg)
Pasture	4.9	27.9	455.7	29.4	9.8	1.3
Supplement	2.4	32.2	331.4	5.5	3.1	45.6
Total	7.3	60.1	787.1	34.9	12.9	46.9
Requirements		53.6	366.3	35.5	21.1	72.5
Balance		+6.5	+420.8	-0.6	-8.2	-25.6
Overall nutrient balance postcalving (calving to two months after calving)						
Pasture	9.2	52.4	855.6	55.2	18.4	2.5
Supplement	2.7	36.2	372.9	6.2	3.5	51.3
Total	11.9	88.6	1228.5	61.4	21.9	53.8
Requirements		109.5	1350.8	42.7	32.9	119
Balance		-20.9	-122.3	+18.7	-11	-65.2

after 90 days for supplemented and control groups are shown in Table 7. Postpartum ovarian cyclicity resumed before 90 days in a greater proportion of animals that were supplemented than those in control group although the association between the groups

(supplemented and control) and days to initiation of cyclicity (before or after 90 days postpartum) was not significant ($P > 0.05$). Postpartum first visual oestrus was observed earlier in a larger proportion of supplemented animals compared with animals in the control

Table 6: Least square means (LSMs \pm Standard error) for selected plasma minerals and urea nitrogen

Parameter	Group			P-value
	Control	Supplemented	SEM	
Blood plasma Ca (mmol/L)	2.70 ^a	2.65 ^a	0.03	ns
Blood plasma P (mmol/L)	1.52 ^a	1.65 ^b	0.03	*
Blood plasma Cu (μ mol/L)	6.28 ^a	7.85 ^b	0.31	***
PUN (mmol/L)	3.32 ^a	4.11 ^b	0.07	***

Means with different superscript within each row are significantly different

Table 7: Proportions of cows in different categories of days from parturition to resumption of ovarian cyclic activity (DPOA) and days from parturition to first visual oestrus (DPO) for supplemented and control groups

Parameter/ Category	Groups						χ -square p-value
	DPOA	N	Overall (%)	N	Supplemented (% cows)	N	
\leq 90 days PPI	27	56.5%	16	50.0%	11	36.7%	
> 90 days PP	35	43.5%	16	50.0%	19	63.3%	
Total	62	100.0	32	100.0	30	100.0	ns
DPO							
< 90 days PP	17	28.8%	10	34.5%	7	23.3%	
90-150 days PP	22	37.3%	17	58.6%	5	16.7%	
> 150 days PP	20	33.9%	2	6.9%	18	60.0%	
Total	59	100.0	29	100.0	30	100.0	***

I PP = postpartum; ns = Not significantly different ($P > 0.05$); *** = $P < 0.001$

group (Table 7) and the association between the groups (supplemented and control) and days to postpartum first visual oestrus was significant ($P < 0.001$) with significant strength of association (Cramer's V < 0.001). The overall mean DPO (\pm SD) was 125.0 ± 9.7 days while the supplemented group had significantly ($P < 0.05$) lower DPO of 103.2 ± 11.9 days than the control group (146.8 ± 15.3 days).

Discussion

The mean ME in pasture (5.7 MJ/kgDM) in the present study was below the 7.95 MJ/kgDM observed by Mussa (1998) in the same area and less than the expected range (7.0 to 11.0 MJ/kgDM) for tropical native or improved grasses when cut between 2-8 weeks regrowth (Wan Hassan *et al.*, 1981;

Doto *et al.*, 2004). The probable reasons for the variations among the reported ME values were differences in species of grasses, stage of maturity, seasons, and years when the pastures were cut and analysed. Values of ME as low as 7 MJ/kgDM are observed for mature pasture or unfertilised grass with low leaf content but well fertilised actively growing tropical grass pasture can provide 9 MJ ME/kgDM (Moss, 2004). The observed mean (9.3%) CP content of pasture in the present experiment was lower than 10.46% CP reported by Mussa, (1998) in the same area but was within the range of values for most tropical pastures containing mainly grasses which is 7-12% CP (Aminah and Chen, 1989). This is in contrast to a well fertilised actively growing tropical grass pasture containing 30-40% green leaf which can provide 14-19% CP (Moss, 2004).

The observed low mean ME with high CP in pastures in the present study could partly be due to the *in vitro* digestibility method used. The method always underestimates digestibility values of forages when compared to *in vivo* data (Tilley and Terry, 1963). In addition, it has been suspected that the lower digestibility values obtained at the DASP laboratory of Sokoine University of Agriculture have been partly due to poor nutritional status of rumen liquor donor animals, which leads to low numbers of microbes in the liquor and hence low digestibility (Yona, 2004). Underestimation of the *in vitro* digestibility will lead to lower ME content.

Pasture Ca content (0.6%) in the current experiment was higher than 0.33% reported by Mussa (1998) for the same area but the P contents (0.2%) were similar in the two studies. The probable reasons for the difference in pasture Ca levels between the current and Mussa's report could be differences in stage of maturity, seasons and years of observation where pattern and amount of rainfall play a role through their effects on plant growth, maturity as well as leaching of Ca in soil. The pasture Cu concentration in the current experiment (0.27mgCu/kg) was far less than the critical dietary levels of 5mgCu/kg (Cunnigham, 1992).

The observed oestrus detection rates of 68% in current study were higher than the critical value of 65%. Values below 65% are considered as indicative of heat detection problems (Laing *et al.*, 1988).

The effects of supplementation are exerted through increased intake of energy, protein and minerals to improve the reproductive process. The greater proportion of supplemented animals showing shorter DPOA and DPO than unsupplemented animals, and shorter mean DPO observed in the supplemented than the control group of animals in the current study could be due to better nutrition in the supplemented than the unsupplemented animals. Reduction of DPO in the current experiment was similar to findings by Nkya *et al.* (1999) where supplementation of concentrate comprising maize bran (70%), cottonseed cake (28%) and minerals (2%), resulted in reduction of post-partum anoestrus period from 86.3 to 71.2 days and calving to

conception from 102.4 to 80.4 days.

Higher levels PUN in the supplemented animals could have contributed to improving the DPOA, since dietary protein stimulates DMI (Butler, 1998), may decrease the incidence of silent heats and increase conception rates in lactating cows (Van Saun *et al.*, 1993; Donna *et al.*, 1997).

The improved RP in the supplemented animals could partly have been a result of the increase in dietary and plasma P in the same animals similar to increase in fertility levels and growth in cattle reported in many parts of the world after P supplementation (McDowell, 1976; Engels, 1981; Bauer *et al.*, 1982). Low P content in pasture (0.2%) observed in current study signifies a risk of infertility in unsupplemented dairy cattle. This is because reproduction is likely to be impaired when dietary P concentrations are extremely low (<0.25%) (Wu *et al.*, 2000), where rumen microbes may have inadequate amounts of P for maximum growth, resulting in less microbial protein (Durand and Kawashima, 1980; Petri *et al.*, 1988) and possibly lowered ration digestibility (Durand and Kawashima, 1980). Under these extreme conditions, low dietary P could conceivably have an indirect effect on RP through impact on the cow's energy balance (De Vries *et al.*, 1999). Furthermore, since cell growth, division and differentiation are essential for gamete formation and embryo development, fertility may be affected by P deficiency because P is a component of deoxy- and ribonucleic acids and plays a vital role in energy utilization and transfer, which are essential functions in cell growth, division and differentiation (Hafez, 1980; Underwood and Suttle, 1999).

The improved DPOA and DPO for supplemented animals in the current study could also be attributed to higher plasma since the rate of conception in ruminants is directly related to rate of uptake of Cu (Grace and Clark, 1991) and suppressed oestrus has been observed in cows grazing on Cu-deficient pastures (Underwood, 1977). Furthermore, supplemented cows showed more standing heats, improved conception on first service (Kropp, 1992) and reduced calving to conception interval and matings per conception (Manickam and Balagopal, 1993).

Overall supplementation reduced the number of days from parturition to first visual oestrus from 146.8 to 103, which is within the recommended range of 85 - 115 days after calving (Roberts, 1971). However, the overall nutrient balance for the supplemented animals was negative for Ca, P and Cu prior to calving and ME, CP, P and Cu after calving. This suggests that at the current level of feeding, the nutrients were adequately mobilized and used for reproductive function although the same level might not be sufficient for milk production.

Conclusions and Recommendations

From the study results, the null hypothesis that supplementation with concentrates will not improve reproductive performance of smallholder dairy cattle in Rungwe district was rejected and the alternative hypothesis accepted.

It was concluded from pasture nutrient analyses, daily dietary nutrient balance and the observed response of cattle to supplementation that there was a need for concentrate and mineral supplementation of dairy cows and heifers in Rungwe district. Although the supplementation improved the reproductive performance, plasma urea nitrogen, P and Cu, the levels of plasma P and Cu were still below those normally recommended for dairy cattle under these conditions.

Since only one level of supplementation was studied it would have been of interest to include more levels in order to come up with clear conclusions in relation to appropriate plasma P and Cu concentrations and reproductive performance. Furthermore, an assessment of the economic viability of supplementation is recommended.

Impact

The study is of importance to smallholder dairy cattle farmers who seek to upgrade their indigenous breeds by crossbreeding the local short horn zebu to exotic breeds. The study shows the inadequacy of natural pastures alone to meet the nutrient requirements of cattle for improved reproductive performance due to the increased demands especially after calving,

for higher milk production when compared with indigenous zebu cattle. Hence the need for strategic supplementation of dairy cows and heifers with concentrates and minerals.

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

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Abstract

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

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

PREVALENCE DES ENDO-, ECTO- ET HÉMO-PARASITES DES LÉZARDS AGAMA (*Agama agama*) A MAIDUGURI DANS L'ÉTAT DE BORNO

Résumé

Cent lézards agama (*Agama agama*) ont été étudiés dans le but de rechercher la présence d'ecto-, endo- et hémoparasites à Maiduguri en utilisant les techniques de diagnostic parasitologique classiques. De l'ensemble de ces lézards, quarante-quatre (44) étaient mâles tandis que cinquante-six (56) étaient femelles. Quarante (40) étaient adultes, tandis que 20 étaient juvéniles. Les résultats obtenus ont montré que tous les lézards étaient positifs pour l'un ou l'autre parasite. Les endoparasites (100%) étaient plus abondants car ils ont été détectés chez tous les cent lézards examinés. Cependant, l'infection aux hémoparasites a été notée chez vingt (20) mâles et seize (16) femelles, représentant respectivement 55,6% et 44,4% de l'infection hémoparasitaire. Aucune variation statistique significative au niveau de l'infection n'a été détectée entre les sexes ($P < 0,05$). De même, aucun des lézards étudiés n'était positif pour les ectoparasites. La répartition des parasites sur la base de l'âge des lézards examinés montre que les adultes étaient plus infectés ($P < 0,05$) par rapport aux juvéniles, avec des taux d'infection de 80 (80%) et 31 (86,1%) respectivement pour les endoparasites et les hémoparasites. Deux (2) espèces d'endoparasites, à savoir *Trichuris* spp (70%) et *Ascaris* spp (30%) ont été notées au cours de l'étude, tandis que les espèces d'hémoparasites comprenaient *Plasmodium* (47,2%), *Haemoproteus* spp (30,6%) et *Leucocytozoon* spp (22,2%). Les résultats peuvent être d'une importance épidémiologique dans la zone d'étude en raison du rôle des lézards comme réservoirs des parasites identifiés. De même, une enquête plus approfondie dans la

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zone d'étude, faisant appel à des techniques avancées telles que la sérologie et les techniques moléculaires, est nécessaire pour s'assurer du statut des lézards comme porteurs / réservoirs de maladies telles que la toxoplasmose et la pentastomose, qui sont très importantes chez les reptiles.

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

Introduction

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

species for the presence of endoparasites and ectoparasites. A prevalence rate of 76.1% was encountered for endoparasites among the 331 investigated lizards, while only one ectoparasite (Trombiculid) parasite was encountered. Similarly, the presence of haemoparasites have presence of haemoparasites has been demonstrated in the blood of lizards under both natural and experimental conditions and using both conventional parasitological techniques and serology (Schall, 1996; Al Sadoon and El Bahrawy, 1998).

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

Despite several studies conducted by

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

Materials and Methods

Study area

Maiduguri, where the study was conducted is the capital of Borno State and the largest urban settlement in the State. Borno State has an area of about 69,436 Km². It lies within latitude 11°03'2" North and 11°04'2" N and longitude 13°02'0" E and 13°02'5" E, located between Sudan savannah and Sahel Savannah Zones, with an ambient temperature of 40-45°C. It is characterised/characterized by short rainy season of about 3-4 months (June-September) followed by prolonged dry season of about more than 8 months (Udoh, 1981)

Sampled Animals

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

consisted of forty four (44) males and fifty six (56) females, 80 adults and 20 juveniles.

Experimental Procedure

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

Statistical Analysis

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

Ethical Consideration

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

Results

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

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

Discussion

In the current study, parasites belonging to the haemoparasitic and endoparasitic groups were encountered, with none belonging to the ectoparasitic group. Among endoparasites recovered, parasites belonging only to the

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

The prevalence of 100% reported in this study for endoparasitism in male and female lizards investigated, is in agreement with the findings of Adeoye and Ogunbanwo, (2007) who reported an overall prevalence of 95.5% among 310 Agama lizards examined, with a sex

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

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

Different superscripts within columns differed significantly ($P < 0.05$)

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

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

Different superscripts within columns differed significantly ($P < 0.05$)

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

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

Different superscripts within columns differed significantly ($P < 0.05$)

based prevalence of 97.6% and 94.1% for males and females respectively. Variations, however is in the species of helminths encountered in the two studies. In the current study, only two nematode species namely; *Trichuris* spp and *Ascaris* spp were encountered, while Adeoye and Ogunbanwo, (2007) reported four nematode species (*Strongyluris brevicaudata*, *Parapharyngodon awokoyai*, *Capillaria* spp. and *Oxyuris* spp) different from those encountered in this study, in addition to other parasites belonging to Cestoda, Trematoda and Pentastomida classes. Also, a prevalence rate of 76.1% was reported for endoparasitism by Rataj *et al.*, (2011) in Slovenia among 331 lizards examined. This indicates a high degree of similarity with the current study. The differences however, are

in the species of endoparasites encountered which differ from the current study. Unlike the current study where the endoparasites recovered were limited to two species (*Trichuris* spp and *Ascaris* spp) from the class Nematoda, the parasites encountered in the former study belonged to six different classes distributed across eighteen different species. Similarly, unlike the current study where no ectoparasite was encountered, the trombiculid mite (*Geckobia* sp.) was reported by Rataj *et al.*, (2011) accounting for 24% parasitism among the 139 reptiles studied. In the current study, free ranging *Agama* lizards were used, which differed from the study conducted by Rataj *et al.*, (2011) who used, as his test subjects captive reared animals from a Zoological garden. Another reason for the variation may be the

specie of reptiles used in the two studies. Unlike the current study where only lizards were used, three different reptilian species consisting of snakes, chelonians and crocodiles were used in the former. The comparative susceptibility of each of these species along side lizards to ectoparasites need to be investigated. Also the result of the current study varied from the earlier report of Ameh (2005) who investigated ectoparasitism among seventy (70) wall geckos (also a reptile) in Jos, north central Nigeria and reported a prevalence of 70%. Jos, due to its location on the Plateau is known to have a near temperate climate, thereby making cold blooded animals like reptiles to be inactive and cluster together most of the times. This aids in sharing of vectors of diseases faster and more efficiently, thereby making disease transmission faster, effective and efficient. Furthermore, the zero prevalence of ectoparasitism in this study may be partly due to the high ambient temperature in the area. The long duration of dry season which is a typical phenomenon in Maiduguri, is a known environmental condition which does not favour the development and survival of parasitic stages of parasites (Mbaya *et al.*, 2006) and that could have been partly responsible for relative low prevalence of endoparasitism and the zero prevalence reported for ectoparasitism in comparison to similar studies in other study areas. Most of the protozoan parasites reported by previous authors workers (Akinboade, 1981; Wekheand Olayinka, 1999; Fernando and Udagama-Randeniya, 2009; Rataj *et al.*, 2011) were limited to tissue parasites, which are at variance with the haemoparasites exclusively encountered in the current study.

Conclusion

Conclusively, findings from this study have demonstrated that lizards (*Agama agama*) harbour both endo ecto and haemoparasites in Maiduguri. The occurrence of haemoparasites among lizards is being reported for the first time in Borno State, and the North eastern part of Nigeria. However further studies need to be done using advance techniques

like serology and molecular methods so that the exact roles of lizards in relation to other diseases like Toxoplasmosis and Pentastomosis could be determined, since they have been reported previously in reptiles. Similarly the zoonotic potential of the recovered parasites require further investigation.

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ANTIBIOTICS SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM BROILER AND LAYER CHICKEN WITH COLISEPTICEMIA IN SUDAN

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Abstract

A total of fifty seven *E. coli* strains isolated from 43 broiler and 14 layer farms with *colisepticemia* in Khartoum and Gezera state were investigated for antimicrobial susceptibility to ten antibiotic agents of Veterinary and human significance. Antibiotic activity against the isolate were determined by Disc diffusion test. Antimicrobial resistance of isolates was found for *Gentamycin* (26%) and *Ciprofloxacin* (39%) as less resistant antibiotic, *Lincomycin* (98%) and *kanamycin* and *amoxicillin* (95%) as more resistant antibiotics. Multiple drug resistances were observed in all isolates. Twenty nine different resistance patterns were demonstrated. Results obtained confirmed the presence of antibiotic resistant to poultry pathogen in poultry farms in Khartoum and Gezera state. It is recommended that antibiotic use in the management of colibacillosis in the farms should be based on the result of susceptibility tests because other than poultry health problems transmission of resistant *e coli* to human can occur.

Key words: antibiotic susceptibility, *Colisepticemia*, *Escherichia coli*, Chicken Khartoum and Gezera ,Sudan

TAUX DE SENSIBILITE AUX ANTIBIOTIQUES DES SOUCHES D'ESCHERICHIA COLI ISOLEES CHEZ LES POULETS DE CHAIR ET LES PONDEUSES SOUFFRANT DE COLISEPTICEMIE AU SOUDAN

Résumé

Un total de cinquante-sept souches de la bactérie *E. coli* isolées chez 43 poulets et 14 pondeuses souffrant de colisepticémie à Khartoum et dans l'Etat de Gezera a été étudié pour rechercher la sensibilité antimicrobienne à dix agents antibiotiques d'importance vétérinaire et humaine. L'activité antibiotique contre l'isolat a été déterminée par un test de diffusion sur disque. La résistance des isolats aux antimicrobiens a été identifiée pour la gentamycine (26%) et la ciprofloxacine (39%) - antibiotiques auxquels les souches étaient moins résistantes, et pour la lincomycine (98%) et la kanamycine et l'amoxicilline (95%) - antibiotiques auxquels les souches étaient plus résistantes. Des résistances à plusieurs médicaments ont été observées dans tous les isolats. Vingt-neuf profils de résistance différents ont été démontrés.

Les résultats obtenus ont confirmé l'existence de la résistance aux antibiotiques des pathogènes de volailles dans les élevages de volaille à Khartoum et dans l'État de Gezera. Il est recommandé que l'utilisation d'antibiotiques pour le traitement de la colibacillose dans les fermes soit fondé sur le résultat des tests de sensibilité, car outre les problèmes de santé de la volaille, la transmission de l'*E. coli* résistante à l'homme peut se produire.

Mots-clés : Sensibilité aux antibiotiques ; Colisepticemie ; *Escherichia coli* ; Poulets ; Khartoum et Gezera ; Soudan

Introduction

As a result of the increase in production of poultry ,egg and poultry Meat has become one of the important sources of protein in Sudan , resulting in an increased per capita consumption. *Colisepticemia* is one of the important E.Coli syndromes responsible for significant economic loss in poultry. *E Coli* is an organism that usually cause Secondary bacterial infection and may also be a primary pathogen in birds (Gross. 1994) , both Broilers and layers are susceptible to the disease (Foley et a.,2000 and Barnes and Gross, 1997) .In Sudan both incidence and severity of *Colibacillosis* have increased rapidly (Omer et al., 2010) .Biosecurity measures ,vaccination and use of competitive exclusion product were used to prevent and control the disease in some areas but with limited value (Gomis et al.,2003; La Ragione et al.,2001).Presently Antibiotic therapy is an important tool to control outbreaks of *Colibacillosis* (Freed et al., 1993). Uncontrolled widespread use of antibiotic agents in poultry industry are a regular practice in Sudan where *Colibacillosis* are common and severe on poultry farms. The permitted uses of antibiotic agent vary among countries and regions (Carlton, 2008) antibiotic misuse and/or improper usage is an important factor that help the emergence , selection and spreading of antibiotic-resistant *E Coli* and other microorganisms in both veterinary and human medicine (Van Den Bogaard et al., 2001 and Witte,1998). Previous Studies showed that *E Coli* of poultry are commonly resistant to one or more antibiotics especially if used over a long period(Allan 35.et al.,1993) .Recent reports showed an increased resistance to antimicrobial agents which are commonly used for treatment (Yang et.al.,2004;Cormican et al., 2001).The increased antibiotic resistance in avian pathogenic E.Coli in the country is worrying and indicates that widespread use of antibiotics for treatment ,prophylaxis and as feed additives may lead to serious problem for both human ,animal health and the environment .In view of the significance of *Colibacillosis* and appearance of drug resistant strains and difficulties in treatment of E. coli infection in poultry farms in Sudan.This study was carried out to determine the susceptibility

of *E coli* strains isolated from broiler and layer chicken with *colisepticemia* to antibiotics used in poultry industry in the country.

Materials and Methods

Area of study

Khartoum and Gezera state which are considered one of the most important States in order to contain the largest number of poultry farms in Sudan.

Description of samples

Fifty seven outbreaks of colisepticmia (43 broiler and 14 layer farm) were investigated. Affected flocks were of different location, breeds, type and production system Table(1) from each outbreak five affected bird were subjected to Pathological and bacteriological examination.

Isolation and identification

Samples which include liver and heart were cultured on 52.MacConkey Agar (Oxoid) and sheep blood agar. Bacterial colonies that were lactose fermented were tentatively identified as *E. coli*. isolates were confirmed with different biochemical tests using (API bioMerieux).

Antibiotic susceptibility test

E Coli susceptibility to 10 antibiotic agents was determined by the disk diffusion method (Kirby-Bauer,1966).Sensitivity to the *E coli* .strains were determine against *ampicillin* AMP (10ug), *Gentamycin* GM (10ug) *lincomycin* L(2ug), *Doxycycline* D (30ug), *streptomycin* S (10ug), *kanamycin* K (30ug), *amoxicillin* AMX(30ug), *ciprofloxacin* CP(5ug), *tetracycline* TE (30ug), and *Nitrfuention* NT(300ug). The diameters of the zones of inhibition were interpreted with reference to NCCLS (2001).

Results

E Coli was isolated in a pure culture from all outbreaks investigated. In broiler chicken Perhepatitis and percaditis was observed at necropsy ,a significantly higher incidence of field outbreaks (53%) were found in Cobb breed reared in an open system farms

Table 1: Distribution of *colisepticemia* outbreaks

State	Breed	Farm design	
		Open	close
Khartoum	Cobb	14	4
	Ross	2	5
	Hubbard	1	3
	Hisex	3	5
	Hyline	2	0
	Lohman	1	0
Gezera	Cobb	10	1
	Ross	0	1
	Hubbard	1	1
	Hisex	2	0

Table (1) . Mortality rate in broiler range from 1%- 12% highest rate was reported in an open system farms, and 1.9% in the layers. Highest percentage of *E coli* isolated from broiler chicken showed resistance to lincomycin (100%),Amoxycillin(95%) . Kanamycin (93%),Tetracycline(86%),Doxycyclin and nitrofurantoin (81%) 70.Ampicillin(79%),Streptomycin(51%) and Ciprofloxacin(42%).Low levels of resistance was against Gentamycin (23%) percentage of isolates susceptibility to the antibiotic agents were shown in table(2). In layer chicken main lesions observed include Perhepatitis,percarditis, salpingitis and egg peritonitis .antibiotic susceptibility tests results revealed that highest percentage of *E coli* isolates were resistant to Ampicillin and Kanamycin (100%),Amoxycillin ,Lincomycin and Tetracycline(93%),Nitrofurantoin 76.(86%),Doxycycline ,Streptomycin and Gentamycin(36%) while Low levels of resistance was against Ciprofloxacin (29%).percentage of isolates to the antibiotic agents were shown in table(3). Resistance of 81%, 51%and 42% broiler isolates to Doxycycline Streptomycin and Ciprofloxacin respectively were significantly higher than those isolated from layer 36%,36% and 29%..All the 57 isolates showed multiple resistances to at least 6 antibiotic agents. Twenty nine different patterns of resistance were obtained to the antibiotic agents used in this study .The most common resistance patterns of antibiotic for broiler and layer were shown in table (4 &5).

Discussion

The present study demonstrates that *E Coli* is the causative agent of the septicemia and mortality of birds in a commercial broiler and layer farms investigated in Khartoum and Gezera state. Clinical sign and Lesions observed in the affected birds are considered as typical signs of *Colisepticemia* caused by highly virulent *E coli* strains as reported by (Krishanmohan Reddy and Koteeswaran, 1994).Mortality rate observed during this study were in agreement with Zanella et al. (2000), who reported 5-10% mortality due to *E. coli*.infections. However, these findings disagree with Omer et al. (2008) who reported 1 % mortality rate of the disease in broilers. from this study it was observed that there were many factors might play a major role in the occurrence of the outbreaks such as farm design, bad management and poor hygienic measures as observed in number of farms investigated it was also observed that the breed of chickens might be one of the factors associated with the susceptibility and severity of *Colibacillosis* as cobb breed was found more susceptible (53%) to the disease in broiler chicken. Multiple antibiotic resistances was observed in all of the 57 *E Coli* isolates, such high incidence of multidrug resistance might be due to indiscriminate use of antibiotic at the present time.resistant to six or more antibiotic agents was found similar to finding of previous studies done in Sudan and other countries (Omer et al.,2010; Bass et al., 1999; Miles et

Table 2: Percentage of antibiotic susceptibility of isolated E Coli strains from broiler chicken with coli septicemia in Khartoum and Gezer state Sudan.

Antibiotic agent	Susceptible %	Resistant%
<i>Ampicillin</i>	21	79
<i>Kanamycin</i>	7	93
<i>Amoxicillin</i>	5	95
<i>Lincomycin</i>	0	100
<i>Nitrofuration</i>	19	81
<i>Tetracycline</i>	14	86
<i>Doxycycline</i>	19	81
<i>Streptomycin</i>	49	51
<i>Ciprofloxacin</i>	58	42
<i>Gentamycin</i>	77	23

Table 3: Percentage of antibiotic susceptibility of isolated E Coli strains from layer chicken with coli septicemia in Khartoum and Gezer state Sudan

Antibiotic agent	Susceptible %	Resistant%
<i>Ampicillin</i>	0	100
<i>Kanamycin</i>	0	100
<i>Amoxicillin</i>	7	93
<i>Lincomycin</i>	7	93
<i>Nitrofuration</i>	14	86
<i>Tetracycline</i>	7	93
<i>Doxycycline</i>	64	36
<i>Streptomycin</i>	64	36
<i>Ciprofloxacin</i>	71	29
<i>Gentamycin</i>	64	36

al 2006., Zahraei, Salehi and Farashi Bonab., 2006 Guerra et al. 2003). Yang et al (2004) in china noted that 80% of E .coli isolated from the livers of chicken was resistant to eight or more antibiotic agents. The multidrug resistant *E Coli* is continuously increasing which were also reported by Hussain et al (1982) and Nazir (2004). *E Coli* isolates from both broiler and layer chicken were found resistant to Lincomycin, Amoxicillin, Kanamycin, tetracycline, Nitrofuration and Ampicillin. nearly similar result were reported by Rahman et al 2008., Omer et al 2010 and Daini and Adesemowo, 2008) . up to now these antibiotics were extremely used in poultry industry, for this reason these antibiotics are inactive against pathogenic E coli strains at the present time. Results showed that 84% and 88% of isolates

was resistant to *Ampicillin*, and *Tetracycline* nearly similar result were reported by Rahman et al 2008., Omer et al 2010, Ozawa et al., 2008 and Daini and Adesemowo, 2008) . Resistance to *streptomycin* in layer 36% disagree with the results recorded by Al-Ghamdi et al., 2001 and Rahman et al (2008). During this study 42% of *E.coli* isolated from broiler chicken were resistant to Ciprofloxacin while 29% were found resistant in layer chicken these results do not agree with the results of a study conducted by (Omer et al., 2010) who report 100% resistant to ciprofloxacin in Kassala state. Different resistance pattern to the drug among organism isolated from different regions and area might be related to properties of pathogenic bacteria and to the difference in the rate and usage of antibiotics (Afshin Zakeri

and Pedram Kashefi., 2012), 23% and 6% of broiler and layer isolates were found resistant to *gentamycin* different results were recorded by Rahman 2008 and Daini and Adesemowo (2008) who report 54% of E. Coli. Isolated from Nigeria chicken were resistant to Gentamicin .Resistance against *Gentamycin* and ciprofloxacin, was 30% and 48% respectively indicating that these drugs may still be effective in the treatment of E. coli infections in poultry farm. Isolation of ciprofloxacin resistant E. coli strains was significantly high in broiler farm 42%. This result may be due to extremely use of Ciprofloxacin for treatment of the disease in poultry because of its very good effect against E. coli. Pandey. et al (1998) reported that the majority of E Coil strains were sensitive to gentamicin and *ampicillin* and were resistant to *tetracycline* which supports the present finding but there is some variation in the sensitivity tests results with the earlier reports because some antibiotics became resistant due to the regular used in poultry feed and treatment of diseases .Prasad et.al 1997 concluded that the highest sensitivity to Quinolone antibiotic is because they are recently introduce in poultry

industry and of the limited use by the poultry farmers which is also suggestive for the present study.

in conclusion finding of these study and other results were compared and clearly demonstrate the increasing problem of drug resistance in poultry population . High and increasing resistance to some common antibiotics in poultry farms, probably due to use of antibiotics as feed additives for growth promotion, prevention and treatment of diseases, alternative of biosecurity measures and use of unsuitable antibiotics for treatment of diseases. Use of more new drugs may cause serious problems such as human health problems and increase economic costs of poultry production..Very little data is available on the epidemiology, prevalence or the mechanisms of antimicrobial resistance in animal feed pathogens in Sudan Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is Strongly needed in the country because in addition to animal health problems, transmission of resistant clones and resistance plasmids of E. coli from food animals (Especially

Table 4: Percentage of antibiotic susceptibility of isolated E Coli strains from Chicken with coli septicemia in Khartoum and Gezer state Sudan

Antimicrobial agent (ug)	% Avian isolâtes (n=57)	
	R	S
B-lactum		
<i>Ampicillin</i> (10)	84	16
<i>Amoxcillin</i> (20)	95	5
Aminogluco-side:		
<i>Gentamycin</i> (10)	26	74
<i>Kanamycin</i> (3)	94	6
<i>Streptomycin</i> (10)	47	53
Tetracycline:		
<i>Doxycycline</i> (30)	70	30
<i>Tetracycline</i> (30)	88	12
Nitrofurans:		
Nitrofurantion (300)	83	27
Lincosamidise:		
<i>Lincomycin</i> (2)	98	2
Quinolones:		
<i>Ciprofloxacin</i> (5)	39	61

Table 5: Pattern of antibiotic susceptibility in broiler

No. of isolate	Resistance	Sensitive
7	Amp, K, AMX , L, NT, TE, D, S	Cp, G
5	Amp, K, AMX, L, NT, TE, D	CP, G, S
3	Amp, K, AMX, L, NT, D, CP	G, S, TE
3	Amp, K, AMX, L, NT, TE, D, CP,	G, S
3	Amp, K , AMX, L, NT, TE , G	CP, D, S
2	K, AMX, L, NT, TE,	CP, Amp, D , S, G

Amp Ampicillin/ **K** Kanamycin/ **AMX** Amoxicillin / **L** Lincomycin / **NT** Nitrofurantoin/ **D** Doxycycline/ **TE** Tetracycline / **S** Streptomycin/ **CP** Ciprofloxacin/ **G** Gentamycin

Table 6: Pattern of antibiotic resistance of *E. coli* isolated from layer

No of Isolate	Resistance	Sensitive
3	Amp, K, S, AMX, L, TE, NT, G	CP, D
2	Amp, K, S , AMX, L, TE, NT	CP, D, G
1	Amp, K, AMX, L, TE, NT, S, D	CP, G
1	Amp, K, AMX, L, TE, NT, D	CP, G, S

Amp Ampicillin/ **K** Kanamycin/ **AMX** Amoxicillin / **L** Lincomycin / **NT** Nitrofurantoin/ **D** Doxycycline/ **TE** Tetracycline / **S** Streptomycin/ **CP** Ciprofloxacin/ **G** Gentamycin

poultry) to humans can occur.

Resistance to existing antibiotics is widespread and is a major challenge for both veterinarians and physicians. Awareness programmes to poultry farmers was required to know how to select the suitable antibiotics preferably after antibiotic sensitivity testing and sensible use of such antibiotics at an optimum dose for sufficient duration to ensure effective treatment and control of various diseases caused by *E. coli* in poultry. Because of the wide variations in drug resistance patterns of the *E. coli* isolates, it is highly recommended that antibiotic use in the treatment of *Colibacillosis* of poultry in the farm should be based on the result of susceptibility tests.

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IDENTIFICATION AND STUDY OF THE ANTIBIOTIC SENSITIVITY OF BACTERIA ASSOCIATED WITH CLINICAL MASTITIS OF SMALL RUMINANTS IN THE REGION OF DAKAR

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Abstract

Mastitis is one of the most important diseases in dairy farming. It is responsible for very important quantitative and qualitative economic losses. This condition is often overlooked in small ruminants and treatment is usually done without bacteriological analysis. The objective of this study was to investigate the bacterial causes of clinical mastitis in small ruminants and study their sensitivity to antibiotics. Hence, a study was conducted from December 2006 to September 2007 in urban and peri-urban area of Dakar, on 103 milk samples collected in small ruminants with clinical mastitis. Bacteriological analysis was used to isolate the vast majority of staphylococci, especially *S. aureus* (30%). The antibiogram revealed sensitivity of staphylococci and bacilli to gentamicin (100%). *S. aureus* had a good sensitivity to the association Trimethoprim-Sulfamethoxazole (66.67%), Doxycycline (66.67%), amoxicillin (66.67%) and ampicillin (62.5 %). On the contrary, considerable resistance to *S. aureus* to penicillin, the Norfloxacin and colistin was noted. In the light of these findings, recommendations were made for the treatment and prevention of clinical mastitis.

Keywords: Goat-Sheep-Clinical mastitis-Bacteria-antibiotics-Dakar.

IDENTIFICATION ET ETUDE DE LA SENSIBILITE AUX ANTIBIOTIQUES DES BACTERIES ASSOCIEES A LA MAMMITE CLINIQUE DES PETITS RUMINANTS DANS LA REGION DE DAKAR

Résumé

La mammite est l'une des pathologies les plus importantes en élevage laitier. Elle est responsable des pertes économiques quantitatives et qualitatives très importantes. Cette affection est souvent négligée chez les petits ruminants et le traitement se fait en général sans analyse bactériologique. L'objectif de ce travail est de rechercher les causes bactériennes des mammites cliniques chez les petits ruminants et d'étudier leur sensibilité aux antibiotiques. Ainsi, une étude a été réalisée de décembre 2006 à septembre 2007 en zone urbaine et périurbaine de Dakar sur 103 prélèvements de lait réalisés chez les petits ruminants présentant une mammite clinique. L'analyse bactériologique a permis d'isoler en grande majorité des staphylocoques, en particulier *S. aureus* (30%). L'antibiogramme a montré une grande sensibilité des staphylocoques et bacilles à la gentamycine (100%). *S. aureus* a une bonne sensibilité vis-à-vis de l'association Triméthoprime-Sulfaméthoxazole (66,67%), la Doxycycline (66,67%), l'Amoxicilline (66,67%) et l'Ampicilline (62,5%). Par contre, une résistance importante de *S. aureus* a été notée à la Pénicilline, la Norfloxacin et la Colistine. Face à ces résultats, des recommandations ont été faites pour le traitement et la prévention des mammites cliniques.

Mots clés : Chèvre-Brebis- Mammite clinique- Bactéries-antibiotiques-Dakar.

Introduction

Faced with a growing population and the need to bridge the gap in animal protein, most Sahelian countries have opted for the development of short-cycle livestock farming, such as small ruminants which have showed a greater ability to withstand drought conditions which are getting tougher (Bourzat, 1980). In Senegal, small ruminants represent the largest livestock and are the second most consumed meat (Direl, 2006). However, many constraints hinder the development of this farming. Among the health constraints, mastitis are one of the most important disease group (Bergonier *et al.*, 1997; Bergonier *et al.*, 2002; Bergonier *et al.*, 2003; Jubb *et al.*, 1993; Radostits *et al.*, 1997 Mork *et al.*, 2007). They cause significant economic and health consequences which are often overlooked. Additional losses associated with clinical mastitis are due to the cost of treatment incurred and the culling of chronic cases (Watson & Buswell 1984). In very severe cases, deadly gangrenous mastitis are observed. Qualitative and quantitative alterations of the milk affect the growth and survival of lambs and kids (goats). The quality of colostrum necessary for the immune protection of newborns is affected because it contains low levels of immunoglobulins.

In addition, human foodborne outbreaks related to an increase in goat milk consumption can be noticed. Improving our dairy breeds and the preservation of human health therefore require thorough understanding of clinical mastitis control measures. In most of our countries, clinical mastitis is not well treated; there is an abusive use of antibiotics without research for the actual bacterial agents responsible and without making use of an antibiogram. This has resulted in ineffective treatment and antibiotic resistance problems. Our work therefore aims to identify the bacterial agents responsible for clinical mastitis in small ruminants, and to determine their sensitivity to commonly used antibiotics.

Material and Methods

Study area

The study was conducted from December 2006 to September 2007 on various sites of the urban and peri-urban area of Dakar: Gueule tapee, Fann, Hann, Foirail abattoir, Sicap Mbao, Keur Massar and Pikine, in close collaboration with veterinarians working in private clinics.

Animal Material

The survey was conducted on 61 females, consisting of 20 goats (Sahelian goat and West African Dwarf goat) and 41 sheep (Touabire races, and Djallonké Ladoum) received for consultation in veterinary clinics and belonging to small herds.

Methods

Clinical examination and milk sampling

After recording the health history and performing the general examination of animals received for clinical care due to mastitis, a special examination of the breasts was made. Hence, inspection and palpation of the udder have enabled the detection of inflammation, hardening of the udder and the presence of sores and ticks.

Then milk from inflamed udder quarters was collected aseptically in sterile tubes after prior hand washing, cleaning of teats and discarding of the first streams of milk. The tubes were identified and kept cold for delivery to the bacteriology laboratory of the Inter-State School of Veterinary Sciences and Medicine (EISMV) in Dakar.

Of the 61 animals, samples were collected via the whole mammary gland in 42 animals and in the remaining 19, only the most affected quarter was sampled. A total of 103 milk samples, that is 70 in ewes and 33 in goats, were collected in the various sites (Table 1).

Bacteriological analyses

Milk samples were analysed by standard isolation and identification bacteriological tests. The evaluation of the sensitivity of the main isolated pathogens was carried out with ten (10) antibiotics including those used in the treatment of mastitis by veterinary practitioners in Dakar. The antibiotic discs used were: *ampicillin* (AM), *amoxicillin* (AMX), *colistin* (CS), *Doxycycline* (DO), *erythromycin* (E), *gentamicin* (GM), *norfloxacin* (NOR), *penicillin*

Table 1: Distribution of samples per study site

Sites	Number of samples
Fann-Gueule tapée	05
Foirail de l'abattoir	54
Hann	12
Sicap Mbaou	10
Pikine	12
Keur Massar	04
Keur Moussa	06
Total	103

(P), Trimethoprim + sulfamethoxazole (SXT) and tetracycline (TE).

The conventional agar diffusion method was used and the interpretation was made by following guidelines from the antibiogram committee of the French Society for Microbiology (CASFM).

Data Processing and analyses were performed with Excel 2003.

Results

General data concerning the sampled population

Clinical signs helped diagnose 61 small ruminants with clinical mastitis, including 41 sheep and 20 goats, with a predominance of Touabires and Sahelian goats (Table II).

Farm characteristics

The animals were raised in makeshift pens. They were fed on various feedstuffs which include groundnut straw or hay, groundnut meal and sometimes cottonseed meal, and leftovers such as rice. Deworming and health care were not frequent. The majority of animals (96.72%) were multiparous.

Table 2: Distribution of clinical mastitis in females by race

Species	Races	Nombre
Sheep	Touabir	37
	Ladoum	2
	Djallonké	2
In-total 1		41
Goat	Sahel	16
	Djallonké	4
In-total 2		20
Total (1+2)		61

Clinical examination Results

Clinical examination revealed acute mastitis on 27% (16/60) and chronic mastitis on 73% (44/60) of the subjects. Cases of acute mastitis were marked by symptoms such as: fever, lethargy, anorexia, gnashing of teeth, and local to locoregional signs such as swelling and breast asymmetry, congestion, pain during palpation, necrosis and adenopathy. Chronic mastitis resulted in local signs which included induration of mammary gland parenchyma, affected half udder being non-functional or festering.

Bacteriological results

Out of the 103 milk samples examined, 72 samples (69.9%) were positive to culture and different groups of bacteria were isolated (Table III). Of the 90 pathogens isolated, a predominance of gram-positive cocci (63.33%) was obtained, followed by gram-positive bacilli (20%), gram-negative non-enterobacteria bacilli (16.67%) and 4.44% of Enterobacteriaceae.

Table 3: Major groups of bacteria isolated

Bacterial groups	Number	Frequency (%)
Coques G+	57	63,33
Bacilli G+	18	20
Bacillus G-		
Non- Entero-bacteries	11	12,22
Enterobacteries	4	4,44
Total	90	100

By classifying bacteria isolated according to animal species, we could notice a predominance of Gram-positive cocci in the ewe as well as in the goat, notwithstanding a higher frequency in the ewe. On the other hand, the frequencies of isolation of Gram positive and negative bacilli were higher in the goat (Table IV).

By observing the isolated bacterial species by animal species we found that:

In goats, among Gram-positive cocci, *S. aureus* was predominant at 27.59%, followed by 10.35% CNS (*S. xylosus*, *S. hominis* and *S. capitis*) and 6.89% of micrococci. In the

Table 4: Bacteriological results according to animal species

Bacterial groups isolated	Species and frequencies of isolation (%)	
	Sheep	Goat
Cocci positive Gram	70,49	48,26
Bacilli positive Gram	16,39	27,59
Bacilli negative Gram	13,11	24,14
Total	100	100

Table 5: Frequency of bacterial species isolated from goat

Description	Bacterial species	Number	Frequency (%)
Cocci positive Gram	<i>Staphylococcus aureus</i>	8	27,59
	<i>S. xylosus</i>	1	3,45
	<i>S. hominis</i>	1	3,45
	<i>S. capitis</i>	1	3,45
	<i>Micrococcus spp</i>	2	6,89
	<i>Aerococcus spp</i>	1	3,45
Bacilli positive Gram	<i>Bacillus cereus</i>	7	24,14
	<i>Bacillus spp</i>	1	17,24
Bacilli negative Gram	Non Enterobacterias	5	6,89
	Enterobacterias	2	6,89
Total		29	100

Table 6: Frequency of bacterial species isolated from sheep

Description	Bacterial species	Number	Frequency (%)
Cocci positive Gram	<i>Staphylococcus aureus</i>	19	31,15
	SCN	17	27,89
	<i>Streptococcus spp</i>	2	3,28
	<i>Aerococcus viridans</i>	2	3,28
	<i>Micrococcus spp</i>	3	4,91
Bacilli positive Gram	<i>Bacillus cereus</i>	5	8,19
	<i>Bacillus spp</i>	5	8,19
Bacilli negative Gram	Non Enterobacterias	6	9,84
	Enterobacterias	2	3,28
Total		61	100

group of Gram-positive bacilli, *Bacillus cereus* represented the majority and stood at 24.14%. Among Gram-negative bacilli 17.24% were non-Enterobacteriaceae and 6.89% were Enterobacteriaceae (Table V).

In Ewes, for the Gram-positive cocci group, 31.15% were *S. aureus*, 27.89% of CNS, 3.28% streptococci and 4.91% micrococci. In the group of gram-positive bacilli, *Bacillus cereus* and *Bacillus spp* were isolated with the same frequency (8.19%). Among Gram-negative

bacilli, 9.84% were non-Enterobacteriaceae and 3.28% were Enterobacteriaceae (Table VI).

Sensitivity of principal bacterial germs isolated to antibiotics used

Sensitivity to 10 antibiotics was tested on 3 main pathogens isolated in our samples, ie, *S. aureus*, CNS and *Bacillus cereus*. The results showed a good sensitivity of the three types of bacteria to gentamicin (100%), efficacy of trimethoprim-sulfamethoxine, doxycycline,

amoxycilin, ampicillin and tetracycline against *S. aureus* and CNS but less effective against *Bacillus cereus*. High frequencies of resistance of *S. aureus* to colistin (Polypeptides) (88.89%), penicillin (β lactams) (79.63%) and norfloxacin (Fluoroquinolones) (72.22%) were obtained (Figure 1, Table VII).

Discussion

A prevalence of 18% of clinical mastitis observed in our study is very high compared to the 5% reported by Bergonier et al. in 2002. This high prevalence can be explained by the conditions in which small ruminants are raised in our country (poor hygiene, lack of health care, inadequate treatment of infections).

Clinical signs are those conventionally described at different stages of development of clinical mastitis in ruminants (Bergonier et al., 1997; Jubb et al., 1993; Radostits et al., 1997).

Out of the 103 milk samples analysed,

31 (30%) were culture-negative. This is significantly lower than the 44% found by White and Hinckley (1999) in goats in the United States, the 34% found by Hama (2006) in goats in Togo and Mauritania, but it is higher than the 10% observed by Issa Ibrahim (2005) on cow's milk in Niger. The negative result of these cultures could be explained by the fact that, the samples were obtained after antibiotic treatment which could have rendered the milk sterile. One might also be lead to think that the isolation and identification techniques used could not detect all pathogens present in these samples. This is the case when mycoplasma, fungi and caprine arthritis encephalitis virus are involved in the occurrence of clinical mastitis.

90 bacteria were isolated from the 72 culture-positive samples. A predominance of staphylococci followed by bacilli was noted; hence corroborating the results obtained by Kalogridou-Vassiliadou (1991), on dairy goats.

Table 8: Effectiveness of antibiotic seven families on the major groups of bacteria isolated.

Families antibiotic	<i>S. aureus</i>		SCN		<i>B. cereus</i>	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
β -Lactamines	55,56	44,44	36,21	63,79	12,12	87,88
Tétracyclines	62,5	37,5	68,42	31,58	27,27	72,73
Aminosides	100	00	100	00	100	00
Fluoroquinolones	33,33	66,67	36,84	63,16	00	100
Sulfamides	66,67	33,33	78,95	21,05	45,45	54,55
Macrolides	50	50	52,63	47,37	36,36	63,64
Polypeptides	8,33	91,67	15,79	84,21	9,10	90,9

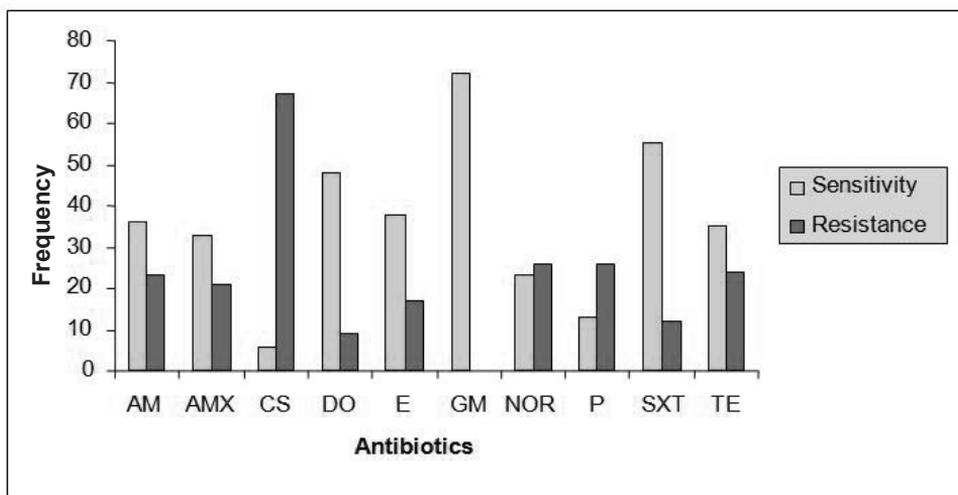


Figure 1: Sensitivity of isolated bacteria to antibiotics tested

Gram positive cocci are the most numerous with a frequency of 63%. Among these cocci, *Staphylococcus aureus* was predominant with a percentage of 30%. These results are comparable to those of other authors who have shown that *S. aureus* was predominant among bacteria which cause clinical mastitis in small ruminants (Ryan et Greenwood 1990; Lafi et al., 1998; Hinckley et White, 1999; Bergonier et Berthelot 2003; Mork et al., 2007). Similarly, *S. aureus* is frequently isolated in gangrenous clinical mastitis (Le Guillou, 1989).

Among other bacterial germs identified, CNS were observed with a prevalence of 22%. This prevalence is in the range of 10 to 53% as reported by Bergonier et al. (1997). CNS long considered as minor pathogens are nowadays cited as the major causes of subclinical mastitis among both cattle and small ruminants (McDougall et al., 2002; Contreras et al., 1995; Ryan et Greenwood 1990), but they are also associated with clinical mastitis.

To explain the high percentage of CNS, although opinions are controversial, some authors think that the freezing of samples at -20°C for several weeks, is the cause of these high percentages. Indeed, the work of Schukken et al. (1989), carried out on dairy cows, show that preservation of milk samples by freezing at -20 °C for 4, 8 to 12 weeks, would decrease the frequency of isolation of Enterobacteriaceae, and increase the frequency of CNS. According to Sanchez et al. (2003), on the other hand, the freezing of goat's milk decreases the frequency of isolation of Enterobacteriaceae, but it has no effect on that of CNS.

As regards Gram positive (20%), *Bacillus* spp were isolated with 13% being *Bacillus cereus*. This germ which is hardly associated with mastitis is becoming more and more frequent and its presence could be related to a lack of hygiene in farms as *B. cereus* is a soil-dwelling germ and acts as an opportunistic pathogen. In dairy cows, it is associated with gangrenous mastitis caused by contamination of teats during intramammary treatments (Schiefer et al. 1976). As relates to public health, it was observed that 5-7% of epidemic infectious diseases transmitted by food are caused by milk and dairy products (Cerf, 2002). The pathogens which cause these

diseases are coagulase-positive staphylococci in particular *S. aureus*, *Salmonella*, *Listeria* as well as toxin producing *Bacillus cereus*.

According to work done by several authors (Smith et Roguinsky 1977; Poutrel et Lerondelle 1983; Hunter, 1984; Manser, 1986; East et al., 1987; Ryan et Greenwood, 1990; Contreras et al., 1995), other bacteria such as Gram-negative bacilli are also associated with mastitis in small ruminants, but at a smaller scale. Contreras et al. (1995) link these bacteria to an environmental source because of poor hygiene during milking and a poorly maintained building. This high frequency of Gram-negative bacilli in our study may be explained by the fact that almost all animals examined live in open air farms, with earthen floors and a very low rate of picking of faeces. Finally, micrococci, streptococci and enterobacteriaceae were isolated at negligible frequencies, this corroborates the work of Bergonier et al. (1997).

On the ground, the most commonly used antibiotics in local treatment belong to the family of beta-lactam antibiotics (*Ampicillin*, *Amoxicillin*, *Cloxacillin*). These antibiotics are available on the Senegalese market in two presentations: ointment and intramammary infusion. These presentations which are intended to be applied locally on the breast are more expensive, therefore most treatments of clinical mastitis in the field are administered parenterally. The association *Penicillin-Streptomycin*, *tetracyclines* are among the most administered antibiotics by this route. In addition to these basic antibiotics, a supportive treatment was often carried out, with the use anti-inflammatory and anti-oedematous drugs. *Diurizone* (association between dexamethasone and an anti-oedematous) and *phenylarthrite* are generally administered.

Referring to the results of the antibiogram, aminoglycosides are by far the most effective. Indeed, with 100% efficacy against the growth of *S. aureus*, gentamicin is the antibiotic of choice to treat mastitis caused by this organism. Our results corroborate those of Hama (2006) who describes the efficacy of gentamicin as excellent against staphylococci. However, it is important to note that good in vitro sensitivity does not guarantee healing

in vivo. In this regard, concerning mastitis caused by staphylococci, bacteriological cure rates obtained in vivo reach more than 60 to 70% (Bouchot *et al.*, 1985). According to the same authors, this inconsistency is due to the intracellular localization of these bacteria, they are almost always encapsulated, a state which may render access by antibiotics difficult. According to some authors, in addition to its tendency to live inside the cell, *S. aureus* in the living organism adopts a protective L-form and tends to find refuge in microabscesses located within the breast hence making it difficult to cure all cases of mastitis caused by *S. aureus* by using antibiotics (Sears *et al.*, 1987, Smith & Sherman, 1994).

Tetracyclines and trimethoprim-sulfonamides combinations are antibiotics considered to have a good efficacy with 62% and 67% respectively.

The family of beta-lactam antibiotics has a lower efficacy (55%). This is due to the high resistance of bacteria isolated to penicillin (62%), which had the effect of lowering the good results obtained with *ampicillin* and *amoxicillin*. Our results on the resistance of *S. aureus* to penicillin agree with the 5-90% range of frequency of resistance obtained in comparative studies in several European countries (De Oliveira *et al.*, 2000). This resistance might be explained by the ability of hyperproduction of beta-lactamases by strains of *S. aureus* (Franklin, 1999).

With a frequency of resistance for *S. Aureus* at 92%, and 67% against polypeptides and fluoroquinolones respectively, these families are the most inefficient. Despite their wide spectrum of activity, first-generation Fluoroquinolones such as norfloxacin, are only active on Gram-negative bacteria. That is why, in our study, the sensitivity of staphylococci and Gram positive bacilli to colistin (polypeptide) and norfloxacin (quinolone) is low.

The trend in the frequency of sensitivity and resistance of CNS to antibiotics tested is comparable to that of *S. aureus*. The results of a Finnish study, conducted on cattle by Myllys *et al.* (2001) revealed a multidrug resistance of CNS to penicillin, erythromycin and occasionally to the association trimethoprim-sulfamethoxazole. Our results show that,

apart from resistance to penicillin, CNS are sensitive to erythromycin and the association trimethoprim-sulfamethoxazole. However, it is important to note a very poor efficacy of all the antibiotics tested, against *Bacillus cereus*. With the exception of gentamicin, no other antibiotic has an efficacy greater than 50% on this bacterial specie. This result is not surprising when one considers that *B. cereus*, because of its production of beta-lactamases, is naturally resistant to beta-lactams. Moreover, the results with norfloxacin on *B. cereus* can be explained by its natural resistance.

Conclusion

Our results showed that both acute and chronic mastitis, are not uncommon in herds of small ruminants raised in Dakar. Many bacteria are responsible for mastitis. Among bacteria identified, some indicate lack of hygiene and others may have a negative impact on human health. While some antibiotics are effective against these bacteria, others are less effective. As a result, the treatment of clinical mastitis must be based on antibiogram results and animal owners should be sensitised on the use of bacteriological analyses. This is why, further studies should be conducted to better understand these diseases and so as to propose appropriate treatment plans, in order to improve the productivity of these animals and to reduce the risk for consumers of both goat's and sheep's milk of in our country.

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EFFECT OF THE GILL FLUKE *GYRODACTYLUS* ON HAEMATOLOGY OF INFECTED African Catfish *CLARIAS GARIEPINUS* IN CULTURE

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Abstract

This observational study was made on the African Catfish *Clarias gariepinus* to determine the effect of the gill fluke *Gyrodactylus* species on the Catfish haematological parameters in a culture environment. A total number of 120 fish samples were collected from small scale fish farms in Ibadan Metropolis. Fish were screened for parasitism with the gill fluke *Gyrodactylus* then separated into positive and negative samples. Twenty specimens were selected randomly from each group into Group A (twenty fish from those parasitized with *Gyrodactylus* selected in one chance in two) and Group B (twenty from those fish that had no gill fluke in one chance in three). Packed Cell Volume (PCV), Total Erythrocytes Count (RBC), Total Leukocytes Count (WBC), and the differential white blood cells count) of group A, were evaluated and compared with that of group B, using one way analysis of variance (ANOVA). The effect of the parasite on the PCV of infected fish was significant at 5% ($L= 0.025$), while there was no meaningful change on the values of the RBC, WBC, Neutrophils and Lymphocytes. It was observed that neutrophils and lymphocytes constituted the bulk of the circulating white cells both in infected and free fishes. There was no eosinophils however few specimens among the infected group had monocytes in response to parasitism with *Gyrodactylus*. A good number of infected fish were found to have spur cell in their erythrocyte pool which was most likely ascribed to the presence of the parasites. The presence of spur cell in the circulating erythrocyte could then be a rapid diagnostic tool indicating the presence of *Gyrodactylus* infestation from a blood film of the affected fish.

Key words: *Clarias gariepinus*, gill fluke, haematology.

EFFET DU PARASITE *GYRODACTYLUS* SUR L'HEMATOLOGIE DU poisson-chat d'Afrique *CLARIAS GARIEPINUS* INFECTE EN MILIEU DE CULTURE

Resume

Ce papier décrit une étude faite pour déterminer l'effet de la douve des branchies de l'espèce *gyrodactylus* sur les valeurs haematologiques du poisson chat africain (*Clarias gariepinus*) dans son milieu naturel de culture. Un total de 120 échantillon de poissons a été collectés des petits et moyens étangs dans la ville d'Ibadan au Nigéria. Les poissons étaient examinés pour la présence de la Douve; puis séparés en deux groupes A et B. Le groupe A représentant les poissons parasités par la douve et le groupe B les poissons négatif. L'échantillonnage de vingt poissons sélectionnés au hasard était ensuite effectué dans chaque groupe a la probabilité d'une chance sur deux pour le groupe A et une chance sur trois pour le groupe B. Le PCV (pack Cell Volume), le nombre total des érythrocytes (RBC) et celui des leucocytes (WBC) dans la circulation sanguine était déterminé, puis le différentiel des globules blanc. Les valeurs obtenues pour le groupe A était comparées aux valeurs du groupe B, utilisant la method simple de l'analyse de la variance (ANOVA). Le parasite a eu un effet significatif sur le PCV a 5% ($L=0.025$). Aucune variation importante n'était observée sur les valeurs du RBC, WBC, les neutrophils et les lymphocytes. Il a été observé que les leucocytes étaient constitués en grande partie des neutrophils et des lymphocytes dans cet espèce. Les eosinophils n'étaient pas present par contre les monocytes étaient trouvés dans le frottis de certains poissons infectés. Un bon nombre de poisson infectés possédaient des cellules passible a la destruction par l'effet du spur de horseman. La présence de ces cellules dans la circulation sanguine de ce poisson pourra par conséquent être consider ée comme une méthode rapide de diagnostic indiquant la présence de la douve des branchies chez le poisson chat Africain a partir du frottis sanguin du poisson infecté.

Mots clé: *Clarias gariepinus*, douves des branchies, haematologie

Background of the Study

Diagnosis, control and prevention of fish diseases in developing African Countries have not been relatively attended to. In Africa, much concentration has been on fish taxonomy and more recently on techniques in aquaculture systems and management. Small scale aquaculture production of *clarias gariepinus* is well established in Nigeria which is among the largest producers of the African Catfish in the continent (FAO, 1994) and high outputs from such productions are expected to make significant impact on food security and poverty alleviation in the country. Farm culture of the African catfish is still constrained by availability of high quality feed amongst other management difficulties and high mortalities due to a variety of diseases. There are more than twenty cutaneous and systemic bacterial diseases, more than thirty viral diseases and more than one hundred external and internal parasitic diseases of commercial importance in finfish species (Klontz, G.W. 1985). Some of these infectious diseases are very serious in view of the high mortality rates they can cause in fish species.

Even though the African catfish *clarias gariepinus* is said to be highly tolerant to environmental extremes (Hogedorn, 1981; Hetch *et al*, 1988); resistant to many diseases conditions and high density culture (Richter, 1976; Haylor, 1991), many parasitic infections have been reported in this specie both in cultured and wild fish (Awachie, 1966; Ukoli, 1970; Ugwufor (1987), Anthony (1982) among which include the monogenean trematodes. The parasitic trematodes cause little harm in free ranging fish in their natural environment. The parasites are known to be browsers i.e. feed on the superficial layers of the skin and gills and then leave. In confinement/captivity in ponds and tanks where there is poor sanitation and deteriorating water quality overcrowding, high ammonia or nitrite, organic pollution and low oxygen tension, fish immunity is severely lowered by these stressors and so is the resistance to parasitism (Dobson and May, 1987; Iwama *et al*, 1996). Heavy monogenean infestation can be observed since this parasite can rapidly reproduce with the doubling time

for the viviparous gyrodactylids being as little as 24 hours. Their reproductive rate is enhanced by temperature which is less controlled in ponds and raceways. Their feeding activity is irritating and often causes skin cloudiness and focal reddening resulting from excess mucous production, epithelial hyperplasia or hemorrhage (Kataba, 1985). Individual worms cause proportionately greater damage, incite excess mucus production and pruritus (Bakke *et al* 2002; 2007). The monogenean of genus *gyrodactylus* has been reported to cause high mortalities among catfish populations in confinement in aquaculture systems in Ibadan. Diagnosis of monogenean requires necropsy and mainly euthanasia since the organism does not resist for long on dead fishes. Prognosis of treatment of diseased fish is usually guarded given that they are less resistant to high concentrations of the chemical agent such as formalin 25-250mg/l(ppm), hydrogen peroxide 300- 560mg/l(ppm); potassium permanganate 2-10mg/l (ppm) which are the approved medication for farmed fish (UF/IFAS, 2003). Small scale fish farms depend highly on fingerlings from other commercial farms for stocking of their ponds. A good source of infection has been traced to these commercial farms (Boerger, *et al* 2005), and there is usually reluctance to cull the highly priced brood stock for diagnosis. Thus in addition to other biosecurity measures which involve proper siting of the pond, adequate selection of material used for construction and species to be cultivated; providing to the fish a conducive environment for growth and resistance to disease, it will be important to introduce routine hematological analysis. In large scale fish farms where cost of production is high and there is obvious need for sustainability, haematological analysis will be a comparatively good diagnostic tool; Not only for the monogenean trematodes also for other systemic conditions that could cause severe outbreaks with high mortalities among fish populations.

Control of monogeneans at the level of fingerling production units will enhance production in catfish industries. It is therefore, for enhanced productivity in catfish farming industry, to develop capacity for fish diseases and control measures.

Materials and Methods

A total number of 120 fish specimens were obtained from randomly selected fish ponds in Ibadan town. Individual fish was measured for its standard and total length, weighed and the variables recorded.

2mls of blood was collected from each fish sample and preserved with EDTA for haematology. The fish was then euthanized. The opercula were cut off and removed using scissors or scalpel blade. Gill arches were cut and removed. These were examined grossly for paleness, haemorrhages and other lesions. Samples of the gill lamellae were obtained from the arch near its base and smears of gill tissues prepared. A cover slip was applied and viewed under low power microscope for the monogenean trematode. Some positive samples for gill fluke were stained with giemsa solution and mounted into a permanent slide.

A. Haematological analysis.

From every blood sample collected haematocrit or Packed Cell Volume (PCV), total erythrocyte count (RBC), total leukocyte count (WBC) and the differential white blood cell count were obtained and a thin blood smear was prepared.

Blood smear:

A drop of blood was placed on one end of a slide, using another slide, drawn into a thin film. The film was air dried, fixed in absolute methanol for 5 minutes, air dried again, stained for 30 minutes in giemsa solution, washed gently with tap water and air dried. Blood films were observed at high power under phase contrast microscope.

Haematocrit or Packed cell volume (PCV):

Refrigerated blood sample was brought to room temperature and agitated by gentle inversions.

Tubes were filled by capillary diffusion to about 2/3 of its length, the outside carefully dried with a piece of gauze and the opposite end of the tube sealed with special clay. The sealed tubes were then placed in a high speed micro haematocrit centrifuge with the sealed edge near the outside rim, and spun at 2000 rpm for

5 minutes. The spun micro-haematocrit tube was then placed on microhaematocrit reader and PCV read off in percentage.

Total erythrocyte count:

The haemocytometer method was used for the determination of total red cell count-procedure. A chemically clean red cell pipette was used to draw blood to the 0.5 mark of the pipette. The later was now plunged into a test tube containing diluting fluid. Normal saline solution was used (0.85% NaCl).

This was aspirated to the 101 mark of the pipette and blood and fluid mix properly by inversion of the tube. Thus aspirated blood was diluted at the proportion of 1:200. Diluted blood was discharged into haemocytometer counting chamber. After allowing several minutes for red cells to settle to a single layer (red cells requires more setting time than leukocytes). A cover slip was applied and total number of cells in five squares in the centre of the counting chamber was evaluated and this value was multiplied by 10⁶ to have total erythrocytes per 1 μ l of blood (as 10⁶/ μ l).

Total leukocyte count:

Haemocytometer method was used. The procedure was the same as in total erythrocyte count except for the following:

- The diluting fluid was solution of glacial acetic acid tinted with methylene blue
- The white cell pipette was used; it allows a 1:20 dilution instead of the 1:200 dilution in erythrocyte count.
- The cells are counted in the 16 squares within the larger ruled area in the corner and total number of cells counted multiplied by 10³ to obtain total number of leukocytes per μ l of blood (bx10/ μ l).

Differential Leukocyte Count:

Thin blood film stained with Giemsa was placed under the microscope with oil immersion. Leukocytes were counted from the tail end of the smear to the base (meander system for counting). Individual cells were recorded using a multiple unit counter. Distorted, degenerated and abnormal cells were recorded as atypical. A total number of

100 cells were counted and the differential cell count given in percentage. Cells counted were Lymphocytes, Neutrophils, Eosinophils, Monocytes and Mast cells.

B. Data analysis:

The one way analysis of variance was used to compare mean values of two independent groups of sample estimates.

Results and Anaysis

The monogenean trematode was found to parasitize 31% of the total fish sample examined, and 25% of visited ponds. Infected fish had a mean weight of between 130g and 220g, the range of actively growing population.

The *Clarias gariepinus* erythrocytes in the circulating pool are nucleated unlike the red cell in other animal species. The lymphocytes

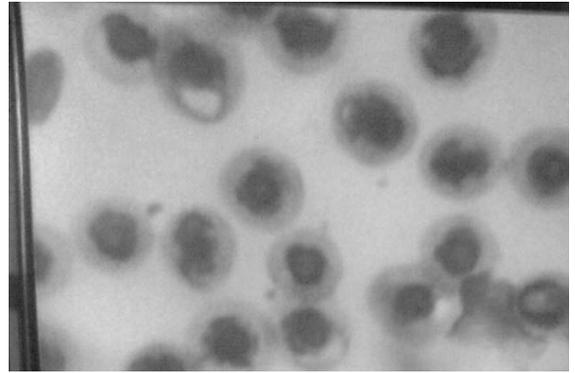


Figure 3: Blood picture of fish non infected with gill fluke.

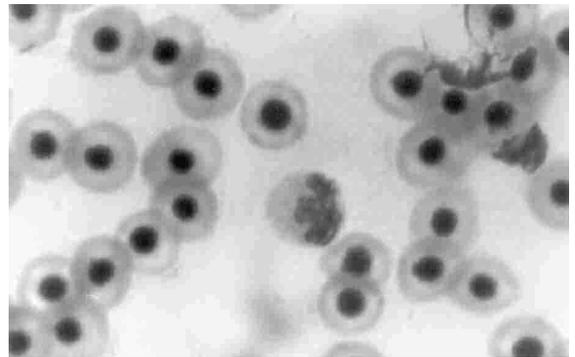


Figure 4: Blood picture of non infected fish (x 1000)

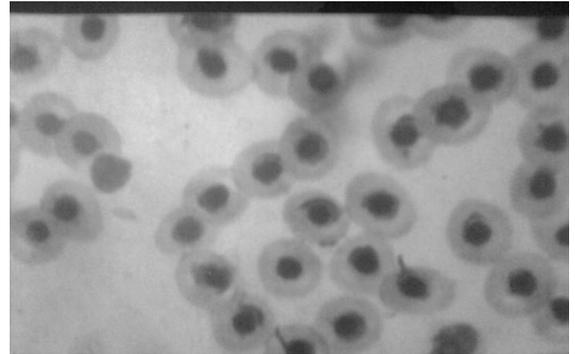


Figure 5: Blood film of an infected fish showing spur cells(x 1000)

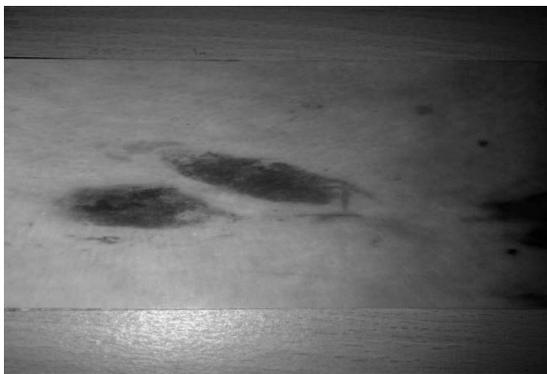


Figure 1: Monogenean termatodes attached to the gill lamellae of parasitized fish, two parasites on a field (x250)

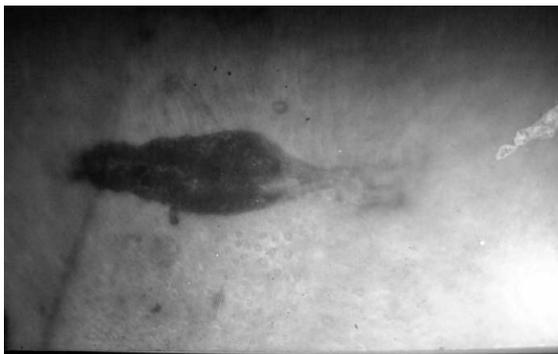


Figure 2: Gill fluke attached to the gill lamellae.(x250)

and neutrophils were found to make the bulk of the circulating white cell series with the mean values of 73.8%- 82.1% for the lymphocytes then 17.7%-24.5 % for the neutrophils. There was higher numbers of Lymphocytes in infected fish (mean value: 82.1%) than in non infected fish (73.8%) Eosinophils were totally absent in the parasitized fish while the Monocytes, which are large phagocytic cells and are said to increase in number in reaction with the presence of

Table 1: Biodata of the randomly selected *claris gariepinus* fish infected With the monogenean trematode *gyrodactylus* spp

S/N	Wt (gm)	Sl (cm)	Tl (cm)	Pcv (%)	Rbc (M/dl)	Wbc (M/dl)	Lym (%)	Neut (%)	Eos (%)	mon (%)
1	320	28.5	32.5	33	3.08	5.55	85	15	-	-
2	280	29	33	27	3.76	5.6	93	7	-	-
3	200	26	29	23	2.20	7.49	96	4	-	-
4	380	34	39	24	3.4	6.5	79	21	-	-
5	360	31	35	30	4.16	10.4	84	16	-	-
6	280	29	33	31	4.60	8.10	83	17	-	-
7	200	26.5	31	10	1.84	11.2	79	21	-	-
8	340	31	35	31	3.92	10.00	60	40	-	-
9	160	20	23	31	4.86	11.2	86	14	-	-
10	130	20	24	30	3.96	6.8	81	19	-	-
11	220	26	30	35	3.88	6.2	79	20	-	1
12	220	25.5	28	24	1.68	8.2	80	20	-	-
13	160	24	27.5	23	1.84	9.8	68	31	-	1
14	200	29	31	21	2.40	8.8	75	24	-	1
15	260	28	32	24	2.42	8.6	86	14	-	-
16	200	28	32	26	2.52	7.8	92	8	-	-
17	340	31	35	31	3.92	10.00	81	18	-	1
18	300	26	28	33	3.62	4.8	90	10	-	-
19	320	28.5	32.5	33	3.08	5.55	79	21	-	-
20	200	26	29	24	4.32	11.20	86	14	-	-

Note: Wt(gm)= Weight in grams

Sl(cm)= Standard length in centimeters

Tl(cm)=Total length in centimeters

PCV(%)= Packed Cell Volume in percentage

M/dl= Million per deciliter of blood

WBC= white blood cells

RBC= Red blood cells

Neut (%)= percentage of neutropils in 100 white cells counted

Lym(%)= percentage of lymphocytes in 100 white cells counted

Eos(%)= percentage of eosinophils in 100white cells counted

infectious agent in the system, were observed to be recurrent among the infected group (0.2%) even though in small numbers.

Discussion

Although some screening and diagnostic test are referred to as "Gold standard" there are a few perfect test and always a number of different clinical assessments and considerations for making a cost efficient decision in the management of production

diseases. In every disease control system early diagnosis is very important for implementation of control measures such as isolation and rapid treatment of affected stock, culling, control of spread to other farms. Control of monogenean trematodes which are found to be a potential drawback in both large and small scale fish farming sectors in Ibadan will be very efficient if routine hematological analysis could be carried out in fish farms.

Table 2: Biodata of the randomly selected uninfected *clarias gariepinus* fish

S/N	Wt (gm)	Sl (cm)	TI (cm)	Pcv (%)	Rbc (M/dl)	Wbc (M/dl)	Lym (%)	Neut (%)	Eos (%)	mon (%)
1	200	24	27	36	2.12	10.5	79	21	-	-
2	190	23.5	26	27	2.68	8.2	65	35	-	-
3	170	24	26.5	12	1.68	6.4	80	20	-	-
4	120	23	25	21	3.00	10.8	58	42	-	-
5	100	21	23	27	1.84	9.8	73	27	-	-
6	160	24	27	25	2.0	6.6	81	19	-	-
7	180	23	26	34	1.38	11.4	84	16	-	-
8	200	26	29	28	4.0	6.0	68	32	-	-
9	200	26	29	27	3.96	5.3	75	25	-	-
10	170	27.5	31	34	4.8	10.25	89	11	-	-
11	200	22	25	25	3.84	5.5	67	30	-	3
12	240	20	30	26	3.92	5.6	75	25	-	-
13	190	25	27	19	3.32	4.08	30	40	-	-
14	200	30	32.5	18	3.72	6.4	68	32	-	-
15	160	19	21	24	2.48	3.4	83	18	-	-
16	180	22	25	10	2.4	3.6	82	18	-	-
17	190	21	24	15	1.48	3.0	70	30	-	-
18	360	30	34	28	5.24	8.6	83	17	-	-
19	440	35	40	27	4.16	9.1	81	18	-	-
20	600	39	43	29	4.40	9.8	86	14	-	-

The presence of fluke attached to the gill lamella as presented in figures 1 and 2 are the obvious method for diagnosis of this parasite in the fish population. This method is used when clinical disease is overt and except that the attendants may be very observant, it will be reported when there are already mortalities within the populations. At this stage, it may too late to effect any treatment giving that approved therapy is poorly tolerated by diseased fish.

Routine haematological analysis will give a picture of health status of individual fish specimen and at statistical values reflect the health in the pond/farm.

Low PCV values and total erythrocytes count to the levels of 24.6% and 3.17 (million / dl) respectively as obtained in the mean values of infected fish in Table 1, are indicative of anaemia. In infestation with *gyrodactylus* there are numerous petechial haemorrhages in gill tissues, thin blood smears reveal erythrocytes liable for destruction by horseman spur as

presented in Figure V. These represent the ways by which red cells are lost in *gyrodactylus* infestation and the obvious resulting anaemia. The relatedness to infestation is confirmed by the significance of statistical test ANOVA with $L = 0.025$ at 5 %.

The presence of high numbers of lymphocytes with mean values of 82.1% as seen as mean value in Table 1 infected then monocytes in lieu eosinophils in differential white blood cell count as could be seen in most parasitic diseases of food animals will be suggestive of gill fluke infestation. Effects of this parasites on the fish can then be said to be brought to view through haematological analysis and closely definitive diagnosis made from blood picture of affected fish.

Conclusion

The effect of parasitism on the host is governed by the degree of parasitism, virulence of the organisms, organs or tissues invaded the

nature of damage and host reaction. The effects of the parasitic infection on the host tissues are manifested by direct injuries, irritations caused by their activities, feeding and multiplication; release of injurious substances that may lead to hypersensitivity. The body intervenes through cellular and humoral response to mitigating these effects. The mechanism by which these numerous factors may affect the haematocrit in this fish species may be the object for new investigations. Already monogeneans have been reported in blood and also observed to feed on tissue. The presence of spur cells in peripheral circulation of infected fish is an indication of red cells programmed for destruction. These two parameters will significantly affect haematocrit and as such justified the statistical findings. When transportation of molecular oxygen from gill tissue is reduced by decrease of available circulating red cells, when surface area for gaseous exchange of the gill filament is equally reduced by the effect of the parasite, irreversible damage will result in death by asphyxiation. In a highly priced group such as the brood stock that is used for fingerling production, the knowledge of haematological indices of diseased fish shall be very important where euthanasia may not be welcomed for diagnostic purposes. Inference to these values would be relevant with regards to monogenean infestation. Presence of monocytes which comprise a small proportion of the WBC population in fish increase in number when foreign substances are present in tissues or blood stream. Believed to originate in the kidney, monocytes concentrate at and phagocytize foreign particles. They are capable of killing a variety of pathogens including bacteria and helminth larvae (Secombes, 1996). Control of monogeneans at the level of fingerling production units will enhance production in catfish industries.

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BOVINE MASTITIS IN SELECTED DISTRICTS OF BORENA ZONE, SOUTHERN ETHIOPIA

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Abstract

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

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

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

Résumé

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

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

Introduction

At least 137 infectious causes of bovine mastitis are known to date. In large animals the commonest pathogens are *Staphylococcus* sp Coliform bacteria and Actinomyces pathogens (Du Preeze, 2000; Quinn *et al.*, 2004). Many other organism including Actinomyces pyogenes, Pseudoaomonas aeruginose, Nocardia and asteroids, Clostridium perfringens may also be associated with mastitis (Radostits *et al.*, 2007).

Mastitis can be manifested as clinical or sub-clinical form. Although the exact reasons why a cow develops clinical or subclinical mastitis are not well understood, different studies suggest that it is likely to be influenced by the pathogen involved as well as the immune status of the cow (Sordillo *et al.*, 1997; Bradley and Green, 2001; Zadoks *et al.*, 2003). Changing patterns of control methods have apparently also changed the importance of some organisms relative to others, such that the classic causes of contagious mastitis (e.g. *Streptococcus agalactiae*) are now considerably less important than they were, while the incidence of mastitis caused by the so-called 'environmental bacteria', such as *Streptococcus uberis*, has increased.

According to Lobago *et al.*, (2001) of the major disease of cross bred cows in Addis Ababa milk shed, mastitis was the second most frequent diseases of intensification next to reproductive health problem in urban and peri-urban dairy production system. However, very little information exists regarding the magnitude of the disease in pastoral areas. The pastoralists in Borana region almost exclusively depend on their livestock for their living; as a source of their daily food, earnings to meet their house hold expenditures and all other social needs including those for veterinary care. Some reports indicate that mastitis is affecting the livelihood of several communities by reducing milk yield (Biffa *et al* 2005; Getahun *et al*, 2008). Since production loss is not greatly affected by the type of causative organism (DeGraves and Fetrow, 1993), determination of its prevalence and identification of the risk factors are considered as the main objectives of this study.

Materials and Methods

Study Area

Borana zone is located to southern part of Ethiopia, under Oromia regional state. The Zone consist thirteen districts and borders Kenya in the south and Somali in the southeast. The altitude of the zone ranges between 943 and 2,400 meters above sea level with average annual rain fall of 400 and 1100mm exhibiting a bimodal rainfall (long and short rainy seasons). The long rainy season extends from March to May whereas the short rainy season occurs from mid September to the mid November. The annual temperature varies between 19 – 42°C. The area is known for being the origin of the Boran cattle breed, a known dual purpose animal. The pastoralists usually follow their animals depending on the availability of forage and water for their animals. Milk is the main source of food in addition to being the source of income particularly during the rainy season when it produced in excess.

Sample collection and mastitis test

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

Each quarter was clinically inspected for apparent lesion. Visible abnormalities of udder and teat (injuries, blindness, swelling, asymmetry of the quarters) and abnormal secretions were recorded. Factors associated to prevalence of mastitis such as age, parity number, body condition, stage of lactation, the presence of ticks were also recorded. Age was categorized as young adults (2 - 4 years), adults (5 - 8 years), and old (> 9 years). Parity number was also categorized into three: 1 - 2, 3 - 4 and > 4. Lactation stage was classified as < 2 months, 3 - 6 months and > 6 months; body condition was determined according to Richard (1993).

Milk samples were then collected aseptically before milking time as described by

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

Microbiological tests

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

Data analysis

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

Results

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

Risk factors

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

Bacterial isolates

From both clinical and sub-clinical cases 281 randomly selected milk samples were collected for bacterial culture. *Staphylococcus* and *streptococcus* species were the most frequently isolated bacteria accounting for 37.0% (104/281) and 25.9% (73/281), respectively. List of bacteria isolated from the milk samples are given in table 3. About 3.6%

Table 1: Quarter level prevalence of mastitis in Boran cows

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

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

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

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

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

(10) of the culture didn't show any growth of bacteria while 6.8% (19) samples showed mixed growth.

Discussion

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

The level of quarter prevalence found in this study compares with previous studies both in Ethiopia and elsewhere (Shirmeko, 1996; Hunderra *et al.*, 2005). Higher incidence of blind teat means a great economic significance for the pastoralists who depend on milk for living. Blind teats often arise as a result of chronic untreated mastitis or tick infestation. Different studies also confirm that the risk of clinical and subclinical mastitis increased significantly with advancing age, lactation, and parity of the cow (Abera *et al.*, 2012; Biffa *et al.*, 2005; Mungube *et al.*, 2004; Kerro and Tareke, 2003; Molalegne *et al.*, (2010). Absence of dry period therapy, presence of udder/or teat injury and pressure of milk on teat orifice makes easy entrance of bacteria to teat canal in early lactation. Further, the susceptibility of the mammary gland to new infection during both early and late dry period also increase prevalence of bovine mastitis (Radostits *et al.*, 2000; Molalegne *et al.*, 2010).

Similar bacterial isolates were also reported from other studies in Ethiopia in which *Staphylococci* and *Streptococci* species accounted for 50-89% of the total isolates of bacteria from mastitic milk (Abera *et al.*, 2012; Kerro and Tareke, 2003; Almaw *et al.*, 2008). However, higher occurrence of *Staphylococcus* species were found in this study compared to 23.1-31.1% reported from different localities within Ethiopia (Tolossa, 1987; Zerihun, 1996; Addisalem and Mersha, 2012). The presence of these agents on the skin and mucous

membranes; and adaptation of some of them to survive in the udder and become contagious gives them a relatively higher chance to be shed into the milk that also serves as source of infection for healthy cows (Quinn 3., 2004; Carter and Wise, 2004; Radostits *et al.*, 2007). The occurrence of environmental pathogens like coliforms is associated with associated with poor quality management of housing and bedding (Radostits *et al.*, 2007)

Conclusion

This study showed that a high prevalence of bovine mastitis which is a major health problem of dairy cows in the study area. The vast majority being subclinical in occurrence imply a higher economic loss from its adverse effect on productivity. This is more significant as the pastoralists are highly dependent on milk for their living. There were a number of identified risk factors affecting the prevalence of mastitis considerably. The fact that the most important bacteria isolated in this study were both contagious and environmental pathogens indicates the existence of poor management and hygienic conditions like absence of good housing management, lack of effective teat preparation and disinfection before and after milking, and absence of prompt identification and treatment/control of clinical mastitis. Further study should aim at creating awareness about the importance of subclinical mastitis among the pastoralist and evaluation of its economic impact.

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Impact

Mastitis is one of the most economically important diseases of dairy animals. Particularly in the pastoral areas, milk is the primary source of food. It is also source of income hence loss of milk production has a direct economic implication. The fact that many animals acquire subclinical form of the disease mean the loss is

mostly unrecognized. The condition is further complicated with different management problems such poor milking hygiene due to lack of awareness. This study is the first step towards identification of the magnitude of the problem, risk factors involved and the most commonly implicated bacterial agents.

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INTEGRATED CROP-LIVESTOCK SYSTEMS IN NEWLY RESETTLED AREAS OF GOROMONZI DISTRICT IN ZIMBABWE

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Abstract

Crop-livestock integration has been a foundation of agriculture for years. A survey was conducted to assess crop-livestock integration in resettled areas of ward 17, Goromonzi district in Zimbabwe. Five villages and 25 families per village were randomly selected in the ward. A semi-structured questionnaire was administered to collect information on household demography, level of education, farming history, livestock and crop management. Data were analyzed using the statistical package for the Social Sciences (SPSS) version 16. The youngest respondent was 27 while the oldest was over 77 years old. Ninety percent of the respondents produced maize. Cattle were the most common livestock species with an average herd size of 9.8 and providing draught power and manure. Maize stover was the crop residue widely used for feeding livestock. It was concluded that there was evidence of crop-livestock integration in Goromonzi and recommended that farmers improve the nutritive value of residues for feeding livestock.

Key words: Cattle, crops residues, draught power, livestock, maize, newly resettled farmers

SYSTEMES INTEGRES CULTURES-ELEVAGE DANS LES ZONES DE REINSTALLATION RECENTE DU DISTRICT DE GOROMONZI AU ZIMBABWE

Résumé

L'intégration cultures-élevage est un fondement de l'agriculture depuis des années. Une enquête a été menée pour évaluer l'intégration cultures-élevage dans les zones de réinstallation de la circonscription 17, dans le District de Goromonzi au Zimbabwe. Cinq villages et 25 familles par village ont été choisis de manière aléatoire dans la circonscription. Un questionnaire semi-structuré a été administré en vue de recueillir des informations sur la démographie des ménages, le niveau d'éducation, l'expérience de l'agriculture, la gestion de l'élevage et des cultures. Les données ont été analysées en utilisant la version 16 du progiciel statistique pour les sciences sociales (SPSS). Le plus jeune répondant était âgé de 27 ans tandis que le plus âgé avait plus de 77 ans. Quatre-vingt-dix pour cent des répondants produisaient du maïs. Les bovins étaient l'espèce animale la plus courante avec une taille moyenne de troupeau de 9,8 et fournissant la puissance de traction et le fumier. Le maïs-fourrage était le résidu de récolte largement utilisé pour l'alimentation du bétail. Il a été conclu qu'il existait des preuves d'intégration cultures-élevage à Goromonzi, et il a été recommandé aux éleveurs d'améliorer la valeur nutritive des résidus pour l'alimentation des animaux.

Mots-clés : Bovins ; Résidus de culture ; Puissance de traction ; Elevage ; Maïs ; Agriculteurs nouvellement réinstallés

Introduction

Integrated crop-livestock systems (IC-LS) have been a foundation of agriculture for hundreds of years (FAO, 2010). These are mixed farming systems in which crops and livestock are integrated on the same farm. Such production systems are widespread in rain-fed Sub-Saharan Africa. They are more important than any other system in terms of their contribution to the total output of animal products and contribute to enhancing the livelihoods of the poor through provision of food, income generation, provision of draught power and employment (Lenné and Thomas, 2006).

The word integrated is derived from the Latin verb “integrare” which means to make whole, to complete by addition of parts and combine parts into a whole (Edwards *et al.*, 1988). This comes from the recognition that crop and livestock subsystems may function independently in certain farming systems with their products being only additive. However, an output from one subsystem in an integrated farming system, which otherwise may have been wasted becomes an input to another subsystem resulting in a greater efficiency of output of desired products from the area under a farmer’s control (Edwards *et al.*, 1988).

Integrated crop-livestock system is the predominant system of production and subsistence in communal farming systems of Zimbabwe (Masikati, 2010). In Zimbabwe, the farming system is mainly based on maize, sorghum, groundnuts and cowpeas as staple crops, combined with the use of communal rangelands and fallow land for livestock production (Masikati, 2010). Livestock are a key component in smallholder cropping systems in Zimbabwe and other parts of the world. They provide critical inputs to cropping such as manure and draught power for cultivation, weeding and transport of produce (Ndlovu *et al.*, 2004). While animals provide manure and draught power for cultivation, forages are part of the cropping cycle and together, they form part of the same production unit (Niog, 1998). In most systems, crop residues are fed to animals especially during the dry season. The residue used most frequently is maize stover

(Sibanda, 1986). According to Masikati (2010), agricultural production systems as currently practiced by farmers in the semi-arid tropics of sub-Saharan Africa are different from those used in the past. In Zimbabwe, the past decade has seen a lot of newly resettled farmers, from varying backgrounds, engaging in various forms of agricultural production activities. This study was aimed at assessing crop-livestock systems in newly resettled areas of Zimbabwe.

Materials and Methods

Study site

Zimbabwe is divided into five agro-ecological or natural farming regions according to Vincent and Thomas (1960). Natural region II covers approximately 15 % of the land area. The study was conducted in Goromonzi district which lies in natural farming region IIa. The region receives, on average, between 1000 - 1200 mm of rainfall per annum and is suitable for intensive farming based on crops and/or livestock production (Gambiza and Nyama, 2000). Goromonzi falls under Mashonaland East province and is located 32 km South East of Harare, the country’s capital city. The selection of the district was based on the relative abundance of newly resettled farmers.

Selection of households

The district is divided into wards and ward 17 is dominated by newly resettled farmers who were resettled under the A1 scheme, a system designed for smallholder agricultural production. Purposive sampling was used to select this particular ward. Five villages namely; Belmont, Scott, Belvedere, Eton and Rochester were randomly selected in this ward. From the five villages, twenty-five households were randomly selected and interviewed.

Data collection

A semi-structured (pre-tested) questionnaire was used to collect survey data. This was administered by five trained enumerators. The questionnaire was designed to capture information on; household demography, level of education, history before resettlement, activities in new farming area, livestock and

crop management, uses of livestock, livestock feeds and feeding strategies and forms of assistance received, among others. Rapid rural appraisals (RRA) as well as interviews with key informants were also employed. Data were collected from September to October 2010.

Statistical analysis

Data were analyzed using the statistical Package for the Social Sciences (SPSS) version 16. Descriptive statistics were generated to represent respondents' opinions on various aspects of livestock and crop production.

Results and Discussion

The youngest head of household was 27 while the oldest was over 77 years old. The majority of the respondents were between 27 and 36 years of age and only 10% of the respondents were aged 77 or older.

It is interesting to note that there is quite a remarkable change in the types of crops grown before and after resettlement (Figure 1). About 45% of the respondents

produced maize before resettlement while up to 90% of the respondents produced maize after being resettled. Besides producing maize for subsistence purposes, this is probably because maize has a ready market since it is a very important staple crop in Zimbabwe. The observation is also supported by Kassam and co-workers (2009) who noted that maize is among the main food crops produced by farmers while Scoones and Wolmer (2000) noted the same in Ngundu, Zimbabwe. In yet another study, Ndlovu and co-workers (2004) observed that over 60% and 45% of the land was put to maize in Masvingo and Sanyati, respectively. In this study, only 1% of respondents produced tobacco before resettlement compared with 51% after resettlement while sugar beans, soya beans and paprika were only introduced by the same farmers after resettlement (Figure 1). The introduction of the three crops mentioned earlier is probably due to the proximity of the farming area to Harare which can absorb produce and provide income for various farming and socio-economic activities.

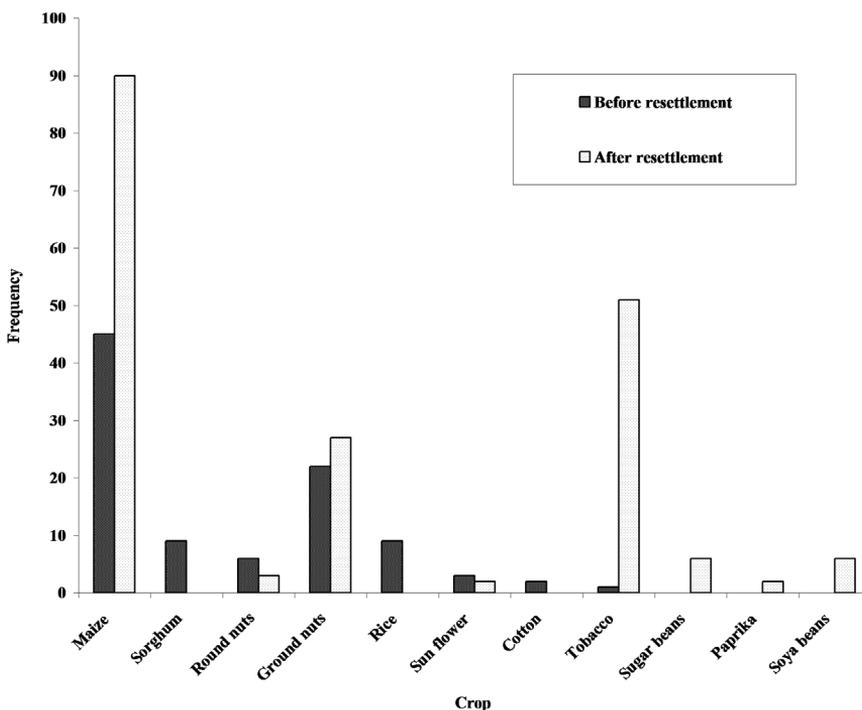


Figure 1 Crops grown before and after resettlement in Goromonzi district.

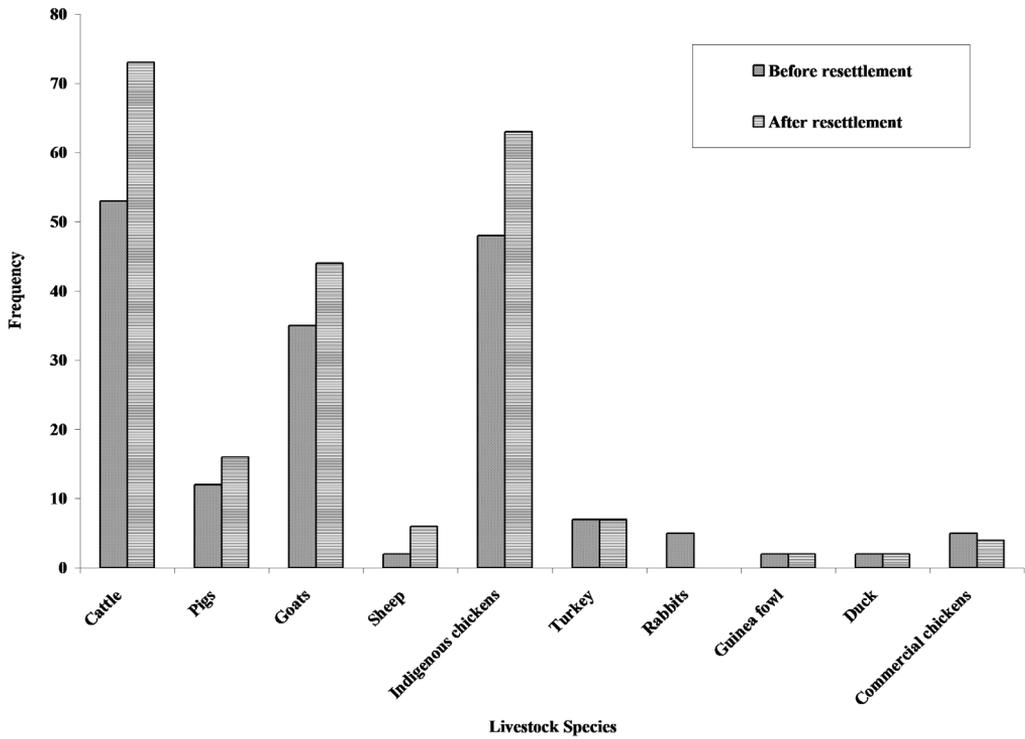


Figure 2 Livestock species kept before and after resettlement in Goromonzi district

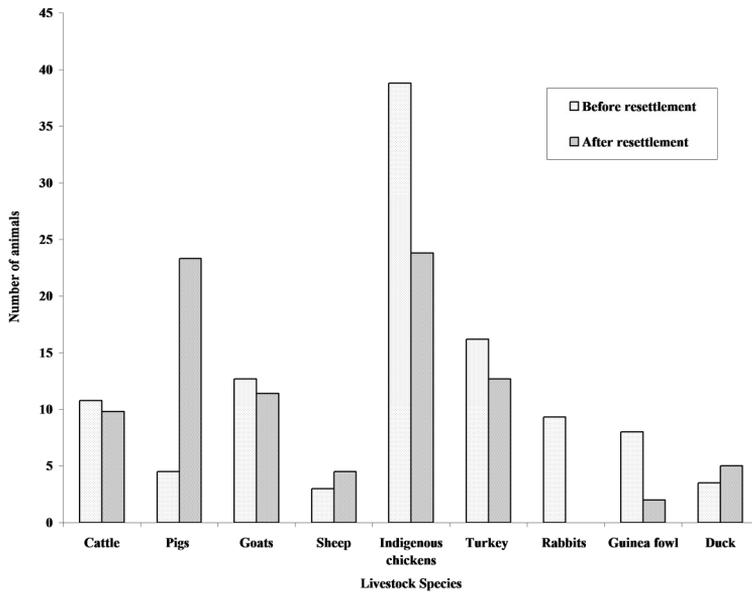


Figure 3: Average herd/flock sizes for common livestock species in Goromonzi district

It was observed in this study that cattle were the most common livestock species kept followed by indigenous chickens and goats, respectively (Figure 2). This observation concurs with findings by other researchers, for example a study by Ndlovu and co-workers (2004). They noted that cattle are the major livestock species in crop-livestock systems (Ndlovu *et al.*, 2004). Also, according to the Cereal Knowledge Bank (2007), cattle are the most important and widespread source of manure while the droppings of small animal species and domesticated birds tend to be used as a fallback when other sources of fertilizer are in short supply. Figure 2 also shows that there was a general increase in the proportions of farmers keeping various livestock species notably; cattle (20%), indigenous chickens (15%) and goats (9%), after resettlement.

Average herd sizes for cattle, pigs, goats and sheep after resettlement were 9.8, 23.3, 11.4 and 4.5, respectively while flock size for indigenous chickens averaged 23.8 (Figure 3). The number of cattle per household observed in this study is not very different from the observation made earlier in Chikombedzi where herd size averaged 9.2 (Scoones and Wolmer, 2000). The average herd size is however double the herd size for Ngundu (4.6) and almost double the average for Chipuriro (4.4) as observed in the study by Scoones and Wolmer in the year 2000. The average chicken population observed in this survey is similar to observations by Mlambo and co-workers (2011) in Zhombe communal areas of Zimbabwe where flock size averaged 20.7. Masimba and co-workers (2011) also noted 22.7 as the mean number of chickens per household for Gutu district in Zimbabwe. However, average flock sizes observed for Zimbabwe was 17.5 (Muchadeyi *et al.*, 2004).

Though only a marginal increase was noted in the proportion of farmers keeping pigs, this study showed that there was a marked increase in herd size for pigs from an average of 4.5 to 23.3 pigs per household before and after resettlement, respectively. This is probably because of a deliberate move by those few farmers towards commercializing production as noted with crop production where there was the introduction of crops such as paprika

and sugar beans. Even more profound was the increase in average commercial chicken flock sizes from 106 to 2 900 before and after resettlement, respectively as observed in the current study.

Farmers in the area used cattle as a source of draught power, manure, milk and meat among other things (Figure 4). Up to 72% of the respondents used cattle as a source of draught power while 52% indicated that livestock, in general, were a source of manure. In a similar study, it was noted that, 82% of total draught power in the developing world, comes from livestock (Masikati, 2010). Other researchers have stated that animals are kept to compliment cropping activities through the provision of manure for soil fertility, draught power for cultivation, transport, cash and food (Peden *et al.*, 2009; Ndlovu *et al.*, 2004) as confirmed by this study. Khabo and Agyare (online) also stated that the use of manure in crop production is widely practiced in most communities that rear cattle. Milk and meat were also identified by 45 and 29% of the farmers respectively, as products obtained from keeping cattle. The generally low proportion that indicated meat as a product obtained from livestock production perhaps reflects the importance of livestock, particularly cattle, in small-holder farming communities. Cattle ownership is regarded as a sign of wealth. This is only one of the many factors, including complex ownership patterns, limiting both off-take and flexibility regards the slaughtering of cattle.

This survey showed that cattle were the most common source of manure (Figure 5). Other important sources were indigenous chickens, goats and pigs (Figure 5). A study that was carried out by Khabo and Agyare (online) revealed that the common sources of manure in northern Ghana were cattle, sheep and goats, fowls, and pigs with cow dung being the most important among them. A similar study recognized cattle as the most important and widespread source of manure, adding that droppings of small animals and domesticated birds tend to be used as a fallback when other sources of fertilizer are in short supply (Cereal Knowledge bank, 2007).

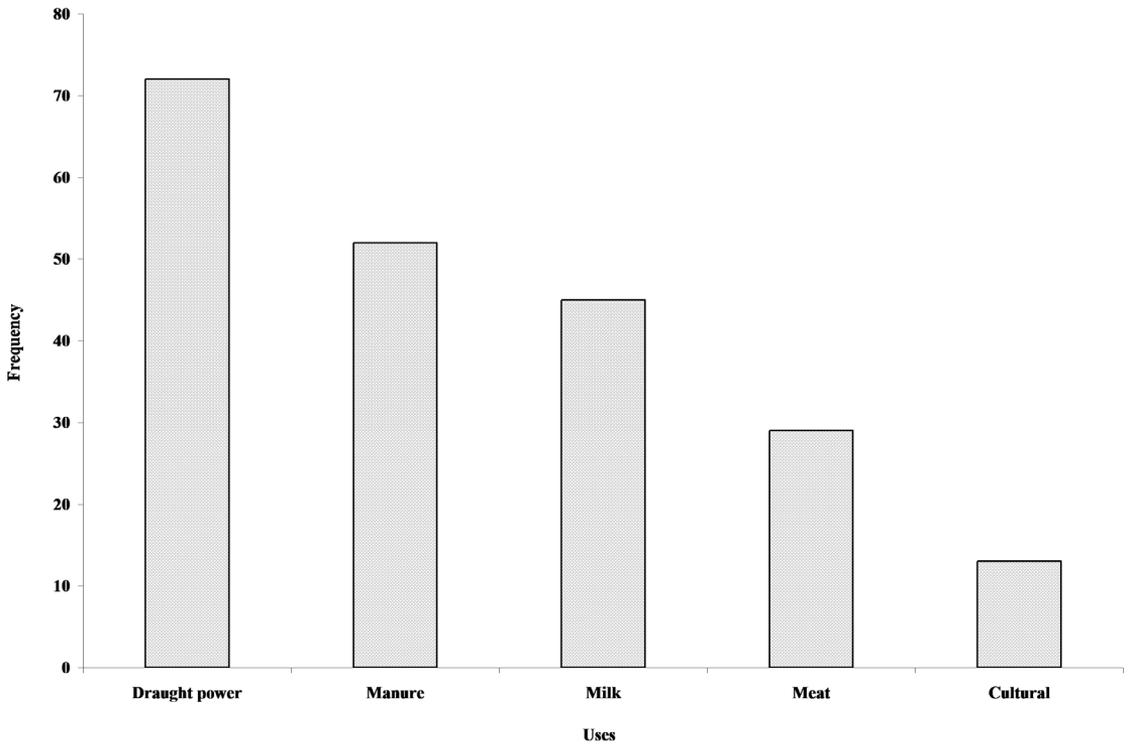


Figure 4: Common uses of cattle in crop-livestock systems in Goromonzi district

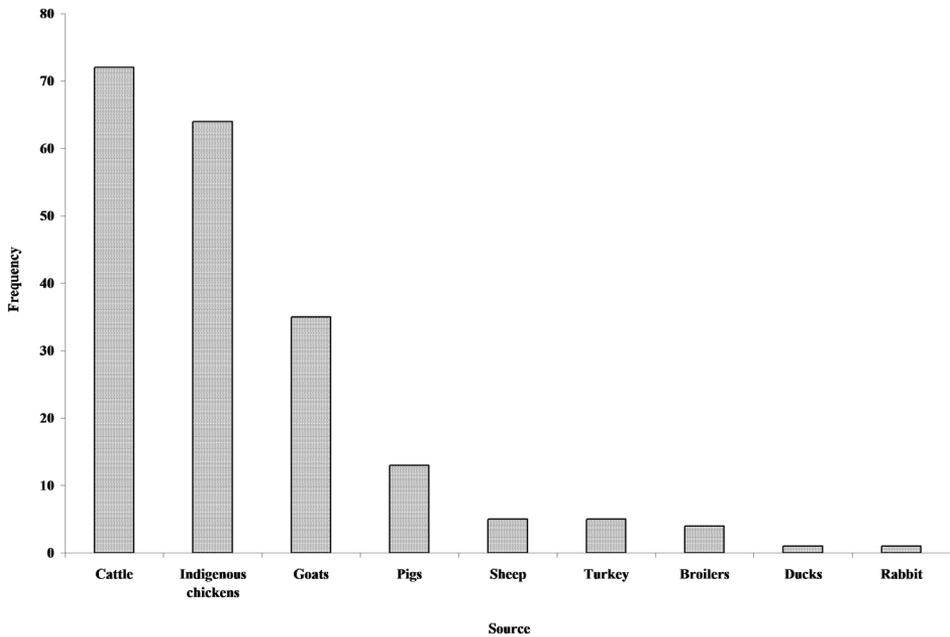


Figure 5: Common sources of manure

This study indicated that farmers in the Goromonzi district used crop residues to feed their livestock. Other researchers have demonstrated that the use of crop residues is indeed common in different parts of Africa. For example, the practice was noted in Ethiopia, Mali and Zimbabwe (Scoones and Wolmer, 2000). The crop residue that was most commonly used by farmers in Goromonzi is maize stover. Respondents indicated that maize stover and other crop residues were not modified in any way before feeding them to various livestock species. This agrees with observations by various researchers including; Lukuyu *et al.*, (2009) and Sibanda (1986). The former noted that maize stover was the most commonly used crop residue in; Kenya, Uganda and Rwanda while Sibanda (1986), made similar observations for different areas in Zimbabwe. In a study that was conducted in Nkayi district in Zimbabwe, it was noted that crop residues are the most common feed source for alleviating feed shortages during the dry season notably residues from maize, groundnuts, cowpeas and sorghum (Masikati, 2010).

Conclusions and Recommendations

There is evidence of crop-livestock integration in Goromonzi district as demonstrated by the widespread use of crop residues as livestock feed and the use of livestock waste as sources of manure. The most commonly used crop residue is maize stover while cattle are the most common source of draught power and manure. It was recommended that farmers improve nutritive value of crop residues before feeding them to livestock. This can be achieved through technologies such as urea treatment or through intercropping with forage legumes during the production of the relevant crops.

Implications

The findings of this study confirm the existence of a relationship between livestock and crop production. It is clear from the study that these two entities exist in a single unit and each system depends on the other as there is exchange of resources. This is essential since

it leads to efficient use of resources which could otherwise be deemed waste in some cases. However, more can be done regarding innovative ways of utilizing resources.

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EFFECT OF PARENTAL LIVEWEIGHT ON THE REPRODUCTIVE PERFORMANCE OF JAPANESE QUAIL (*Cortunix cortunix japonica*)

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Abstract

The data used for this study consisted of 3,195 records on parental live weight obtained from 400 females and 200 males. The records were further categorized into four mating groups (LM x LF, HM x HF, LM X HF and HM x LF) based on the parental live weight. Egg weight, Egg length, Egg circumference and body weight from day old to 7 weeks were measured on eggs and chicks from the four crosses and the data was analyzed using SAS (2003). Percent fertility, hatchability, hatch and mortality was estimated and compared over the four crosses using GLM procedure. It was observed from this study that the mating group (LM X HF) with the highest egg weight had the highest egg length and egg circumference. Significant differences exist statistically ($p < 0.05$) in all the crosses for post hatching weight at week 2 to week 7 with LM x LF having the highest performance. Progressive increase in body weight with increase in age in all the four crosses was also observed. Percent fertility and hatchability in LM x LF and LM X HF crosses were not significantly different from each other but were significantly different ($p < 0.05$) from HM x HF and HM x LF crosses. High mortality was obtained from HM x HF and HM x LF crosses. This study concluded that light male and light female crosses gave chicks with better post hatching performance and lowest mortality rate than all other crosses.

Keywords: Parental live weight, Egg weight, Hatchability, Fertility, Crosses, Japanese quail

EFFET DU POIDS VIF DES PARENTS SUR LA PERFORMANCE DE LA FONCTION REPRODUCTIVE DES CAILLES JAPONAISES (*Cortunix cortunix japonica*)

Résumé

Les données utilisées pour cette étude comprenaient 3.195 dossiers sur le poids vif des parents obtenus pour 400 femelles et 200 mâles. Les dossiers ont été répartis en quatre groupes de copulation (LM x LF, HF x HM, LM X HF et LF x HM) sur la base du poids vif des parents. Le poids des œufs, la longueur des œufs, la circonférence des œufs et le poids corporel de 1 (un) jour à 7 semaines ont été mesurés pour les œufs et les poussins issus des quatre croisements, et les données ont été analysées en utilisant SAS (2003). La fécondité en pourcentage, le taux d'éclosion, l'éclosion et la mortalité ont été estimés et comparés pour les quatre croisements en utilisant la procédure GLM. Il a été noté à partir de cette étude que le groupe de copulation (LM x HF) ayant le plus grand poids d'œuf avait la plus grande longueur d'œuf et la plus grande circonférence d'œuf. Des différences significatives existent statistiquement ($p < 0,05$) dans tous les croisements pour le poids post-éclosion à la 2ème – 7ème semaine, le croisement LM x LF ayant les meilleures performances. L'accroissement progressif du poids corporel parallèlement à l'augmentation de l'âge chez tous les quatre croisements a également été observé. Le taux de fécondité et le taux d'éclosion chez les croisements LM x LF et HF LM X n'étaient pas significativement différents les uns des autres, mais étaient significativement différents ($p < 0,05$) de ceux des croisements HM x HF et LF x HM. Une mortalité élevée a été notée parmi les croisements HM x HF et LF x HM. Cette étude a constaté que les croisements de mâles de poids léger et de femelles de poids léger donnaient des poussins ayant une meilleure performance post-éclosion et le plus bas taux de mortalité par rapport à tous les autres croisements.

Mots-clés : Poids vif des parents ; Poids d'œuf ; Taux d'éclosion ; Fécondité ; Croisements ; Caille japonaise

shown on Table 1.

LM x LF, HM x HF, LM X HF and HM x LF were 11.8g, 3.13cm and 7.82cm; 10.93g, 3.17cm and 7.81cm;

11.49g, 3.21cm and 7.89cm; and 10.87g, 3.15cm and 7.77cm respectively.

From the crosses, significant differences ($p < 0.05$) were obtained in egg weight, egg length and egg circumference. It was seen that the egg weight and egg length of the HM x HF and HM x LF were not significantly different from each other but they were different from LM x LF and LM X HF with LM X HF having the highest egg weight and egg length of the four crosses. The egg circumference of LM x LF, HM x HF, and HM x LF were not statistically significant from each other but different from LM X HF which has the highest value. It was observed from this table that the mating group

with the highest egg weight has the highest egg length and egg circumference which indicates that egg weight is influenced by egg length and egg circumference. This result agrees with the findings of Farooq *et al.*, 2001.

The means of hatching and post hatching body weight are shown in Table 2. The hatching weight LM x LF, HM x HF, LM X HF and HM x LF were 6.64, 4.78, 6.46 and 4.11 g respectively. At day old and 1 week, body weight from LM x LF and LM X HF were not significantly different ($p < 0.05$) but were different from HM x HF and HM x LF. Progressive increase in body weight was observed as the age of the birds increased in the four crosses ranging from 6.64 – 183g, 4.78 – 123.64g, 6.45 – 168.2g and 4.11 – 101.3g respectively. This indicates physiological growth as the age of the birds increased. These results agree with the findings of Oruwari and Broody (1998) and Adeogun *et al.* (2003). Significant differences exist statistically ($p < 0.05$) in all the crosses for post hatching weight at week

Table 1: Means \pm SEM of External Egg Quality Traits.

Traits	LM x LF	HM x HF	LM x HF	HM x LF
Egg weight(g)	11.1814 \pm 0.03 ^b	10.9326 \pm 0.06 ^c	11.4973 \pm 0.03 ^a	10.8667 \pm 0.10 ^c
length Egg (cm)	3.1335 \pm 0.005 ^c	3.1651 \pm 0.008 ^b	3.2054 \pm 0.004 ^a	3.1547 \pm 0.01 ^b
Egg circumference (cm)	7.8258 \pm 0.01 ^b	7.8067 \pm 0.01 ^b	7.8922 \pm 0.009 ^a	7.77067 \pm 0.02 ^b

^{abc}Means with different superscript along rows are significantly different ($p < 0.05$)

Table 2: Means \pm SEM for hatching and post hatching body weight from different crosses

Age (Weeks)	LM x LF	HM x HF	LM x HF	HM x LF
0	6.6402 \pm 0.07 ^a	4.7830 \pm 0.19 ^b	6.4579 \pm 0.07 ^a	4.1067 \pm 0.29 ^c
1	17.6178 \pm 0.20 ^a	10.8260 \pm 0.46 ^b	16.7548 \pm 0.20 ^a	9.0767 \pm 0.72 ^c
2	35.8360 \pm 0.40 ^a	22.526 \pm 0.97 ^c	33.550 \pm 0.40 ^b	19.213 \pm 1.52 ^d
3	68.321 \pm 0.75 ^a	41.182 \pm 1.73 ^c	62.704 \pm 0.73 ^b	34.987 \pm 2.72 ^d
4	91.825 \pm 1.01 ^a	59.112 \pm 2.46 ^c	84.911 \pm 0.98 ^b	49.027 \pm 3.79 ^d
5	120.334 \pm 1.31 ^a	80.464 \pm 3.32 ^c	111.157 \pm 1.27 ^b	67.420 \pm 5.20 ^d
6	150.834 \pm 1.64 ^a	100.321 \pm 4.15 ^c	138.684 \pm 1.58 ^b	83.600 \pm 6.52 ^d
7	183.080 \pm 1.20 ^a	123.641 \pm 5.10 ^c	168.292 \pm 1.92 ^b	101.260 \pm 7.99 ^d

^{abc}Means with different superscript along rows are significantly different ($p < 0.05$)

Table 3: Percent fertility, hatchability, hatch and mortality from crosses (The GLM Procedure)

Traits	LM x LF	HM x HF	LM x HF	HM x LF
Fertility	96.40 ^a	89.72 ^b	93.58 ^a	88.64 ^b
Hatchability	98.34 ^a	78.44 ^b	90.66 ^a	70.62 ^c
Hatch	90.46 ^a	70.38 ^c	85.88 ^b	65.1 ^d
Mortality	2.54 ^b	10.19 ^a	4.48 ^b	12.7 ^a

*Means with different superscript along rows are significantly different ($p < 0.05$)

2 to week 7 with LM x LF having the highest performance which suggest that optimum body weight of Japanese quail could be achieved using light males.

Percent fertility, hatchability, hatch and mortality from the four crosses are shown in Table 4. There was no significant differences statistically ($p < 0.05$) in the percent fertility of HM x HF and HM X LF crosses but they were significantly different from LM x LF and LM x HF with LM x LF having the highest fertility. Percent hatchability in LM x LF and LM X HF crosses were not significantly different but they were significantly different ($p < 0.05$) from HM x HF and HM x LF crosses. Percent hatch in the four crosses were significantly different ($p < 0.05$) from each other with LM x LF having the highest value. For percent mortality, there were no statistical differences in LM x LF and LM X HF but they were significantly different from HM x HF and HM x LF crosses. Hatchability of fertile eggs was significantly lowest ($p < 0.05$) in HM x LF cross, this agrees with the findings of Sahan and Ipek, 2000. Fertility and hatchability of LM x LF and LM X HF crosses were better than the other two crosses which suggest that light males are better used for breeding purposes (Narahari et al, 1998; Ipek et al., 2003; Zoccarato et al., 2004). The high mortality obtained from HM x HF and HM x LF crosses shows that most of the chicks obtained from these crosses are probably not strong as indicated in their body weights thus the lower survival.

Conclusion

From this study, the progressive increase in body weight observed in the crosses from day old to 7 weeks indicates a physiological growth of the birds as the age of birds increased. Parental live weight had a significant effect on fertility, hatchability, hatching weight and post hatching performance of Japanese quail. Parental live weight affected egg weight, egg length and egg circumference in all crosses. Light male and light female crosses gave chicks with better post hatching performance and lowest mortality rate than all other crosses. The use of light males is therefore recommended to poultry breeders

for optimum productivity.

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REPLACEMENT VALUE OF SORGHUM MILLING DUST FOR MAIZE IN THE DIETS OF WEANER RABBITS

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Abstract

A study was conducted to determine the replacement value of sorghum milling dust for maize in rabbit production. Five weeks feeding trial involving thirty-two, six weeks old New Zealand White weaner rabbits weighing 584.38g on the average was arranged in Completely Randomized Design and fed four diets containing 0 (control), 10, 20 and 30% malted sorghum milling dust (MSMD).

The MSMD was high in crude protein (10.30%) and crude fibre (20.60%). The body weight gain, feed efficiency and protein efficiency ratios of rabbits fed 10 and 30% MSMD were higher than the control and 20% MSMD. Rabbits fed diet containing 30% MSMD recorded the highest daily weight gain of 37.14 g while the least value of 27.14 g was observed for rabbits on control diet. The inclusion at 30% MSMD resulted in a very high utilization of the diet by the rabbit and least cost of feed/kg weight gain.

Key words: Sorghum dust, weaner rabbit, maize

VALEUR DE REMPLACEMENT DES DRECHES DE SORGHO POUR LE MAIS DANS LES REGIMES DE LAPINS SEVRES

Résumé

Une étude a été réalisée dans le but de déterminer la valeur de remplacement des drêches de sorgho pour le maïs dans la production de lapins. Un essai alimentaire de cinq semaines portant sur trente-deux lapins blancs de Nouvelle-Zélande sevrés, âgés de six semaines et pesant en moyenne 584.38g, a été organisé dans un schéma complètement aléatoire : les lapins ont été répartis en quatre groupes et ont reçu les régimes contenant respectivement 0 (témoin), 10, 20 et 30% de drêches de sorgho maltées (MSMD).

La MSMD avait une teneur élevée en protéines brutes (10,30%) et en fibres brutes (20,60%). Le gain de poids corporel, les ratios indice de conversion et coefficient d'efficacité protéique des lapins recevant de la MSMD à 10 % et à 30% étaient supérieurs à ceux du groupe témoin et ceux des lapins recevant de la MSMD à 20%. Les lapins soumis au régime contenant de la MSMD à 30% ont enregistré le gain pondéral quotidien le plus élevé de 37,14 g, tandis que la plus petite valeur de 27,14 g a été observée chez les lapins recevant un régime témoin. L'inclusion de la MSMD à 30% a entraîné une très forte utilisation du régime par les lapins et le plus faible ratio coût d'aliments / kg de gain pondéral.

Mots-clés : Drêches de sorgho ; Lapin sevré ; Maïs

Introduction

In order that, cheap animal protein could be made available to average people in developing world at affordable prices, the need to enhance livestock production cannot be overemphasized. To achieve the above mentioned objective, proper management techniques aimed at improving the wholesomeness of the animal in terms of health and nutrition must be adopted. Analysis showed that all the efforts directed at the improvement of conventional animal protein sources resulted in little improvement in terms of livestock population, growth, off take percentage, slaughter weight or dressing percentage (Obioha, 1992). The outlook according to Obioha is due to exploding population, urbanization, inflation and depletion of the national livestock due to extraneous circumstances such as the effect of draught. The extremely high cost of feed presently being experienced by livestock industry in the developing world has increased the feeding cost to about 70-75% total cost of production particularly in poultry and rabbits. This development arises from the explosion of population growth, which results in competition for the available grains by man and livestock. The interest of Animal Scientist in recent years has been directed towards search for cheaper locally available alternatives which are nutritionally viable and non-toxic. These alternatives have been found in agro-industrial by-products and farm residues. Agro-industrial by-products have been rendered into valuable protein and energy supplements for partial or complete substitution of expensive conventional feedstuffs in the diet of livestock without having adverse effect on growth and productivity of the animal, thereby reducing the cost of production.

Malted sorghum milling dust consists of material separated during grain or seed processing and milling in the brewery industry. It consists of 15% ash and almost the same nutrient composition as in sorghum grain (AFCO, 1999). Presently, this by-product has no commercial value but is sufficient enough to be fed as whole-feed to animals because research has indicated it contains nutrient required by

rabbits. The main objective of this investigation was to determine the replacement value of sorghum milling dust for maize in the diets of weaner rabbits.

Materials and Methods

The study was carried out at the rabbitry unit of the Teaching and Research Farm, University of Agriculture, Abeokuta. Dried sorghum milling dust (which consists of material separated during grain or seed processing and milling in the brewery industry) was collected from Sona Breweries Plc, Sango-Ota, Ogun State.

Thirty-two New Zealand White weaner rabbits of both sexes weighing 584.38g on the average were used. They were raised on wooden cages with metal mesh floor. The housing and equipment used were thoroughly washed and fumigated with disinfectant at the rate of one cup full per litre of water. The rabbitry unit was well ventilated; water and feed were supplied ad libitum. The individual rabbits were given 100 g of their daily ration at 0900hours. The left over were collected, weighed and recorded daily. The feeders and drinkers were made from clay.

The ration contained two variable components, maize and MSMD and other fixed ingredients. Four experimental diets were formulated with three as test diets containing different replacement values of MSMD, while the last diet was the control diet without malted sorghum milling dust. MSMD had a direct variation replacement value with maize in the diet formulation and were incorporated into the diet at 0, 10, 20, and 30%. The rabbits were allocated to the diets on weight equalization basis in a Completely Randomized Design. Each treatment group was sub-divided into 2 replicates with each consisting of 4 rabbits. The study lasted for 5 weeks. Feed intake was determined daily by subtracting the feed left-over from the feed supplied. Changes in body weight were recorded on weekly basis. Weight gain and feed conversion ratio were determined. Data obtained were analysed using Completely Randomized Design (CRD) and significantly ($P < 0.05$) different means were separated using New Duncan's Multiple Range

Test (Duncan, 1955).

Results

Table 1 shows the proximate composition of the experimental diets. It reveals that the moisture, crude protein, crude fibre, ether extract, ash content of the diets containing 10, 20 and 30% MSMD were higher than those in the control diet. As the levels of MSMD increased the percentage crude protein of the experimental diet increased while crude fibre and nitrogen free extract decreased with increase in the level of MSMD inclusion.

In table 2, the average daily weight gain increased with increase in the percentage of MSMD in the diets with the highest level recorded at 30% substitution level which was significantly ($P < 0.05$) different from the values obtained in rabbits fed the control diet. The rabbits on 20% substitution level of MSMD diet had the highest feed intake though this did not result to higher body weight gain when compared with rabbits fed control and two other experimental diets (10% and 20% substitution levels), respectively. The feed efficiency of rabbits fed 30% MSMD diet is significantly ($P < 0.05$) different from rabbits fed 10% MSMD diet and the protein efficiency ratio of rabbits fed the control diet was significantly ($P < 0.05$) higher than those fed the MSMD experimental diets.

Total feed intake and cost of feed/kg weight gain were significantly ($P < 0.05$) influenced by malted sorghum milling dust inclusion level in the diet of weaner rabbit (Table 3). Rabbits on diets containing 0 and 20% MSMD had similar total feed intake which were significantly higher than those on 10 and 30% MSMD. Total cost of feed consumed values ranged from 143.38 (rabbits on 30% MSMD substitution level) to 177.63 (control diet). Cost/kg feed and the cost of feed/kg body weight gain decrease gradually as the percentage of MSMD inclusion increased.

Discussion

In livestock industry, sorghum has been used primarily as feed grain because its nutritive value is similar to maize. Smith (1995) opined

that sorghum compares well with other feed grains in total carbohydrate, which indicates its suitability as a feed grain. Malted sorghum milling dust has a fibre content of 20.60% which is required for the normal functioning of the rabbit caecum and for the production of vitamin B. The diet contained crude protein (23.15 – 23.35%) and the crude fibre (16.80 – 17.80%), therefore, the diets were suitable for rabbit feeding which agreed with the findings of Omole (1982) that a diet of 18 – 22% crude protein is optimal for efficient rabbit production. Also, Cheeke (1983) reported that the crude fibre requirement for rabbit production is between 15- 17%. Ekpenyong and Biobaku (1986) also reported that a fibre level of 28–32% did not result in caecal impaction in rabbits. Cheeke (1983) has reported that although rabbits digest fibre poorly, dietary fibre is useful in preventing enteritis and for chewing.

MSMD also contains adequate macro and micro minerals that is vital for optimal performance of the rabbits. The crude protein content of sorghum dust is about 10.30% whilst the rabbit requires about 12% for its maintenance ration. So, MSMD needs low level of supplement in order to meet up the required level. The significant higher growth rate observed in rabbits fed the 30% MSMD diet that has the highest crude protein of 23.36% was in agreement with the findings of Omole (1982) and Butcher et al. (1983) who reported that rabbits were able to gain more body weight with diets that are higher in protein.

The feed intake (57.43 to 61.14 g/day) was similar to those obtained by Abubakar et al. (2006) who reported dry matter intake of 50.70 to 60.67 g/day for rabbits on diet containing malted or un-malted sorghum. Also, Doma et al. (1999) reported intake of 53.50 to 65.46 g/day for rabbits fed diets containing 25.30 to 50.70% maize and varying levels of cowpea shell and maize cobs. Adegbola and Okonkwo (2002) also reported 53.00 to 66.85 g/day feed intake for rabbits on diets containing 24.40 to 61.00% maize and varying levels of cassava leaf meal. Results of this present study varied from the observation of Dada (1993) that reported similar effects on weight gain, feed intake and

Table 1: Percentage composition of experimental diets on dry matter basis

Composition	Diets			
	1 (0% MSMD) Basal diet	2 (10% MSMD) diet	3 (20% MSMD) diet	4 (30% MSMD) diet
Maize	55.50	45.50	35.50	25.50
MSMD	0.00	10.00	20.00	30.00
Soya beans	20.10	20.10	20.10	20.10
BDG	20.00	20.00	20.00	20.00
Fish meal	2.00	2.00	2.00	2.00
Blood meal	1.50	1.50	1.50	1.50
Di' Methionine	0.40	0.40	0.40	0.40
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Determine Analysis				
Moisture (%)	4.84	5.02	5.12	5.24
Crude protein (%)	23.07	23.15	23.30	23.36
Crude fibre (%)	16.80	18.20	17.24	17.80
Nitrogen free extract (%)	31.81	28.84	27.13	24.93
Ether extract (%)	12.28	12.30	12.43	12.50
Ash (%)	9.58	10.83	13.19	14.58

Table 2: Performance of weaner rabbits as influenced by the dietary treatments

Parameters	0% MSMD	10% MSMD	20% MSMD	30% MSMD
Initial weight (g)	587.38± 0.40	588.38±0.48	588.38±0.56	589.38±0.40
Final weight (g)	1537.38±1.64 ^d	1738.38±2.10 ^b	1600.88±2.62 ^c	1889.38±3.90 ^a
Weight gain (g/day)	27.14±0.037 ^d	32.86±0.043 ^b	28.93±0.052 ^c	37.14±0.050 ^a
Feed intake (g/day)	60.57±0.071 ^a	57.43±0.064 ^b	61.14±0.059 ^a	57.43±0.063 ^b
Feed efficiency ratio	2.02±0.040 ^d	2.58±0.052 ^b	2.13±0.043 ^b	2.72±0.054 ^a
Protein efficiency	3.92±0.066 ^a	2.23±0.045 ^c	1.83±0.040 ^d	2.33±0.046 ^b

^{a,b,c,d}: Mean values in a row with different superscripts are significantly different.

Table 3: Cost benefit of replacing maize with sorghum milling dust in the diet of rabbits

Parameters	0% MSMD	10% MSMD	20% MSMD	30% MSMD
Total Feed intake (kg)	2119.95±2.37 ^a	2010.05±1.56 ^b	2139.90±2.25 ^a	2045.75±1.75 ^b
Total cost of feed consumed (N)	177.63±0.27	166.38±0.19	155.13±0.24	143.38±0.30
Cost/kg feed (N)	67.03±0.05	66.29±0.10	57.88±0.08	57.32±0.03
Cost of feed/kg weight gain (N/kg)	186.98±0.30 ^a	144.68±0.35 ^c	153.13±0.27 ^b	110.68±0.32 ^d

^{a,b,c}: Mean values in a row with different superscripts are significantly different.

feed conversion ratio of weaner pigs fed diets containing sorghum dust at replacement value of 50% and 100% for maize.

The cost of feed per kg body weight gain (N110.68 - N153.13) was significantly ($P < 0.05$) lower in diets containing MSMD when compared to N186.98 obtained from the diet with 0% MSMD. This indicates that inclusion of MSMD in the diet helps in the reduction of cost of production. The decrease in cost of feed per kg weight gain in the diets containing MSMD might have resulted from the little commercial value placed on MSMD at present. This observation is in agreement with the findings of Igwebuikwe et al. (1998) who reported a decrease in feed cost per kg weight gain with dietary replacement of costly ingredients with cheaper ones. Also, Dada (1993) reported that feed cost per kilogramme weight gain was lowest in diet where sorghum dust completely replaced maize. Hence, farmers will save a lot of cost by incorporating MSMD into the diets of rabbits since better performance were recorded.

The low levels of feed efficiency ratios of rabbits fed the control and 20% MSMD diets respectively agree with the findings of Onwuka et al. (1992) who reported that rabbit preference for agro by-products is not stable when compared with concentrates. So, the rabbits on diets containing 0% and 20% MSMD had better feed efficiency ratio of 2.13 compared to 2.58 and 2.72 obtained for those on diets containing 10% and 30% MSMD respectively. This implies that rabbits fed diet containing 20% MSMD had better feed utilization. (Ogbonna et al., 2000; Akinmutimi, 2006)

Conclusion

Malted sorghum milling dust can be included in the diet of rabbits, at dietary levels of 30% substitution level for maize as the growth rate is significantly higher than the rabbits in other treatments. The inclusion does not have a deleterious effect on the rabbits. The cost of feed/kg weight is lowest at 30% substitution level, meaning that the 30% substitution level of MSMD diet is cheap and the utilization by the rabbit is very effective. It helps to reduce

the cost of production and eventually leads to maximum profit to the farmer.

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SERO-SURVEILLANCE OF AVIAN INFLUENZA IN SUDAN, 2009-2010

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Abstract

This study reports the evidence of circulation of *avian influenza viruses* (AIV) in domestic poultry in Sudan. A total of 3525 sera samples collected from 14 States from 2009-2010 and were assayed for *avian influenza* (AI) antibodies using ELISA. Sera were collected from commercial (2267), backyard (550) and live birds markets (581) poultry flocks as well as 127 other bird species. Positive sera were further confirmed for the presence of H5, H7 and H9 subtype-specific antibodies, using *haemagglutination inhibition* (HI) assay. The overall prevalence of Influenza A-specific antibodies was 8.3% the highest value was detected in western region (13.6%) and the lowest in eastern region (4.4%). Amongst the whole tested birds, the backyard flocks showed significant ($P < 0.05$) highest values (10.5%) than the commercial (8.6%) or live bird markets (5.5%). Subtyping of all type A positive sera, showed that 1.7% contain antibodies to H5 antibodies with average mean titre of $5.1 \log_2$ and 0.3% of the sera was found to be positive to H7 antibodies with average mean titre of $4.9 \log_2$, while 2.5% were positive for H9 antibodies with average mean titre of $5.2 \log_2$. The other birds species included in this study displayed 7.1% antibodies to AI type A and found negative for either of H5, H7 & H9 subtypes. These results proved the circulation of low pathogenic AIV in the country.

Key words: *Avian influenza*, antibodies, birds, Sudan

SÉRO-SURVEILLANCE DE L'INFLUENZA AVIAIRE AU SOUDAN, 2009-2010

Résumé

Cette étude présente des informations factuelles sur la circulation du virus de l'influenza aviaire (VIA) chez les volailles domestiques au Soudan. Au total, 3525 échantillons de sérum prélevés dans 14 États de 2009 à 2010 ont été testés pour rechercher les anticorps de l'influenza aviaire (IA) en utilisant la méthode ELISA. Des sérums ont été recueillis dans des systèmes d'élevage commercial (2267), de basse-cour (550), et les marchés d'oiseaux vivants (581), ainsi que chez 127 autres espèces d'oiseaux. Les sérums positifs ont été en outre confirmés pour la présence d'anticorps spécifiques aux sous-types H5, H7 et H9 en utilisant le test d'inhibition d'hémagglutination (HI). La prévalence globale des anticorps spécifiques à la grippe A était de 8,3%, la valeur la plus élevée ayant été détectée dans la région de l'Ouest (13,6%) et la plus faible dans la région de l'Est (4,4%). De l'ensemble des oiseaux testés, les oiseaux de basse-cour ont montré des valeurs significativement ($P < 0,05$) plus élevées (10,5%) que les volailles commerciales (8,6%) ou celles des marchés d'oiseaux vivants (5,5%). Le sous-typage de tous les sérums positifs pour le type A a montré que 1,7% contenaient des anticorps H5 avec un titre moyen de $5,1 \log_2$, 0,3% des sérums étaient positifs pour les anticorps H7 avec un titre moyen de $4,9 \log_2$, et 2,5% étaient positifs pour les anticorps H9 avec un titre moyen de $5,2 \log_2$. Les autres espèces d'oiseaux incluses dans cette étude avaient 7,1% d'anticorps pour le type A de l'influenza aviaire avec des résultats négatifs soit pour les sous-types H5, H7 ou H9. Ces résultats ont prouvé la circulation du virus de l'influenza aviaire faiblement pathogène dans le pays.

Mots-clés : Influenza aviaire ; Anticorps ; Oiseaux ; Soudan

Introduction

Influenza A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease in chickens (Alexander, 2000). The highly pathogenic *avian influenza* (HPAI) viruses cause mortality rates up to 100% in several days. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. Low pathogenic *avian influenza* (LPAI) viruses cause subclinical to mild, primarily respiratory disease, which however can result in a serious disease when being complicated by concurrent infections and/or suboptimal environmental conditions. Strains of the H5 and H7 subtype with multiple basic amino acids at the cleavage site of the HA protein that do cause low mortality in experimental infections are also classified as HPAI viruses because the virulence can easily increase by a single mutation. (Alexander, 2000). Live bird markets and wild waterfowl infected with (LPAI) may introduce AI viruses into poultry flocks and these viruses may mutate to become highly pathogenic strains (Swayne and Halvorson, 2003). Conducting a continuous surveillance for AI antibodies, especially those against the H5, H7, and H9 subtypes, is therefore important to detect possible conversions from low to highly pathogenic *avian influenza*.

Historically, outbreaks of HPAI have occurred all over the world in all continents (Alexander and Brown, 2009). In Sudan, an outbreak of HPAI was first reported in 2006, caused principally by the H5N1 strain (Najat *et al.*, 2007). Due to this outbreak, 220 poultry farms were depopulated and around one million birds were destroyed. This event emphasized national surveillance of AIV in the natural hosts.

The poultry industry in Sudan was in the recent years estimated to compromise over 47 million birds (Elamin *et al.*, 2008). This production is based on closed, semi-closed system for commercial purpose, and backyard systems in rural areas which based on scavenging indigenous domestic chickens accompanied by other free range avian species which they usually utilized as a source of protein.

The aim of the present study was to

investigate the occurrence of circulating AI antibodies in birds in Sudan. Also to compare the seroprevalence of the samples collected among regions and among production types. The results of such investigations are very important for the analysis of risk assessment and eradication strategies.

Materials and Methods

This study was a part of National active surveillance system within the project (Supported Programmes of Integrated National Plan of *Avian Influenza*- SPINAP-AI) activity in collaboration with Ministry of Animal Resources & Fisheries and the Veterinary Research Institute (VRI). 3525 Serum samples were collected from birds in fourteen States of Sudan during the period from 2009-2010. The sampling was conducted randomly from selected flocks which had willingness to participate in the study. A number of 3398 sera were from chickens, 127 were from other bird species (domesticated ducks, pigeons, geese, guinea fowl and turkeys) (Table I). All flocks were raised in an open system.

Enzyme Linked Immunosorbant Assay (ELISA)

Indirect ELISA

The commercial indirect ELISA Kit (Biocheck-Gouda, Holland) was used to detect AI type A antibodies in chickens, sera. The test was performed as described by the manufacturer.

c-ELISA

The competitive ELISA Kit (Anigen-Korea) was used to detect AI antibodies in sera of other bird species, sera. The test was applied as described by the manufacturer.

Haemagglutination Inhibition (HI) Test

The sera found positive for AIV type-A, were subjected to HI test to be subtyped. The sera were considered positive if there was inhibition at a dilution of 1/16 or more against 4 haemagglutination unit of antigen (OIE, Manual, 2009). The standardized H5, H7 and H9 panels of antigens (OIE reference laboratory for Newcastle disease virus and *avian influenza*,

Padova) and positive sera (Intervet, Holland) were used (OIE, Manual, 2009).

Data Analysis

The serological results of the sampled animals were analyzed statistically using statistical package (Statistix 9). To compare the proportion of Type A AI antibody between regions and among different types of chicken, the Chi-Square (χ^2 test) was used. The statistical significance level used was $P \leq 0.05$.

Results

ELISA test

Out of 3525 sera samples tested at all States, 294 (8.3%) were proved to be positive for type A influenza virus antibodies. The highest percentage of antibodies (13.6%) was observed in western regions and lowest in eastern (4.4%) (Figure 1). The prevalence of AI type A antibodies in commercial chickens flocks was 8.6% (195 out of 2267) (Table 2). Whereas 10.5% (58 out of 550) of backyard chickens and

5.5% (32 out of 581) of live bird markets were positive AI type A antibodies (Table 3&4).

As shown in Table 5, other bird species displayed 7.1% AI type A antibodies.

There was significant difference ($P \leq 0.05$) in proportions of AI type A in tested birds in different regions ($\chi^2 = 54.03$, $df=3$, P -value = 0.0181).

Also a significant variations ($P \leq 0.05$) detected in values of AI of commercial, backyard, live bird markets & other bird species ($\chi^2 = 10.6$, degree $df=3$, P -value = 0.0181).

HI Test

The seroprevalence of AI subtypes H5, H7 and H9 antibodies in samples collected were 1.7%, 0.3% and 2.5% with mean antibody titre of 5.1, 4.9 and 5.2 \log_2 respectively. These AI subtypes represent 54.8% of positive ELISA sera. The percentage of H5, H7&H9 antibodies in different regions were illustrated (figure 2). The highest percentage of H5 and H7 were recorded in samples taken from the central regions and that of H9 was in western regions. The H5 mean antibody titre in all studied

Table 1: Number of sera collected from 14 States and the different species of birds surveyed.

Region	State	Commercial chickens	Backyard chickens	Live bird markets	Other species	Total
Central	Khartoum	360	80	0	0	440
	Gazeera	103	23	4	0	130
	White Nile	424	40	37	22	523
	Sennar	149	56	82	10	297
	Blue Nile	90	100	131	44	374
	Total		1126	299	254	76
Western	North Kordofan	390	30	90	4	514
	South Kordofan	14	64	52	4	134
	South Darfur	146	15	52	0	213
	West Darfur	39	23	22	27	111
	Total		589	132	216	35
Eastern	Gadaref	275	24	47	0	346
	Kassala	108	25	31	16	180
	Red Sea	69	13	33	0	115
	Total	452	62	111	16	641
Northern	Northern	100	30	0	0	130
	River Nile	0	27	0	0	27
	Total	100	57	0	0	157
Total		2267	550	581	127	3525

Table 2: Number and percentage of positive type A AI antibodies and number & mean antibody titre of positive H5.H7 &H9 Subtypes in tested Commercial chickens, sera

Region	State	Type A		H5		H7		H9	
		No+ve / No. tested	%	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)
Central	Khartoum	55/360	15.3	38	6.6	3	4	25	3.6
	Gazeera	2/103	1.9	0	-	0	-	0	-
	White Nile	24/424	5.7	0	-	4	4	6	4.5
	Sennar	1 /149	0.7	0	0	0	0	0	0
	Blue Nile	1/90	1.1	0	0	0	0	1	4
Western	North kordofan	69/390	17.7	10	5.2	2	5.5	27	5.1
	South Kordofan	0/14	0	0	-	0	-	0	-
	South Darfur	10/146	6.8	0	0	0	0	0	0
	West Darfur	0/39	0	0	-	0	-	0	-
Eastern	Gadaref	13/275	4.7	1	6	0	-	0	-
	Kassala	3/108	2.8	1	4	1	5	0	0
Northern	Red Sea	3/69	4.3	0	-	0	-	0	-
	Northern River Nile	14/100	14	0	-	0	-	0	-
Total		195/2267	8.6	50	5.5	10	4.6	59	4.3

Table 3: Number and percentage of positive type A AI antibodies and number & mean antibody titre of positive H5.H7 &H9 Subtypes in tested Backyard chickens, sera

Region	State	Type A		H5		H7		H9	
		No+ve / No. tested	%	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)
Central	Khartoum	3/80	3.8	0	-	0	-	0	-
	Gazeera	0/23	0	0	-	0	-	0	-
	White Nile	0/40	0	0	-	0	-	0	-
	Sennar	6/56	10.7	0	-	0	-	2	8
	Blue Nile	13/100	13	1	6	1	5	8	5.6
Western	North kordofan	5/30	16.7	5	4	0	-	0	-
	South Kordofan	24/64	37.5	3	5.3	0	-	9	5
	South Darfur	0/15	0	0	-	0	-	0	-
	West Darfur	1/23	4.3	0	-	0	-	0	-
Eastern	Gadaref	3/24	12.5	0	-	0	-	0	-
	Kassala	1/25	4	0	-	0	-	2	6
Northern	Red Sea	1/13	7.7	0	-	0	-	0	-
	Northern River Nile	1/30	3.3	0	-	0	-	1	4
Total		58/550	10.5	9	5.1	1	5	22	5.7

Table 4: Number and percentage of positive type A AI antibodies and number & mean antibody titre of positive H5, H7 & H9 Subtypes in tested Live Bird Markets chickens, sera

Region	State	Type A		H5		H7		H9	
		No+ve / No. tested	%	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)	No+ve	Mean Titre (log ₂)
Central	Gazeera	0/4	0	-	-	-	-	-	-
	White Nile	4/37	10.8	0	-	0	-	0	-
	Sennar	1/82	1.2	0	-	0	-	0	-
	Blue Nile	7/131	5.3	2	5.5	0	-	3	5
Western	North kordofan	8/90	8.9	0	-	0	-	1	4
	South Kordofan	10/52	19.2	0	-	0	-	4	4.3
	South Darfur	0/52	0	0	-	0	-	0	-
	West Darfur	0/22	0	0	-	0	-	0	-
Eastern	Gadaref	0/47	0	0	-	0	-	0	-
	Kassala	0/31	0	0	-	0	-	0	-
	Red Sea	2/33	6.1	0	-	0	-	0	-
Total		32/581	5.5	2	5.5	0	-	8	4.4

Table 5: Number and percentage of AI Type A antibodies in tested sera of other bird species

Region	State	Domestic	
		No.+ve/ No.tested	%+ve
central	Blue Nile	21,2/44	4.5
	North Kordofan	12/4	25
Western	South Kordofan	11/4	25
	West Darfur	31,2,2/27	11.1
Eastern	Kassala	21,3/16	12.5
Total		9/127	7.1

1: Duck, 2:Pigeon,3: Goose

chickens ranged between (5.5-4.8 log₂), (4.3-5.5 log₂) for H7 and (4.7-6 log₂) for H9. As shown in tables (2, 3 & 4) the highest prevalence rate of H5 antibody were detected in commercial flocks (2.2) followed by backyard (1.6%) and least in live bird markets (0.3%).

The percentage of H7 antibodies in commercial and backyard chickens studied, were 0.4 & 0.2% respectively. The highest percentage of H9 were recorded in backyard flocks (4%), followed by commercial (2.6%) and least (1.4%) recorded in live bird markets.

The other domestic species of poultry including pigeons, ducks, geese and guinea fowl tested, were negative for either H5, H7 & H9 subtypes.

Discussion

The data obtained in this study confirmed that anti-influenza type A viruses antibodies were prevalent in all of the studied regions of the country in spite of the relatively low number of samples of some States. Since the last outbreak of *avian influenza* in 2006 in the country, no other outbreaks were reported as well as no clinical signs were observed. In addition, no vaccination programmes were practiced. Thus, the presence of AI antibodies may be attributed to circulating of low pathogenic viruses. In this study, different backyard chickens flocks tested showed considerable levels of antibodies which proved

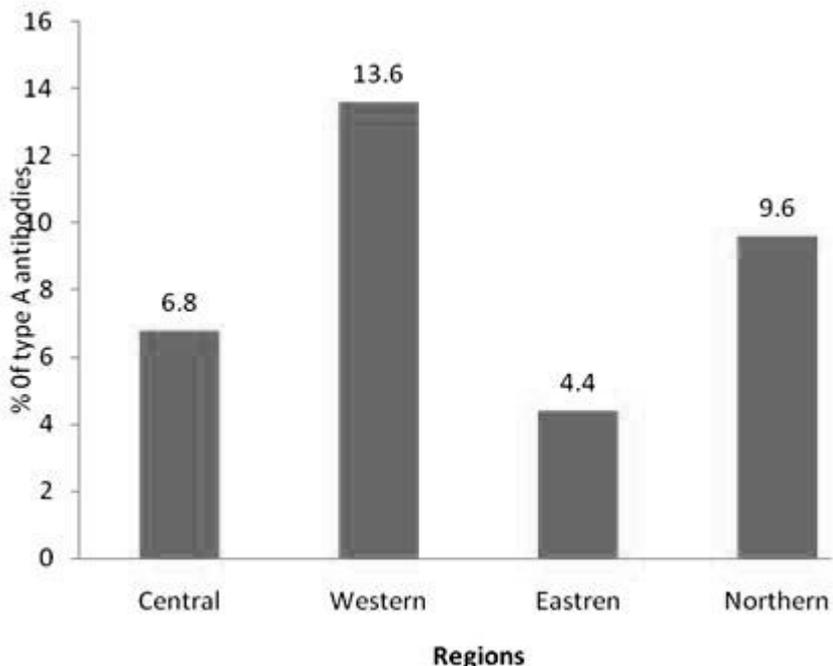


Figure 1: Percentage of AI type A antibodies of all chickens sera in different regions

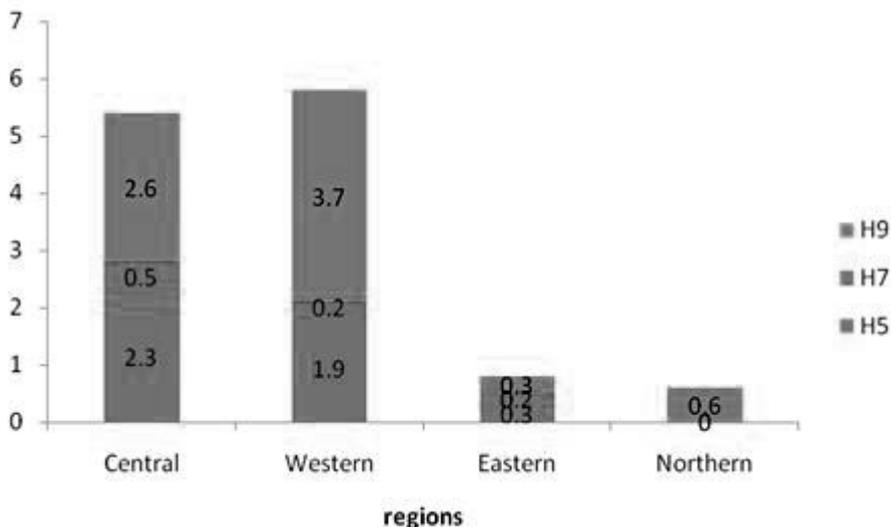


Figure 2: Percentage of H5, H7 & H9 AI subtypes in different regions

that backyard flocks were quite exposed to AI viruses. It is suggested that this group of scavenging birds may play significant roles in the transmission of this virus to commercial poultry farms.

In the present study, (30.3%) 89 out of 294 positive type A, were positive for H9 antibodies which conclude that the vast majority of circulating AI was H9 subtypes. This result was in accordance with previous findings of Wegdan

(2008) who detected highest percentage of H9 antibodies (90.7%) compared to 12.9% of H7 and 2.6% of H5. Infection of poultry with low pathogenic viruses can produce economic losses by causing drop in egg production and reduced weight gain. Furthermore there is a great danger for virus mutations caused by fast and repeated animal passages (Fereidouni *et al.*, 2005). In this investigation, antibodies against H5, H7 & H9 were identified which will raise

attention of possible mutation of these viruses that can lead to highly pathogenic forms of infections.

The results of the current investigation were similar to that conducted previously in Sudan. Manal et al. (2000) detected 18% AI type A antibodies in chickens in Khartoum State and in 2007 after the HPAI outbreak, 17.7% of type A and 12.6% of H5 antibodies were recorded in birds (Iman et al., 2009), while later, 12% were confirmed by ELISA as type A AI antibodies among non vaccinated chickens (Epidemiology of *avian influenza* in Africa (EPIAAF) survey-2008).

Seroprevalence of AIV varied in surveys conducted in different countries. A percentage of (13-20%) AIV antibodies were detected in small holdings in different periods in Bangladesh (Biswas et al., 2009). While Sabarinath et al. (2011) revealed 18.8% AIV antibodies in tested 143 backyard chickens sera. And similarly 13.7% positive AIV antibodies were detected in backyard chickens in Mali by commercial ELISA test (Molia et al., 2010). But a high seroprevalence AIV antibodies (71%) had been reported in sera of breeder broiler chickens in Jordan (Al-Natour and Abo-Shehada, 2005). However, a serosurveillance conducted in Nigeria showed that all studied birds were free from AIV (Owade et al., 2006).

AIV H9 antibodies were detected in Jordan (Dergham et al., 2009) as they found that 65 out of 120 broiler flocks (54.2%) and 47 out of 60 layer flocks (78.3%) were positive for AIV H9 subtype antibodies using HI test.

Although the sample size for other bird species tested was small, interestingly, the results show that 7.1% were influenza type A antibody positive. Other bird species may play a major role in the epidemiology of *avian influenza* in Sudan, so, more samples from these species are required in searching for the virus. Previously, different bird species were investigated for the presence of AI during 2006-2009, the results revealed that the species passer domestics, one duck, 36 Egyptian & 13 geese, were positive to type A AI (Report of avian pathology and diagnosis Department, VRI, 2007).

The present results along with previous studies confirmed the circulation

of AI viruses in poultry in Sudan which necessitate the need to develop and execute a continuous monitoring system to detect LPAI virus infections in domestic poultry. The data provided by this study do not therefore cover the full production potential of the Sudanese poultry industry. It does, however, provide information of AI situation in the country. Further studies including migratory birds will be helpful in identifying the currently circulation of AIV in the country.

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SEROPREVALENCE OF SELECTED AVIAN PATHOGENS OF BACKYARD POULTRY IN SINAR, SUDAN.

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Abstract

A serological survey for Newcastle (ND), *avian influenza* (AI), Gumboro (IBD) and *Infectious bronchitis* (IB) viruses was conducted in 310 serum samples in village chickens in Sinar State, Sudan. The studied chickens had no history of previous vaccination and showed no clinical signs. Results of indirect enzyme-linked immunosorbent assays (I-ELISA) on serum samples showed that birds had antibodies against Newcastle disease virus (ND) (92.6%), *infectious bursal disease virus* (IBD) (91.8%), *Infectious bronchitis virus* (IB) (86.5%) and *Avian influenza* (AI) virus type- A (34.2%). Positive sera of influenza type A were subjected to *haemagglutination inhibition* (HI) testing for AI subtypes H5, H7 and H9. The results showed that 1% of the sera were positive for H5 antibodies with average mean titer of $6 \log_2$ while, 0.3% of the sera were found to be positive for H7 antibodies with average mean titer $4 \log_2$. None of the sera were positive for H9. These results indicate that ND, IBD, IB and AI were endemic in Sinar state and these diseases contribute significantly to local chicken losses in the free range production systems. Also it represents foci of infection to the commercial chickens.

Key words: backyard birds, Newcastle disease, *Infectious bursal disease*, *Infectious bronchitis*, *Avian influenza* antibodies, Sudan.

SÉROPRÉVALENCE DE CERTAINS AGENTS PATHOGÈNES DE L'INFLUENZA AVIAIRE CHEZ LES VOLAILLES DE BASSE-COUR À SINAR (SOUDAN)

Résumé

Une enquête sérologique sur les virus de la maladie de Newcastle (MN), l'influenza aviaire (IA), la maladie de Gumboro (MG) et la bronchite infectieuse (BI) a été réalisée sur 310 échantillons de sérum chez des poulets villageois dans l'État de Sinar au Soudan. Les poulets étudiés n'avaient pas d'antécédents de vaccination antérieure et ne présentaient aucun signe clinique. Les résultats des tests immuno-enzymatiques indirects (I-ELISA) sur des échantillons de sérum ont montré que les oiseaux avaient des anticorps contre le virus de la maladie de Newcastle (MN) (92,6%), le virus de la bursite infectieuse (MG) (91,8%), le virus de la bronchite infectieuse (BI) (86,5%) et le virus de l'influenza aviaire de type A (34,2%). Les sérums positifs pour l'influenza de type A ont été soumis à des tests d'inhibition de l'hémagglutination (HI) pour les sous-types H5, H7 et H9 de l'IA. Les résultats ont montré que 1% des sérums était positif pour les anticorps H5 avec un titre moyen de $6 \log_2$, et 0,3% des sérums était positif pour les anticorps H7 avec un titre moyen de $4 \log_2$. Aucun des sérums n'étaient positif pour H9. Ces résultats ont révélé que la MN, la MG, la BI et l'IA étaient endémiques dans l'État de Sinar, et ces maladies contribuent de manière significative aux pertes de poulets locaux dans les systèmes de production en libre parcours. En outre, elles représentent des foyers d'infection pour les poulets commerciaux.

Mots-clés : Oiseaux de basse-cour ; Maladie de Newcastle ; Bursite infectieuse ; Bronchite infectieuse ; Anticorps contre la grippe aviaire ; Soudan

Introduction

Poultry keeping in the rural areas of the Sudan is one of the most ancient households activity practiced in both transhumant and in settled life areas. Usually the family keeps a variable number of birds, from local breeds, around the homestead and no distinct system of poultry is followed. The birds are kept free around the house compound and use the same shelter as the family.

The importance of village poultry keeping in the Sudan as a factor contributing to the nutritional level of the family is fully realized. Therefore, efforts are being made to promote poultry production under village conditions and to control diseases (Elfadil, 1997).

Village chickens are the most common type of livestock in many rural areas. Even very poor household with few labour resources will normally keep some chickens (Christine et al., 2009). They provide the owners with a form of savings which can help in times of need to meet essential family expenses such as medicines, clothing and school fees. Families can also increase their income by taking advantage of seasonal peaks in poultry demand, such as at religious festivals or celebrations (Johnston and Cumming, 1991). Village chickens are known to be susceptible to most diseases which affect commercial chickens. Intercurrent disease is very important. Survival rates of chicks without disease outbreak ranges from 60-70 percent. The survival rate in the rainy season is generally lower because of high humidity, strong wind, and fluctuating temperatures. These conditions also make the chicks more susceptible to infectious diseases, especially respiratory diseases, thus reducing the survival rates. Due to free-ranging and unconfined type of management, disease control is very difficult and expensive, thus is rarely practiced by the villagers (Aini, 1990). Causes of losses in free range chickens in absence of diseases were due to Malnutrition and mismanagement. Predation and exposure to extreme weather conditions have also been named as another important cause of losses particularly among the youngest age group of chickens (Bell, 1992).

There are many constraints to village chickens production (Sanaiya et al 1999)

including a range of bacterial and viral diseases, internal and external parasites, (Permin and Hansen, 1998). In Sudan the major constraints of household poultry keeping and traditional open house producers are inadequate health care; their major problem is the high incidence of Newcastle disease, and infectious bursal disease (khalafalla et al., 2001).

It is believed that the free range chicken act as potential reservoir of infection to themselves and to the commercial birds (Emikpe et al., 2003). The aim of the present study was to determine the sero-prevalence rate of ND, IBD, IB and AI in free-range chickens in Sinar state.

Material and methods:

Serum collection:

A total of 310 blood samples were collected randomly from different ages of unvaccinated village chickens at Sinar state, no data are available concerning the total population of free-range chickens in this state. Samples were kept at 37 °C for 3 hours before the serum was removed. The separated serum was stored at -20 °C until examined by indirect ELISA and HI test.

ELISA test:

The indirect ELISA kits (flock-check-IDEXX, USA) were used to detect ND, IB, IBD and AI type A antibodies. The tests were performed as described by the manufacturer. In brief, 100µl of undiluted negative and positive control were put into well A1, 2 and A3, 4 (of the coated plates with each antigen) respectively. Then 100µl of diluted samples (1:500) were added into appropriate well and incubated for 30 minutes at room temperature, plates were washed 3-5 times with deionizer water. 100µl of (goat) anti-chicken/ (goat) anti-turkey: horseradish peroxidase conjugate were dispensed into each well and incubated for 30 minutes at room temperature. Plates were washed again as mentioned above. 100µl of Tetramethylbenzidine (TMB) substrate solution were dispensed into each well and incubated for 15 minutes at room temperature, then 100µl of stop solution were dispensed into each well to stop the reaction. The absorbance

of the control serum and serum samples was recorded using the Bio-Tek ELx808 plate reader at 650nm. The software (X chek™ 3.2USA) was used for calculation of the sample to positive (S/P) values. Presence of ND, IB, IBD and AI virus antibodies indicated previous exposure to the virus.

Haemagglutination inhibition test HI:

HI test was performed (on positive AI samples examined by ELISA) as described in the Office International des Epizooties (OIE, 2008). A reference antigen was used for subtypes of AI virus antibodies H5, H7 & H9. Samples with HI-titer above $3 \log_2$ were considered positive. Results: Antibodies prevalence by Indirect ELISA against Newcastle, Gumboro, *Infectious bronchitis* and *Avian influenza* is shown in Fig. (1). ELISA-positive AI sera were tested for H5, H7 & H9. For H5 1% of the sera were positive with average mean titer $6 \log_2$, 0.3% of the sera were positive for H7 with average mean titer $4 \log_2$. None of the sera were positive for H9.

Discussion

Free-roaming chickens are at a disadvantage compared with commercial poultry for maintaining their health because they do not receive vaccinations nor are they afforded treatments typically applied to commercial poultry. These chickens are on a poor plane of nutrition and run in flocks of mixed ages, placing susceptible younger chicks in contact with adults that are potential reservoirs of disease. Additionally, most commercial poultry breeder flocks are maintained free of certain infectious diseases that can be transmitted from the hen to progeny. It is likely that the diseases, to which these chickens are exposed, both singly and in combination, are responsible for the high mortality among the young and decrease reproductive success. On the other hand, their relative low densities, hybrid vigor, and capability to free-roam away from excrement may prevent them from suffering from more substantial clinical disease. Lack of education, geographic isolation, and cost of veterinary services preclude the owners in this community from applying standard preventative-medicine protocols. Morbidity

and mortalities reported are directly linked to the lack of preventative medicine and lack of shelter and to keeping chickens of different ages in the same group and allowing the introduction of new individuals into the flock (Bermudez and Stewart, 2003).

The antibodies obtained in this study could only have been acquired from natural infection since there was no history of vaccination among local chickens and all the birds were over three months of age, thereby ruling out the presence of maternal antibodies.

The free ranging management system allows the uninterrupted cycle of infection as the virus passes from one to the other. The chickens are also prone to acquire infection from wild birds. The local open markets where huge numbers of chickens are gathered might also serve as continuous foci of infection (Aschalew et al., 2005). Also the ease of contact at local open-air markets between chickens from different areas in Sinar state, which are then taken back to various localities, can undoubtedly facilitate the rapid spread and persistence of these diseases among indigenous chickens.

The seroprevalence of ND, IBD, IB and AI as found in this study was high. This indicates that these diseases were highly prevalent among the village poultry population in this area. The high prevalence of these diseases in village chickens in Sinar state may contribute significantly in the epidemiology of these diseases in exotic chickens reared in the same area since there are extensive exotic chicken's farms and ND, IBD, IB were among the most common diseases in these farms. This agreed with Okwar et al., 2011 who stated that the village poultry are being incriminated as an important factor in the epidemiology of the diseases in commercial and exotic chickens.

Newcastle (ND) antibodies detected in this study were the highest sero-prevalence 92.6 % and this result showed the importance of this disease in local chickens. Aini, 1990 reported that Newcastle disease (ND) is regarded as the principle factor limiting rural poultry production in all Africa and Asian countries. ND may kill up to 80% of household poultry in Africa. The scavenging birds may also play significant roles in the maintenance

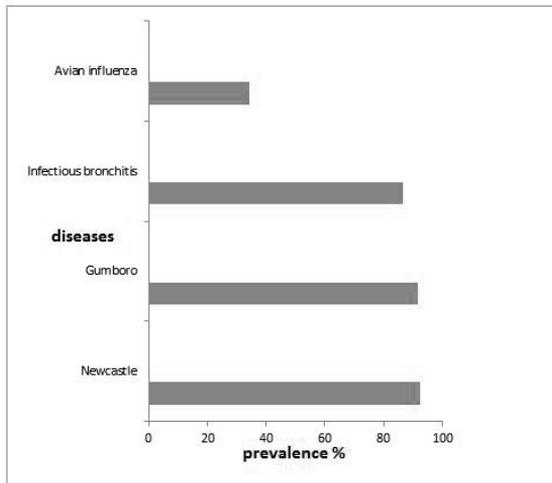


Figure 1: Prevalence of ND, IBD, IB&AI viruses in local chicken's sera

and spread of AI and ND viruses to the more susceptible commercial poultry (Abdu *et al.*, 2002). Newcastle disease, caused by a virus, is documented as the most important disease of poultry in the world, causing devastating losses in both commercial and village chickens; it is capable of causing mortality of up to 100% (Spradbrow, 1997). In Sudan ND seroprevalence was reported by several serological studies in free range chickens, a rate of (16%) NDV antibodies was detected in local chickens in Kordofan region (Elhassan and Kheir, 1989), (12%) in Aldamer province (ElHussein *et al.*, 1995-1996), (18%) in Gadaref & Khartoum States (Khalafalla *et al.*, 2004) and (41.8%) in 14 states of Sudan (Egbal, 2012). Diagnosis of clinical ND in village chickens in Sinar state was based on clinical signs and postmortem examination, so there was no reported cases about ND virus isolation in village chickens in Sinar state. But in a study done by Sana *et al.*, 2004 two isolates of ND virus were obtained from village chickens in Gedarif state.

The high prevalence of infectious bursal disease (IBD) (91.8%) showed the endemic character of this disease and its low pathogenicity. This might indicate a relative resistance of the local birds against the IBD virus (Courtecuisse *et al.*, 1990).

Okoye *et al.*, 1999, mentioned that village chickens are highly susceptible to IBD and this dissemination of the virus by the village chicken contribute to the maintenance of endemicity and spread of this disease to the

commercial birds. IBD infection in the village chicken may represent an important part of the IBD epidemiology because these birds could harbor the IBD virus strain and should not be overlooked in the control and prevention of the disease (Tan DY *et al.*, 2004). A serological study in Sudan which was done by Mahasin, (1998) detected (38.7%) IBD seroprevalence in Khartoum North while Egbal (2002) found A percentage of (36.3%) in local chickens in Khartoum and Gadaref States.

Other field investigations into the diseases including IBV of backyard chickens in the world are relatively rare (Hernandez-Divers *et al.*, 2006), but should be encouraged. Cavanagh and Naqi, 2003, stated that the high prevalence of IBV antibodies in indigenous chickens may be a result of field infection with IB virus. Antibodies to IB virus have been demonstrated in the sera of local chickens in Sudan, although no clinical disease has been reported (Abd El Rahim *et al.*, 1996).

In Zimbabwe the seroprevalence of IB virus among backyard chickens was 86% (Patrick *et al.*, 1994) and this is similar to the finding in this study which showed 86.5% seroprevalence.

Highly Pathogenic Avian Influenza H5N1 was reported in Sudan in 2006 (Najat *et al.*, 2007). Avian influenza was not reported in Sinar state but the result obtained in this study showed a prevalence of 34.2% of AI type-A, this may be due to natural infection as mentioned by Alexander, 2003 that the backyard chickens were reared under semi-scavenging system and were allowed to scavenge with ducks in the yard, in the crop fields near to water reservoirs where domestic ducks, wild ducks and migratory birds used to scavenge over there. And this happened similarly in Sinar state which ducks and water reservoirs are exist. This factor may contribute in natural infection to the backyard chickens.

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PREVALENCE OF MYCOBACTERIUM BOVIS IN CATTLE SLAUGHTERED AT SOKOTO CENTRAL ABATTOIR

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Abstract

This study was undertaken to screen cattle slaughtered at the Sokoto Central Abattoir for antibodies against *Mycobacterium bovis*. By the lateral flow technique (immunochromatography), using monoclonal antibodies for *M. bovis* (BioNote, Inc. Gyeonggi-do, Korea) and by post mortem examination. A total of 194 slaughtered cattle were randomly selected (using systematic sampling technique) and screened. Eight (4.12%) were positive for antibodies against *M. bovis*. No lesions suggestive of bovine tuberculosis were found during post mortem inspection. More females than males appear to have *M. bovis* antibodies ($p < 0.005$). Also cattle of ages between 24-48 months had the highest prevalence in relation to age groups ($P < 0.005$). Four breeds of cattle were encountered during this work although representation was determined by the sampling method. Sokoto Gudali breed had the highest number of cases of bovine tuberculosis ($P > 0.005$). This study indicate an increase (from 0.69% to 4.12%) in the prevalence of bovine tuberculosis in the study area. The public health implications of this findings is that infected animals in the past have been slaughtered and consumed undetected; this will continue to be so long as the relevant authorities do not improve on the current methods of ante mortem screening animals for tuberculosis.

Key words: Antibodies, Cattle, Prevalence, Post mortem examination, Slaughter, Sokoto, Tuberculosis

PREVALENCE DE MYCOBACTERIUM BOVIS CHEZ LES BOVINS ABATTUS A L'ABATTOIR CENTRAL DE SOKOTO

Résumé

Cette étude a été réalisée pour examiner les bovins abattus à l'Abattoir central de Sokoto en vue de rechercher la présence d'anticorps anti *Mycobacterium bovis* par la technique d'écoulement latéral (immuno-chromatographie), en utilisant des anticorps monoclonaux pour *M. bovis* (Bionote, Inc. Gyeonggi-do, Coreia) et par l'examen post-mortem. Au total, 194 bovins abattus ont été choisis de manière aléatoire (en utilisant la technique de l'échantillonnage systématique) et examinés. Huit (4,12%) étaient positifs pour les anticorps anti *M. bovis*. Aucune lésion évocatrice de tuberculose bovine n'a été trouvée lors de l'inspection post-mortem. Il semble que les anticorps de *M. bovis* ($p < 0,005$) ont été détectés chez plus de femelles par rapport aux mâles. En outre, les bovins âgés de 24 à 48 mois avaient la prévalence la plus élevée en ce qui concerne les groupes d'âge ($P < 0,005$). Quatre races de bovins ont été rencontrées au cours de ce travail, bien que la représentation ait été déterminée par la méthode d'échantillonnage. La race Sokoto Gudali avait le plus grand nombre de cas de tuberculose bovine ($p > 0,005$). Cette étude a révélé une augmentation (de 0,69% à 4,12%) de la prévalence de la tuberculose bovine dans la zone d'étude. Les implications de ces constatations sur la santé publique sont que les animaux infectés dans le passé étaient abattus et consommés sans détection préalable de maladie ; et ceci va continuer si les autorités compétentes n'améliorent pas les méthodes actuelles d'examen ante-mortem des animaux pour rechercher la tuberculose.

Mots-clés : Anticorps ; Bovins ; Prévalence ; Examen post-mortem ; Abattage ; Sokoto ; Tuberculose.

Introduction

Tuberculosis is a chronic contagious disease of both domestic and wild animals and humans (Radostits et al., 2002). It is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestines, liver and kidneys (Shitaye et al., 2007). Bovine *Tuberculosis* (BTB) results from infection with *M. bovis*, a gram positive acid fast bacterium in the *M. tuberculosis* complex of the family *Mycobacteriaceae*. It is an important zoonotic disease known to exist in all parts of the world. It has been recognized from 176 countries as one of the important bovine disease causing great economic loss (Martin et al., 1994; Hines et al., 1995; Samad, 2008). According to Majiyagbe and Lamorde (1997) in Nigeria, the loss of livestock exist at a higher rate than in developed countries and such production loss could range between 30% and 40%. Though, primarily a bovine problem, *M. bovis* infects and cause *tuberculosis* in camels, pigs, sheep, goats, horses, dogs, cats, badgers, lions, elephants, deer, primates and man (Ayele et al., 2004).

This study was designed to determine the prevalence of *M. bovis* in cattle slaughtered at Sokoto central abattoir using rapid diagnostic technique (immunochromatography) designed specifically for the detection of *M. bovis* and to compare the rate of detection with post mortem examination of lesions of the disease as described by Biffa et al. (2010).

Materials and Methods

Study area

Sokoto Abattoir

The study was conducted at Sokoto central abattoir using a systematic sampling technique. An average of 140 cattle of both sexes is slaughtered every day at the abattoir. For the purpose of this study, every 10th animal slaughtered was sampled giving a total of 14 samples collected per visit. The study was conducted over a period of seven weeks.

Sample size

Using a prevalence of 0.69% (Ajogi et

al., 1995) and the formular $n = \frac{z^2 p(1-p)}{d^2}$ (Thrustfield, 2002), the sample size was estimated to be a minimum of 105. In order to increase chances of detection 194 samples were collected and analyzed.

Sample collection

About 50mls of blood from blood spillage during slaughter was collected in EDTA test tube at the abattoir. The test tubes were immediately capped washed and labeled. The samples were then transported in an ice packed container to the Public Health and Preventive Medicine laboratory at the Faculty of Veterinary Medicine of Usmanu Danfodiyo University Sokoto, Nigeria, where they were analyzed.

Anigen® Rapid Bovine Tuberculosis Antibodies Test Procedure

Anigen® Rapid Bovine *Tuberculosis* antibodies test kits specific for *M. bovis* antibodies containing the test devices and specimen droppers procured from BioNote, Inc. Gyeonggi-do, Korea were used in detecting the *M. bovis* antibodies using whole blood collected. The test was conducted according to the manufacturer's guidelines as follows:

The test kit was removed from the foil pouch and placed on a flat, dry surface. Three drops of the whole blood diluent were added into the test tube for whole blood dilution. One drop (30µl) of whole blood sample was added with the disposable dropper and mixed for one minute. A capillary tube was then used to add 10µl of the mixed sample to the sample hole marked "S" on the test device and allowed for one minute. Three drops of the developing buffer were then added into the developing buffer hole on the test device.

Interpretation of the Results

The presence of only one purple colour band within the result window indicates a negative result while the presence of two colour bands within the result window, no matter which band appears first, indicates a positive result even if the intensity of the purple band colour is faint, it will be interpreted as positive, if it appears within 20 minutes based on the recommendations of the manufacturer.

Post Mortem Meat Inspection Procedure

On each of the slaughtered animals sampled, detailed meat inspection was conducted as described by Biffa et al. (2010). The procedure involves visual examination and palpation of intact organs such as the liver and kidneys; palpation and incision of tracheobronchial, mediasternal and precrural lymph nodes. Further examinations of other lymph nodes and organs was considered whenever lesions were detected in one of these tissues.

Statistical Analysis

Chi square test (χ^2 -test) was used to

analyze for any significant relationship between the detection of *M. bovis* antibodies and age, sex and breed of cattle.

Results

Eight (8) out of the 194 (valid tests) samples were found to be positive for antibodies against *M. bovis*, representing 4.12% prevalence rate.

No gross lesions of bovine *tuberculosis* were seen at post mortem of any of the sampled animals. No significant statistical association ($P>0.05$) was found to exist between the infection and breed of the slaughtered animals.

Table 1: Age distribution of bovine *tuberculosis* in cattle slaughtered at Sokoto Central Abattoir. (n = 194)

Age (months)	Frequency	Number of animals positive	Percentage (%)
<24	10	0	0.00
25-48	38	3	7.90
49-72	27	1	3.70
73-96	72	2	2.78
97-120	47	2	4.26
Total	194	8	4.12

$\chi_2 = 2.21$ P-value = 0.75 $P>0.05$

No significant statistical association ($P>0.05$) was found to exist between the infection and the age of the slaughtered animals.

Table 2: Sex distribution of bovine *tuberculosis* in cattle slaughtered at Sokoto Central Abattoir

Sex	N = 194	Number of animals positive	Percentage (%)
Male	93	1	1.08
Female	101	7	6.93
Total	194	8	4.12

$\chi_2 = 4.19$ P-value = 0.035 $P<0.05$

A significant statistical association ($P<0.05$) between the infection and the sex of the animals was found to exist

Table 3: Breed distribution of bovine *tuberculosis* in cattle slaughtered at Sokoto Central Abattoir

Breed	N = 194	Number of animals positive	Percentage (%)
White Fulani	16	1	6.25
Sokoto Gudali	53	4	7.55
Azawak	99	3	3.03
Cross breed	26	0	0.00
Total	194	8	4.12

$\chi_2 = 2.37$ P-value = 0.75 $P<0.05$

No significant statistical association ($P>0.05$) was found to exist between the infection and breed of the slaughtered animals.

Discussion

From the results of the study, 8 positive samples were identified in the 194 slaughtered cattle screened giving rise to a prevalence rate of 4.12% of bovine *tuberculosis* in cattle slaughtered at the Sokoto Central abattoir. No post mortem gross lesions of bovine *tuberculosis* were observed during the course of this study.

The prevalence rate of bovine *tuberculosis* as revealed by this study was higher than the 0.49%, 0.69% and 0.5% reported by Dusai and Abdullahi (1994); Ajogi et al. (1995) and Sonfada and Garba (2000) respectively. This may be due to the fact that the studies conducted by the aforementioned researchers were based on identification of the post mortem lesions of the disease in the slaughtered cattle which were confirmed by acid fast staining technique. This, as depicted in this study and as reported by Danbirni et al. (2009), is less sensitive in diagnosing bovine *tuberculosis*. This reason also agreed with the previous report of the National Research Council (1994) that bovine *tuberculosis* is only seen in chronic stage of the infection. The high sensitivity of the immunochromatography is attributed to the fact that it has been designed to detect IgM antibodies (which are the first set of antibodies produced following an infection).

The difference in the sensitivity of the rapid test employed in this study when compared to post mortem inspection for lesions of bovine *tuberculosis* is in total agreement with the findings of Danbirni et al. (2009) who also found no post mortem lesions in cattle that tested positive. This is because post mortem lesions of the disease are only seen at chronic stage.

Age distribution, according to this study, indicates that cattle between the ages of 2 to 4 years had the highest prevalence (7.90%), and followed by those between 6 to 10 years (4.26%). Cattle between the ages of 6 and 8 had the least prevalence of bovine *tuberculosis* (2.78%); this is contrary to the findings of Sonfada and Garba (2000) who reported higher prevalence among older animals (>6 years). The ability of the test kit to detect infections at any age could be the reason for higher prevalence among the cattle between ages 2 and 6 when

compared to the older animals.

A higher prevalence of the disease was recorded among the female animals (6.93%) than the males (1.08%). This could be due to the fact that female animals stay more in the herd for reproduction than the male animals (Sonfada and Garba, 2000). The Sokoto Gudali breed of cattle had the highest prevalence rate of bovine *tuberculosis* (7.55%) followed by White Fulani (6.25%), while the Azawak had the least prevalence rate (3.03%). Sokoto Gudali and Azawak are both animals reared for beef production (as this breed are mostly seen in the abattoir for slaughter) and this could explain why they had higher prevalence rate than the White Fulani which is more of a dairy breed and are rarely found in slaughter house. The control and elimination of bovine *tuberculosis* would only be achieved if strict control measures are adopted for both the animal and human population. Accurate ante mortem diagnosis, which is a pre-requisite to an effective control program using serological methods coupled with other control measures such as public enlightenment, mandatory pasteurization of milk before sale to the public and trace back of infected carcass to the herd with subsequent test and slaughter with full compensation, together with the control measures already in place in the human population would help greatly in reducing the incidence of bovine *tuberculosis*.

The concern for this disease in Sokoto is because the inhabitants are highly dependent on livestock as a source of income and consume milk without pasteurizing.

In conclusion, it is very clear that the results of this study suggest the continuous existence of bovine *tuberculosis* in the study area. It is also suggestive that since no post mortem lesions of bovine *tuberculosis* were observed in serologically positive animals, there is a likelihood that infected animals in the past have been slaughtered and consumed undetected of the disease.

Recommendation

National screening of herds of cattle for the disease with the imposition/strict compliance with the test and slaughter policy.

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SEROLOGICAL AND VIROLOGICAL STUDY OF NEWCASTLE DISEASE AND AVIAN INFLUENZA IN CHICKENS IN RURAL AREAS IN SENEGAL

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Abstract

Serological survey on the prevalence of Newcastle disease (NCD) virus antibodies using *haemagglutination inhibition* test (HI) and virological detection by RT-PCR of highly pathogenic *avian influenza* (HPAI) H5N1, were carried out in 6 regions of Senegal from June to November 2008. Rural chickens were raised in free ranging traditional and backyard systems. A total of 668 birds were sampled and 150 cloacae swabs from wild birds were used for the study. Data on medical care (treatments) and hygiene were collected using a questionnaire. The overall seroprevalence of NCD virus antibodies was 54.4% in these rural chickens but the prevalence of protective Newcastle disease virus antibodies in vaccinated chickens was 70.6% and in unvaccinated chickens 43.8%. These results suggest that for the “vaccinated chickens” group, the vaccination was not well carried out, furthermore the results also imply the possibility of circulation of a wild strain of NCD virus. There was a significant difference in prevalence between regions and the use of antibiotics for vaccinated and unvaccinated chickens. Multiple correspondence analysis classified regions in four sectors in which emphasis has to be laid on vaccination or training. To further understand the epidemiology of NCD in rural chickens in Senegal, characterisation of isolates and risk factor studies should be conducted. No HPAI H5N1 virus was detected in our study. This confirms the negative status of Senegal for HPAI H5N1. Guinea fowls, Ducks, Turkeys and chickens were positive to the HI test.

Keywords: Prevalence, Newcastle disease, *Avian Influenza*, Rural chickens, Senegal.

ETUDE SÉROLOGIQUE ET VIROLOGIQUE DE LA MALADIE DE NEWCASTLE ET DE L'INFLUENZA AVIAIRE CHEZ LES POULETS DES ZONES RURALES AU SÉNÉGAL

Résumé

Une enquête sérologique sur la prévalence des anticorps du virus de la maladie de Newcastle (MN) utilisant le test d'inhibition de l'hémagglutination (IH) et la détection virologique par RT-PCR de l'influenza aviaire hautement pathogène (IAHP) H5N1 a été réalisée dans 6 régions du Sénégal, de juin à novembre 2008. Elle a porté sur des poulets ruraux élevés en systèmes traditionnels de libre parcours et de basse-cour. Au total, 668 oiseaux ont été échantillonnés et 150 prélèvements cloacaux des oiseaux sauvages ont été utilisés pour l'étude. Les données sur les soins médicaux (traitements) et l'hygiène ont été recueillies au moyen d'un questionnaire. La séroprévalence globale des anticorps du virus de la MN était de 54,4% chez ces poulets ruraux, mais la prévalence des anticorps viraux protectifs contre la maladie de Newcastle était de 70,6% et 43,8% respectivement chez les poulets vaccinés et les poulets non vaccinés. Ces résultats laissent entendre que pour le groupe de « poulets vaccinés », la vaccination n'a pas été bien menée, en outre les résultats impliquent la possibilité de circulation d'une souche sauvage du virus de la MN. Une différence significative a été notée au niveau de la prévalence entre les régions et de l'utilisation des antibiotiques chez les poulets vaccinés et non vaccinés. L'analyse des correspondances multiples a classé les régions en quatre secteurs dans lesquels l'accent doit être mis sur la vaccination ou la formation. Pour mieux comprendre l'épidémiologie de la MN chez les poulets en milieu rural au Sénégal, il faudrait

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procéder à une caractérisation des isolats et mener des études sur les facteurs de risque. Aucun virus de l'IAHP H5N1 n'a été détecté dans notre étude. Ceci confirme le statut négatif du Sénégal pour l'IAHP H5N1. Les pintades, canards, dindes et poulets étaient positifs au test IH.

Mots-clés : Prévalence ; maladie de Newcastle ; Influenza aviaire ; poulets des zones rurales ; Sénégal

Introduction

Indigenous chicken make up 80% of total chicken population in Senegal (Traoré, 2006). Indigenous rural chickens are either raised under free ranging or backyard extensive production. In fact, we consider the latter and the former (traditional and backyard) as two separate systems of production in rural areas. The traditional system is characterised by free ranging animals with rudimentary or no housing at all and without medical care, whereas in the backyard system the farmers (women) are better organised (trained as village vaccinators) there is vaccination against Newcastle, there is medical care and diet supplements and above all, the existence of more modern hen housing. These units also possess rapid growth mixed breed cocks. The industrial system is mainly focused around the Dakar region and is estimated at 12 million chicken population. Ninety seven (97%) percent of the latter are made up of imported eggs from Brazil and the remaining 3% from parent breeders in Dakar. Eighty percent of this intensive and modern system is practiced around the Dakar region. Indigenous village chicken plays an important role in the rural economy. It constitutes an important source of income and high quality animal protein in the rural socio-economy with little capital investment. However, the emergence and transmission of infectious diseases such as *avian influenza* and Newcastle disease pose a huge threat to indigenous chicken production.

Newcastle disease (NCD) is caused by an avian paramyxovirus (APMV-1 serotype) that belongs to the genus *Avulavirus*. NCD is endemic and was found to be the most important disease of village poultry in Senegal (Gueye, 1998). It is associated with a high mortality leading to heavy economic loss for the rural population especially in the months of February and May (Arbelot *et al.*, 1997).

Avian Influenza virus is classified as a

member of the Orthomyxoviridae family. To date, all outbreaks of the highly pathogenic form have been caused by influenza A virus of the H5 and H7 subtypes. Before 2003, outbreaks of this virus in poultry seldom occurred (Werner & Harder, 2006) and were reported only in Europe and America (Kalthoff *et al.*, 2010).

The first confirmed outbreak of H5N1 HPAI on the African continent occurred in January, 2006, at a commercial farm in Kaduna State, Nigeria (Saidu *et al.*, 2008). H5N1 HPAI then spread to 6 countries of the West African sub-region; Niger and Cameroon in February, 2006, Burkina Faso and Ivory Coast in March, 2006, Ghana in April, 2007 and Togo in June, 2007. The last outbreaks ended in July and September, 2008 in Nigeria and Togo respectively.

A strong suspicion of commercial farms, a weakness in biosafety and biosecurity both in the rural and/or urban poultry management and in the distribution chain, and the porous nature of the borders seem to be the main factors which contribute to the spread of this disease in this part of the continent.

This study was designed to determine the serological status of NCD virus infections among village chickens in Senegal. In addition, the presence of H5N1 HPAI virus in village chickens and wild birds was detected in order to assess their role in the epidemiology of both diseases in village poultry.

Materials and Methods

Study area

The study was carried out in 6 regions of Senegal (Dakar, Saint Louis, Kaolack, Thies, Kolda, Louga) from June to November 2008. Fresh faecal swab samples were also collected from wild birds in the Djoudj national park.

In each region, the villages and the poultry owners were randomly selected.

Two different types of samples were collected from each chicken, namely blood

samples and tracheal swabs.

Animal management

The traditional system characterised by free range where birds scavenge in the backyard, was used in all the regions. Certain investments in chicken housing were made in the Dakar region or Thies (backyard system). Many avian and non-avian species live in these backyards, with no hygiene and a handful of grains are generally distributed periodically. In some villages of Dakar (region), Thies and Saint-Louis, there was more veterinary care and vaccination against NCD than in other areas (Gueye, 1998).

Questionnaire survey

A small questionnaire was used to obtain information from poultry owners about frequent clinical signs observed, mortality, backyard cleaning, treatment and vaccination against NCD during sample collection.

Collection of serum, swabs and laboratory analysis

Blood samples and tracheal swabs were collected in 668 avian specimens with 638 chickens (vaccinated and unvaccinated against NCD), 15 ducks, 3 pigeons, 3 turkeys and 9 guinea fowls. All the birds were bled via the wing vein into 5 ml sterile tubes. Sera were separated after centrifugation at 3000 t/min for 15 min, then transferred into eppendorf tubes, which were labelled and stored at -20°C until they were used for testing.

Faecal swabs were collected from fresh faeces in 150 wild birds (Spur-winged goose: *Plectropterus gambiensis*; Caspian tern: *Hydroprogne caspia*; Gull-billed Tern: *Gelochelidon nilotica*; Collared pratincole: *Glareola pratincola*) in the Djoudj park.

Newcastle *Haemagglutination inhibition* (HI) assay: HI was done according to CNEVA (Centre National d'Etudes Vétérinaires et Alimentaires, France) technique (PICAUT *et al.*, 1993) in U bottomed-shaped microtitre plates. Newcastle disease vaccine HBI strain obtained from CEVAC vaccine (CEVAC UNI L®: live freeze-dried HBI vaccine; batch 01025DIJ exp: 02-2010; CEVA Santé Animale) was used as antigen after reconstitution. After a two fold serial dilution of test serum,

4 haemagglutinating units (HA) of Newcastle disease virus were added to each well and kept at room temperature for 25-30 minutes. A 1% chicken RBCs (red blood cells) was added to each well and gently mixed and left to settle for about 45 minutes at room temperature. The titre was considered as the highest dilution of serum that caused complete inhibition of 4 HA of antigen. A titre greater than or equal to 1.16 was considered as positive. Furthermore, titres of value 1024 and above could denote a double contact between wild and vaccine viruses. PCR analysis was performed to detect *avian influenza virus H5N1 HPAI (M gene)* in 668 tracheal swabs of domestic birds and 150 faecal swabs of wild birds.

RNA isolation: RNA was isolated from Tracheal and cloacae swabs using commercial RNeasy Mini Kit (Qiagen®, USA) as recommended by the supplier. Total nucleic acid was quantified using a spectrometer at 260 nm wave length. RNA extract were kept at 4°C until used for PCR amplification.

Reverse transcription (RT): One step RT-PCR kit (Qiagen®, USA) was synthesized using the One Step RT-PCR enzyme mix containing Qiagen omniscrypt, Reverse transcriptase, sensiscrypt reverse transcriptase and Hotstar Taq DNA polymerase in a total volume of 50 µl for 30 min at 50°C.

Primers: A set of primers: IA/M1 (5' - AGC GTA GAC GCT TGT C - 3') and IA/M2 (5' - GAC GAT CAA GAA TCC AC - 3') that flanks the region encompassing the cleavage site of the fusion protein gene (M) was used in the study. The expected size of PCR product was 601 bp.

Polymerase chain reaction (PCR): PCR was carried out in a total volume of 50 µl containing 4 µl of the total RNA, 10 µl of 1x PCR buffer Qiagen, 2 µl of 0.1 M DTT, 2 µl of 10mM dNTP, 2 µl One step Qiagen enzyme mix, 0.6µM of primers IA/M1 and IA/M2, 29 µl of RNase free water. The thermocycler conditions were as follows: 15 min at 95°C (initial denaturation), followed by 40 cycles of 1 mn at 94°C (denaturation), 1 mn at 53°C (annealing), 1 mn at 72°C (elongation). The PCR ended with a final elongation for 7 min at 72°C.

Detection of PCR products: PCR products were separated in 1.5% agarose gel in 1x TAE buffer stained with ethidium bromide, compared with molecular mass marker and visualized by ultraviolet (UV) transillumination.

Statistical analysis

Data collected were statistically analyzed using chi-square test ($p < 0.05$) to determine the association between explanatory variables, seropositivity and vaccination status respectively. Statistical analyses were performed using R.2.9.2. A Multiple Correspondence Analysis (MCA) was used to describe the relative position of regions and some important variables such as vaccination, mortality and treatment using the questionnaire data (FactomineR®, Husson et al., 2009).

Results

Newcastle disease

Prevalence and HI titre

Prevalence of antibodies to Newcastle disease by species

The Chi-square test for antibody seropositivity by species indicated that there was a significant difference between species ($p=0.0026$). All the samples from turkeys ($n=3$) and guinea fowls ($n=9$) were positive as shown in table 1. Chickens ($n=638$) and domestic duck ($n=15$) had 54.38% and 33.33% of positives respectively. No pigeon tested positive. Only chickens were vaccinated.

With the low number of samples for other species, all analyses that follow will be based only on chickens.

Prevalence Newcastle disease antibodies virus by region

The overall seropositivity of NCD virus antibodies was 54.4% (CI: 49.6- 57.4). Comparisons between Dakar and other regions indicated that Dakar's positivity is significantly higher than that of Kolda, Louga ($p < 0.001$), and Kaolack, Saint-Louis ($p < 0.01$). No statistically significant difference was observed between Dakar and Thies regions (Table 2).

Prevalence of Newcastle disease virus antibodies in vaccinated and unvaccinated chickens

The antibody seroprevalence with vaccination effect is shown in table 3. Overall, the seroprevalence was 70.6% (CI 95%: 0.6452- 0.7609) in vaccinated birds and 43.8% (CI 95%: 0.3879- 0.4889) in unvaccinated chickens. There was a significant difference between vaccinated and unvaccinated chickens ($p < 0.001$). Almost 30% of vaccinated chickens tested negative to HI.

The percentage of vaccinated chickens with HI ranged between 0% (Saint Louis) and 79.5% (Dakar) as shown in Table 3. The difference was highly significant ($p < 0.001$). No vaccination was used in selected backyard farms in Saint Louis. For unvaccinated chickens, the percentage ranged from 22% (Kolda) to 71% (Thies).

For both vaccinated and unvaccinated groups, the percentage of chickens which have a positive serology is statistically lower in farms which use antibiotics than those which do not use them.

No statistical differences were observed between the presence of other birds, the mortality, the backyard cleaning, the clinical signs (respiratory, diarrhoea, nervous) for vaccinated or unvaccinated chickens. Altogether, in both the vaccinated and unvaccinated groups, farmers clean up their hen houses.

Distribution of Newcastle antibodies HI titre classes by region

The distribution of NCD virus antibody titre according to region is shown in table 4. There were 220 chickens out of 638 (34.5%) with no NCD antibodies in these regions and a quarter of these chickens come from Kolda region.

Higher antibody titre have been observed in 12% (78/638) of the chickens and half come from the region of Thies.

Distribution of Newcastle antibodies titre by vaccination status

The distribution of Newcastle virus antibodies titre for vaccinated and unvaccinated chickens is shown in figure 1. The number of unvaccinated subjects is greater than that of the vaccinated subjects, with HI negative antibody titre ≤ 16 and HI positive antibody titre ≥ 512 .

Multiple Correspondance Analysis (MCA)

The first two factorial axes explain 77% of the total variability of the data. Figure 2 shows that with respect to vaccination, treatment and mortality, we can classify the regions into four different groups:

The first one: High vaccination, Low mortality and low treatment: Dakar

The second: No vaccination, Low mortality and low treatment: Kolda and Saint-Louis

The third: Weak vaccination, High Mortality and High treatment: Kaolack and Louga

The fourth: No vaccination, High Mortality and Weak treatment: Thies

Avian Influenza

All the 668 tracheal swabs from domestic poultry and 150 faecal swabs from wild birds tested negative to gene M of *avian influenza virus H5N1 HPAI* by RT-PCR.

Discussion

The main objective of this study was to determine the seropositivity of the Newcastle disease in traditional poultry and wild birds, which is the main cause of death of these birds and to detect the presence of influenza virus as

well.

The study contains some bias but since birds were randomly sampled, the questionnaire was short and precise, and the laboratory analyses were based on standard methods, we therefore consider the results to be valid.

Serological prevalence of Newcastle in traditional chickens, in our study, was 54.4% and was lower than that obtained by Arbelot et al. (1997), in Dakar and Thies regions (with prevalence 84% in the rainy season and 98% in dry season). However, our NCD seroprevalence estimate of traditional poultry is higher than that recorded in Niger (14%, Courtecuisse et al., 1990), Mauritania (5%, Bell et al., 1990), Zambia (36.9%, Alders et al., 1994), Mali (32.9%, Sylla et al., 2003), and Madagascar (21%, Maminiana et al., 2007).

The prevalence of Newcastle disease virus antibody titre (≥ 16) was 70.6% in vaccinated chickens and 43.8% in unvaccinated chickens. This means that about a quarter of the vaccinated chickens and half of unvaccinated chickens were likely susceptible to the disease. This high prevalence of vaccinated chickens (70% have protective antibodies) is quite important, but some antibody titre higher than 1024 were observed in Saint Louis and Thies, suggesting the possible circulation of wild strains of Newcastle disease in chickens as

Table 1: Newcastle disease prevalence by species, Senegal, 2008

Species	Number of animals	Number of positive/total	Positives (%)
Ducks	15	5/15	33.33
Turkeys	3	3/3	100
Pigeons	3	0/3	0
Chickens	638	347/638	54.38
Guinea fowls	9	9/9	100

Table 2: Overall prevalence of Newcastle disease virus antibodies, Senegal, 2008

Regions	Number of chickens	Positivity (%)	Confidence interval (95%)
Dakar	104	74	64.35-81.91
Kaolack	106	51.9	42.01-61.61
Kolda	121	29.8	21.96-38.84
Louga	110	50.9	41.25-60.49
Saint-Louis	93	51.6	41.07-62.01
Thies	104	72.1	62.32-80.24
TOTAL	638	54.4	49.6 – 57.4

Table 3: Newcastle disease virus HI antibodies in chickens in Senegal, 2008

		Vaccinated (n=252)		Unvaccinated (n=386)	
		HI negative (%)	HI positive (%)	HI negative (%)	HI positive (%)
Region	Dakar	18/88 (20.5)	70/88 (79.5)	9/16 (56.2)	7/16 (43.8)
	Kaolack	20/62 (32.3)	42/62 (67.7)	31/44 (70.5)	13/44 (29.5)
	Kolda	17/34 (50.0)	17/34 (50.0)	68/87 (78.2)	19/87 (21.8)
	Louga	14/46 (30.4)	32/46 (69.6)	40/64 (62.5)	24/64 (37.5)
	Saint Louis	0/0	0/0	45/93 (48.4)	48/93 (51.6)
	Thies	5/22 (22.7)	17/22 (77.3)	24/82 (29.3)	58/82 (70.7)
Respiratory signs	No	21/73 (28.8)	52/73 (71.2)	60/97 (61.9)	37/97 (38.1)
	yes	53/179 (29.6)	126/179 (70.4)	157/289 (54.3)	132/289 (45.7)
Diarrhoea	No	20/68 (29.4)	48/68 (70.6)	19/50 (38.0)	31/50 (62.0)
	yes	54/184 (29.3)	130/184 (70.7)	198/336 (58.9)	138/336 (41.1)
Nervous signs	No	31/115 (27.0)	84/115 (73.0)	54/124 (43.5)	70/124 (56.5)
	yes	43/137 (31.4)	94/137 (68.6)	163/262 (62.2)	99/262 (37.8)
Mortality	No	13/47 (27.7)	34/47 (72.3)	48/78 (61.5)	30/78 (38.5)
	yes	61/205 (29.8)	144/205 (70.2)	169/308 (54.9)	139/308 (45.1)
Backyard cleaning	No	4/21 (19.0)	17/21 (81.0)	25/41 (61.0)	16/41 (39.0)
	yes	70/231 (30.3)	161/231 (69.7)	192/345 (55.7)	153/345 (44.3)
Antibiotics treatment	No	56/166 (33.7)	110/166 (66.3)	136/263 (51.7)	127/263 (48.3)
	Yes	18/86 (20.9)	68/86 (79.1)	81/123 (65.9)	42/123 (34.1)
Other bird species	No	60/186 (32.3)	126/186 (67.3)	132/261 (50.6)	129/261 (49.4)
	yes	14/66 (21.2)	52/66 (78.8)	85/125 (68.0)	40/125 (32.0)
Total		74/252 (29.4)	178/252 (70.6)	217/386 (56.2)	169/386 (43.8)

Table 4: Distribution of Newcastle antibody HI titres by region, Senegal, 2008

Regions	Antibodies HI titre		
	0-8	16-512	≥ 1024
	Negative	Positive	Strongly positive
Dakar	27	69	8
Kaolack	51	47	8
Kolda	85	29	7
Louga	54	55	1
Saint Louis	45	26	22
Thies	29	43	32

indicated by Arbelot et al. (1997).

A suggested hypothesis is that this high titre could be due to chickens which might have survived former Newcastle outbreaks, where there is often a constant transmission of the virus in the backyard via other avian species such as pigeons, guinea fowls and ducks. In our study, the sampled turkeys, guinea fowls, and

ducks, tested positive. These species can serve as a reservoir and could play a role in spreading the disease (Weingartl et al., 2003). However, our results indicated that the presence of other avian species, do not appear to influence the seroprevalence of Newcastle disease antibodies. Similarly, clinical signs (diarrhoea, respiratory and nervous) observed by farmers

could denote the circulation of mesogenic and velogenic viral strains in this production system (Beard and Hansson, 1981). It will be necessary to study Newcastle viral strains circulating in Senegal, and also observe the likely causes of vaccination failures.

Backyard cleaning seemed to be more frequent with farmers who vaccinated, unlike those with unvaccinated chickens and the difference was statistically significant. This can be easily understood because farmers who

vaccinate their animals have a greater tendency to implement good hygiene and veterinary care (prevention and treatment).

Our data suggests that the proximity to large urban areas such as Dakar and Thies offers easier access to the supply of veterinary drugs. This would therefore be an important factor to reckon with, given the peri-urban situation of the farms around these areas (more frequent urban markets, proximity to large villages with birds and genetic improvement by the

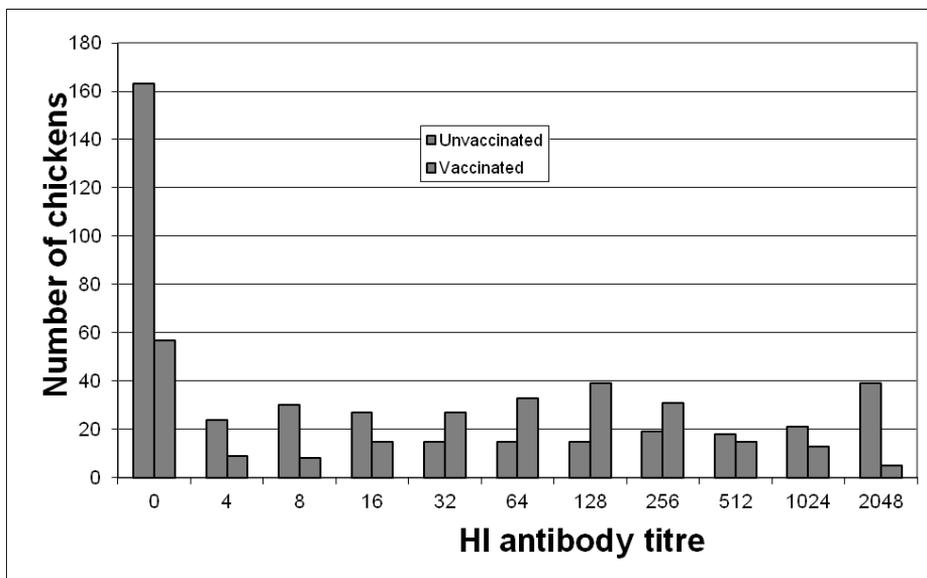


Figure 1: Distribution of Newcastle antibodies titre in vaccinated and unvaccinated chickens, Senegal, 2008.

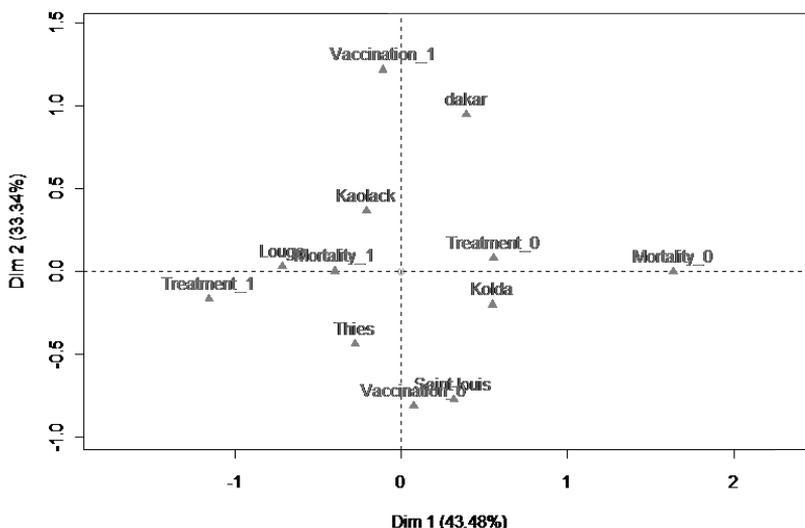


Figure 2: Composition of the principal components (Dim. 1, Dim. 2) and projection of the study sites on the principal components analysis plan.

introduction of cocks). In these two regions, the traditional system was replaced by backyard system where women do get implicated as compared to Kolda, Saint Louis, Louga regions. The income generated by this speculation helps provide for children's education and their personal needs (Gueye, 1998).

Multiple correspondence analysis summarizes our results by classifying the regions into four sectors, with major factors being vaccination, mortality and antibiotic treatment used. According to these sectors, emphasis should be made on vaccination and/or training of farmers. Vaccination and primary hygiene measures should be emphasized upon in other regions through sensitisation of the population, namely in Saint Louis.

Further investigative studies might be done on the phylogenetic strains, which circulated in Senegal and the risk factors associated with Newcastle disease annual outbreaks in rural chickens, in order to build a prediction model for the spread of the disease.

In the sampled regions, there is no trace of the H5N1 HP virus in village birds, as well as in wild birds from the Djoudj Park. These results confirm the negative status of Senegal, to the presence of H5N1 HP virus.

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PARTICIPATORY DISEASE SURVEILLANCE IN THE DETECTION OF TRANS-BOUNDARY ANIMAL DISEASES (TADS) IN BORNO STATE OF ARID NORTH-EASTERN NIGERIA

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Abstract

This paper reports the detection of trans-boundary animal diseases using participatory disease surveillance in Borno State. Participatory epidemiology is an emerging field that is based on the use of participatory techniques for harvesting qualitative epidemiological intelligence contained within community observations, existing veterinary knowledge and traditional oral history. Different techniques of participatory disease surveillance used for this study includes simple ranking, pair wise ranking, proportional piling, transect walk and interview with key informants with the use of check list as a guide were applied in randomly selected 35 villages/communities cutting across all the 3 senatorial districts in Borno State. Analysis of data indicated that the most dominant livestock species in the study area is cattle. The various livestock diseases occurring in the communities visited were identified. The most prevalent disease of cattle is foot and mouth disease (FMD), peste des petits ruminants (PPR) in sheep and goats and Newcastle disease (ND) in poultry. The transboundary animal diseases detected in the study area were FMD, contagious bovine pleuropneumonia (CBPP), bovine tuberculosis, anthrax, PPR and ND. The use of participatory disease surveillance proved to be a useful tool for detecting disease problems from farmers.

Keywords: Participatory disease surveillance, Trans-boundary animal diseases, Borno State, Nigeria.

SURVEILLANCE PARTICIPATIVE DANS LA DETECTION DES MALADIES ANIMALES TRANSFRONTALIERES (MAT) DANS L'ETAT DE BORNO DANS LE NORD-EST ARIDE DU NIGERIA

Résumé

Cet article porte sur la détection des maladies animales transfrontalières en utilisant la surveillance participative des maladies dans l'État de Borno. L'épidémiologie participative est un domaine nouveau, basé sur l'utilisation de techniques participatives pour le recueil de données épidémiologiques qualitatives contenues dans les observations de la communauté, les connaissances vétérinaires existantes et l'histoire orale traditionnelle. Différentes techniques de surveillance participative de maladies utilisées dans cette étude, notamment le classement simple, le classement par paires, la répartition proportionnelle, le transect d'étude, et l'entretien avec des informateurs-clés avec l'utilisation de la liste de contrôle comme guide, ont été appliquées dans 35 villages / communautés choisies de manière aléatoire, à travers toutes les 3 circonscriptions sénatoriales dans l'État de Borno. L'analyse des données a révélé que l'espèce animale la plus dominante dans la zone d'étude était l'espèce bovine. Les différentes maladies animales présentes dans les communautés visitées ont été identifiées.

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Les maladies les plus répandues sont la fièvre aphteuse (FA) pour le bétail, la peste des petits ruminants (PPR) pour les ovins et les caprins et la maladie de Newcastle (MN) pour les volailles. Les maladies animales transfrontières détectées dans la zone d'étude étaient la fièvre aphteuse, la péripneumonie contagieuse bovine (PPCB), la tuberculose bovine, la fièvre charbonneuse, la PPR et la MN. L'utilisation de la surveillance participative des maladies s'est avérée être un outil utile pour les agriculteurs dans la détection des problèmes de maladies.

Mots-clés : Surveillance participative des maladies ; Maladies animales transfrontalières ; État de Borno ; Nigeria.

Introduction

Participatory epidemiology (PE) is the use of participatory approaches and methods to improve our understanding of the patterns of diseases in populations. These approaches and methods are derived from participatory appraisal. The empowerment of people to find solutions to their own development challenges is called Participation (ILRI, 2010). Participatory appraisal (PA) is a family of approaches and methods that enable people to present, share and analyse their knowledge of life and conditions, to plan and to act. It is participatory, flexible, lightly structured, adaptable, exploratory, empowering and inventive. Types of participatory appraisal include rapid rural appraisal, participatory rural appraisal (PRA), farming systems research and participatory impact assessment (ILRI, 2010).

Participatory Disease Surveillance (PDS) is the application of PE to disease surveillance. PDS is a method of disease surveillance where PA approaches and methods are used to combine local veterinary knowledge with conventional methods to establish the presence or absence of a specific disease in a particular area (ILRI, 2010). PE is based on communication and transfer of knowledge, using a variety of methods. There are three main groups of methods firstly informal interviewing including semi-structured interviews with key informants, focus-group discussions or individual livestock keepers. Secondly, ranking and scoring which include simple ranking, pair-wise ranking, proportional piling and matrix scoring. Visualization aspect which included mapping, timelines, seasonal calendars and transect walks was finally applied. These are complemented by secondary information sources which are obtained before going to the study area and as the study is

being conducted. Secondary information sources involve direct observation of animals, farms and villages. Furthermore, the use of area Laboratory diagnostics if available, field diagnostic tests and testing by a regional and/or national laboratory for confirmation. Data are cross checked by probing, triangulation and laboratory diagnostics (Pretty *et al.*, 1995; Mariner and Paskin, 2000; Catley, 2005). Participatory Epidemiology relies on the widely accepted techniques of participatory rural appraisal, ethno-veterinary surveys and qualitative epidemiology (Schwabe, 1984). This information can be used to design better animal health projects and delivery systems, more successful surveillance and control strategies or as new perspectives for innovative research hypotheses in ecological epidemiology (Hussain *et al.*, 2005).

Trans-boundary animal diseases (TADs) are disease of significant economic, trade and/or food security importance for a considerable number of countries, which can easily spread to other countries and reach epidemic proportions and where control/management, including eradication requires cooperation between several countries (FAO, 2004). Common TADs include Contagious Bovine Pleuropneumonia (CBPP), Rabies, Foot and Mouth Disease (FMD), Contagious Caprine Pleuropneumonia (CCPP). Others include Newcastle Disease (ND), Lumpy Skin Disease (LSD), and African Swine Fever (ASF).

Critical reviews of participatory approaches to development by the World Bank (1996) and Aycrigg (1998), Canadian International Development Agency (1997) and United States Agency for International Development (1999) concluded that participatory methods in programme planning, design and delivery yielded the greatest degree of success and sustainability. The failure of

formal data collection methods to produce cost-effective and reliable information for designing rural development projects in developing countries was first recognized in the early 1970s (Chambers, 1983). This problem was related to the behaviour and methods of researchers which limited their capacity to understand the problems of the poorest and most marginalized people in rural communities.

Therefore, this work was carried out using a PDS method to detect the common diseases of livestock with emphasis on trans-boundary animal diseases in Borno State.

Materials and Methods

Study area

Borno State lies between latitude $11^{\circ} 32'$ North and $11^{\circ} 40'$ North and longitude $13^{\circ} 20'$ East and $13^{\circ} 25'$ East; and located in the arid between the Sudan savannah and Sahel savannah vegetation zones, characterized by short rainy season of 3-4 months (June-September) followed by a prolonged dry season of more than 8 months duration (Ibrahim *et al.*, 2006). The state shares international borders with Cameroon, Chad and Niger republics and has Fulfulde, Kanuri and Hausa as the common local languages. The arid zone has rather austere climate conditions with a hot dry season from late January to late June during which average daily peak temperatures especially in April and May, are 34.4 to 37.8°C . The rainy season lasts from late June to mid September and provides an annual average of 46.3 cm rainfall. The cold north-easterly trade wind blowing across the Sahara desert in October to January brings with it cold and desiccant effects on the environment. Thus, nomadic herdsmen who own most of the livestock in the state are constantly on the move in most parts of the year in search of lush grazing ground and water (Geidam *et al.*, 2009). Fatigue imposed on already malnourished animals as a result of movement over long distances, and excessive heat load which is not easily shed, subject animals to excessive stress, which in turn makes them succumb easily to infection by various agents (Brisibe *et al.*, 1996).

Participatory Disease Surveillance (PDS) Team

The PDS team that carried out this

study comprised of 7 Veterinarians, 2 of whom are researchers from the Faculty of Veterinary Medicine, University of Maiduguri, 2 field epidemiologists from the Federal Livestock Department, 1 Desk Officer, Avian Influenza Control Programme and the sanitary mandate officers for Northern and Southern parts of Borno State. Each team member is fluent in at least two of the common local languages (Hausa, Kanuri and Fulfulde).

Data collection

The data generated from this study were collected from 35 villages/communities cutting across all the 3 Senatorial districts within the study area (Fig. 1). The exercise was conducted from 15th October, 2010 to 23rd November, 2010. Before each visitation, prior arrangements were made with each village/community leader for convenience of the farmers in terms of suitability of time and venue of meeting to ensure maximum participation of all categories of farmers. Materials such as geographical positioning system (GPS, GARMIN's eTrex Legend personal navigator), cardboards, counters, permanent markers, digital camera and others were used for the study. The role of note taker, observer, tool applicator and the facilitator was assigned to team members on each visitation. Interviews were conducted using a checklist arranged in the following order; mutual introduction, identification of respondents, livestock species kept, sources of livestock, husbandry systems, problems/challenges, diseases, questions and advice. During interview sessions no mention of TADs was made to avoid bias. The information gathered was scored and ranked using simple ranking, proportional pilling and pair wise ranking. Transect walk was also used as a tool to assess the information gathered during interview.

Data analyses

The data generated in this study was analysed using simple ranking, proportional pilling and pair wise ranking as described in Manual for Participatory Disease Surveillance Practitioners (Anon, 2009).

Table 1: Communities/villages with their local government areas and GPS ordinates where participatory disease surveillance was carried out in Borno State, Nigeria

S/No	Village/Community	Local government area	Latitude N	Longitude E
1.	Wakane	Gwoza	11.08916	13.68624
2.	Ngole	Gwoza	11.02318	13.68624
3.	Rumirgo	Askira/Uba	10.60620	13.02792
4.	Giwi-Wamdeo	Askira/Uba	10.51362	13.11212
5.	Hausari ward	Askira/Uba	10.64759	12.90970
6.	Wamdoe	Askira/Uba	10.52317	13.13441
7.	chanchanbulkuma	Askira/Uba	10.40479	12.54525
8.	Uvu-kofansarki	Askira/Uba	10.49518	13.12118
9.	Abba Tilo	Askira/Uba	10.41807	12.25750
10.	HausariShuwari	Mongono	12.66790	13.60839
11.	Flatari-cross kukawa	Kukawa	12.94756	13.67223
12.	Limanti-cross kukawa	Kukawa	12.94483	13.67261
13.	Mile 90	Kukawa	12.77620	13.66408
14.	Sheep and goat Market	Kukawa	13.09882	13.82102
15.	Bulamari-cross kukawa	Kukawa	12.94456	13.67307
16.	BundirLawanti	Kukawa	12.89499	13.69597
17.	MadariLawanti	Kukawa	12.84785	13.66622
18.	Kekenolawanti	Kukawa	12.79244	13.67589
19.	Kafela	Mongono	12.67238	13.61123
20.	BulaburinBakasi	Mongono	12.66633	13.61012
21.	JegaramLawanti	Mongono	12.67222	13.611285
22.	GajiramLawanti	Nganze	12.49041	13.21545
23.	MainokLawanti	Kaga	11.49769	12.94506
24.	Mainok cattle market	Kaga	11.82784	12.62726
25.	Jakana-BulamaBukartima	Konduga	11.50546	12.46529
26.	Auno	Konduga	11.84580	12.93536
27.	PompomariShuwari IV	Konduga	11.86322	13.10411
28.	Lawanti ward	Dambo	11.09471	12.45261
29.	Nzuda- Malaudti	Dambo	11.06024	12.41876
30.	BulamariSandiya	Konduga	11.13545	12.48721
31.	NyaleriLawanti	Konduga	11.17689	12.51069
32.	BulabulinGaura	Konduga	11.23046	12.54623
33.	DalwaKasu	Konduga	11.39546	13.02043
34.	Gamborijimchilo- Auno	konduga	11.50679	13.02062
35.	KasuwanShanu- Custom	Maiduguri	11.51587	13.10638

Results

The 35 villages/communities and their GPS locations where data was collected are presented in Table 1. Out of these villages/

communities, 13 were from the northern senatorial district and 11 each were from the southern and central senatorial district. The ranking of animal population in Borno State showed cattle as the most populous livestock

species followed by sheep, goats, poultry, donkey, horses, dogs, camel and pig (Table 2).

Disease problems of cattle, their local names and ranking in the communities/villages investigated are presented in Table 3. FMD was ranked as the most common disease. This is followed by CBPP, babesiosis, trypanosomosis, helminthosis, fasciolosis, black quarter, bovine tuberculosis (TB), lumpy skin disease, anthrax, hemorrhagic septicaemia, cowdriosis, brucellosis, ectoparasitism, foot rot, pink eye disease and lastly aman (unidentified disease). The trans-boundary animal diseases of cattle identified based on their occurrence include FMD, CBPP, lumpy skin disease, TB and anthrax. The morbidity, mortality and case fatality rate for the most occurring disease in these

Table 2: Ranking of livestock species based on their population in Borno state, Nigeria

Animal Species	Score	Rank
Cattle	253	1
Sheep	249	2
Goats	238	3
Poultry	185	4
Donkey	96	5
Horses	65	6
Dogs	58	7
Camel	23	8
Pig	10	9

Table 3: Ranking of diseases of cattle, their local names and patterns in Borno state, Nigeria

S/ No.	Disease	Local names of diseases			Score	Rank	MBR (%)	MTR (%)	CFR (%)
		Fulfulde	Kanuri	Hausa					
1.	FMD	Mboru	Tilam		296	1	85	16	19
2.	CBPP		Nzasara	Huhu	221	2	64	46	72
3.	Babesiosis	1. Kahande 2. Tapalaure 3. Boldowol	Chimbrom	1. Shimirau 2. Madachi	186	3	32	9	28
4.	Trypanosomosis	1. Jola 2. Samore	Chiri Kθmatin	Masuwa	151	4	65	29	45
5.	Helminthosis	Balki		Chutan- kori Tsusan- chiki	134	5	-	-	-
6.	Fasciolosis	kaddiowa	Klena	Ciwon hanta	134	5	67	58	87
7.	Black quarter		Zano Atar	Harbin jeji karmamu	123	6	69	69	100
8.	TB			Tari	84	7	25	10	40
9.	Lumpy skin Disease		Namala Ngadan	Gaye	49	8	-	-	-
10.	Anthrax	Wutuduwol		Cefa	43	9	83	52	63
11.	Hemorrhagic septicemia	Bolle	Tsawu		33	10	-	-	-
12.	Cowdriosis		Daukθri	Chiwon- kunne	26	11	-	-	-
13.	Brucellosis	Bakale	kalewena		9	12	100	100	100
14.	Ectoparasites				9	12	-	-	-
15.	Foot rot	Laire			6	13	-	-	-
16.	Pink eye disease		Chimdām		6	13	-	-	-
17.	Unidentified disease		Aman		5	14	-	-	-

MBR= Morbidity Rate, MTR= Mortality Rate, CFR=Case Fatality Rate.

Table 4: Ranking of diseases of sheep and goats and their local names in Borno state, Nigeria

S/ No.	Disease	Local names of diseases			Score	Rank
		Fulfulde	Kanuri	Hausa		
1.	PPR	Nyilbe Ngishu Nyilbil	Kulungu Bema Namasun	Raba	283	1
2.	Foot rot	Katchigel Bunyi Ferore	Kolore Shireta	Zawo	187	2
3.	Mange	Demuwa	Man Kuskun Gurzole	-	147	3
4.	Babesiosis	-	Kōngirkime Chimbrom	Baru	117	4
5.	Orf	-	Ngodogodu Chifu Bunzin	Dumara	99	5
6.	Helminthosis	-	-	-	84	6
7.	CCPP	-	Nganji	Huhu	56	7
8.	Cowdriosis	Bugan Bangele	Losur	Lapsa	48	8
9.	Sheep pox	-	-	-	48	8
10.	Cysticercosis	Budi	Tunukalimbe		12	9
11.	Cutaneous larva migration	-	-	-	12	9
12.	Bloat	-	-	-	6	10

Table 5: Ranking of diseases of poultry and their local names in Borno state, Nigeria

S/No.	Disease	Local names of diseases			Score	Rank
		Fulfulde	Kanuri	Hausa		
1.	NCD	Nyangaurtode	Suum Chuim Kiladuri Miksiri	Zawonkaji Makeke Fakat	310	1
2.	Ectoparasitism	Girwudu	Kuma Kengisu	Girgiri	129	2
3.	Fowl pox	Upulgite	Tuno Tunum Klafu Birkōriye	Chiraki	111	3
4.	Coccidiosis		Yarta	Kashinjini	54	4
5.	Fowl cholera	Charon Iyan	-	-	40	5
6.	Gomboru	-	Britukwa		9	6
7.	Helminthosis	Bidaure	-	-	6	7

communities was also deduced (Table 3). A disease described by the farmers in some of the communities called aman in their local language characterized by body weakness, balanitis and death that usually occurs after drinking water

could not be diagnosed by the team.

The scoring and ranking of diseases of sheep and goats in the communities examined is presented in Table 4. Peste des petits ruminants (PPR) was ranked as the most

occurring disease with a morbidity rate of 73%, mortality rate of 62% and a case fatality rate of 84%. This is followed by foot rot, mange, babesiosis, orf, helminthosis, contagious caprine pleuropneumonia (CCPP), cowdriosis, sheep pox, cysticercosis, cutaneous larva migration and bloat respectively. PPR and CCPP have been identified as the transboundary animal diseases of sheep and goats in these communities.

Diseases of chickens including their scoring and ranking are presented in Table 5. Newcastle disease (ND) occurred more frequently in the study area with a morbidity and mortality rates of 79 and 72% respectively and a case fatality rate of 91%. This is followed by ectoparasitism, fowl pox, coccidiosis, fowl cholera, Gumboro disease and helminthosis (Table 5). ND is the TAD of poultry encountered in the study area.

Discussion

The present study utilizes participatory diseases surveillance in the detection of trans-boundary animal diseases in Borno State. Based on the study carried out, cattle were observed to be the most populous livestock species in the study area followed by sheep, goat and poultry. This finding did not tally with report of Bourn *et al.*, (1994) which reported that poultry outnumbered all other forms of livestock in Nigeria. However, in their report commercial poultry population which constitutes a substantial number in their survey is not commonly produced in this part of the country because of the unfavourable environmental conditions. In cattle FMD was observed to be the most commonly occurring disease, a finding that further consolidates earlier reports that FMD is endemic in sub-Saharan Africa and that widespread outbreaks of clinical disease occur during most years (Sahle *et al.*, 2004; Vosloo *et al.*, 2002). It similarly agrees with the findings of Hussain *et al.* (2005) who in a study conducted in Pakistan rated FMD as the most prevalent disease of cattle. Furthermore, Hussain *et al.* (2005) reported black quarter as one of the significant health hazard recorded in their study a finding that concurred with the observation of the present study in this region. The present study also identified babesiosis, trypanosomosis, fasciolosis, foot

rot, dermatophylosis, hemorrhagic septicaemia, ectoparasitism, cowdriosis, lumpy skin disease, helminthosis, brucellosis and pink eye disease as other diseases found in the area. This finding is inconformity with the report of Brisibe *et al.* (1996) that fatigue imposed on already malnourished animals as a result of movement over long distances, and excessive heat load which is not easily shed, subject animals to excessive stress, which in turn makes them succumb easily to infection by various agents.

PPR was detected to be the most common disease of sheep and goats in the study area, which is in conformity with earlier reports of El-yuguda *et al.* (2008) that showed high prevalence of 47.7% in the study area. Other diseases of sheep and goats detected included foot rot, orf, mange, cowdriosis, helminthosis, ectoparasitism, sheep pox, contagious caprine pleuropneumonia (CCPP), fasciolosis, babesiosis and cysticercosis. The most prevalent disease in poultry recorded during the course of this study was NCD; other diseases recorded were fowl pox, fowl cholera, coccidiosis, gumboro and ectoparasitism. This report was in agreement with the findings of Ndahi and Kwaghe (2011) which rated NCD as the most common disease of poultry following PDS in Plateau State.

The participatory disease surveillance carried out in 35 villages of Borno State detected 7 trans-boundary animal diseases including bovine *tuberculosis* (TB), contagious bovine pleuropneumonia (CBPP), foot and mouth disease (FMD), anthrax, peste des petites ruminants (PPR) and Newcastle disease (NCD). This high number of TADs could be attributed to the common international border being shared by the state. While herdsmen travel long distances in search for lush grazing grounds as reported by Geidam *et al.*, (2009) they tend to cross international borders into Cameroon, Chad or Niger Republics. This information can be used to design better animal health projects and delivery systems, more successful surveillance and control strategies or as new perspectives for innovative research hypotheses in ecological epidemiology as suggested by Hussain *et al.* (2005). The redundant animal diseases control posts should be activated to ensure active surveillance, vaccinations and

quarantine of animals moving in and out of the country.

Conclusion

This study has clearly shown that animal disease information can be generated when farmers are directly involved. The farmers were given chance to present, share and analyse their knowledge of life and conditions involving their animals. Discussing the local names and remedies to some of the problems give them the opportunity to clearly express themselves. The data gathered was analysed using the specified methods in the study and 7 trans-boundary animal diseases were detected including bovine *tuberculosis* (TB), contagious bovine pleuropneumonia (CBPP), foot and mouth disease (FMD), anthrax, peste des petites ruminants (PPR) and Newcastle disease (NCD) in Borno State.

Impact:

This paper describes how farmers and participatory methods of disease Surveillance can add value to national veterinary services by detecting disease problems of villages/ communities which of international importance. The paper suggests approaches to the integration of participatory information collection and community-based service delivery into conventional veterinary service systems. Community-based and participatory methods augment the capacities of the system, rather than replace conventional capabilities. The overall aim is to promote a more effective integrated method of surveillance for developing countries that will enhance the quality of data available for risk analysis and trade decision-making while providing better services to traditional farmers.

Estimation of Morbidity, Mortality and Case fatality rates: The morbidity, mortality and case fatality rates of the diseases in the villages/ communities was deduced using the methods described in the manual for participatory disease surveillance practitioners (Anonymous, 2009). It involved the use of counter (100 bean seeds) to represent the animal population, however, the number of bean seeds was not

known to the farmers. During interviews the bean seeds were placed for the farmers as their animal population to estimate a portion from the beans (animals) that show clinical signs of a particular disease during outbreak, that number out of the 100 beans represents the morbidity rate and expressed in percentage. Similarly mortality rate was estimated by a portion out of the 100 beans (animal population) that die of a particular disease to be presented as percentage. Case fatality rate was estimated by the portion from the animals with clinical signs of disease (out of 100 beans) that died of the disease also percentage as percentage. For all these rates average values for all the villages/ communities represent the rates for the state.

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THE LIVER MICROMORPHOLOGY OF THE AFRICAN PALM SQUIRREL EPIXERUS EBII.

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Abstract

The normal liver histology of the African palm squirrel *Epixerus ebii* was investigated to fill the information gap on its micromorphology from available literature. The liver was covered by a capsule of dense connective tissue- the Glissons membrane. Beneath this capsule is the liver parenchyma where the hepatocytes were supported by reticular fibres. The hepatocytes in the lobules were hexagonal to polygonal in shape. Some hepatocytes were bi-nucleated. Clear spaces in the parenchyma must be storage sites for lipids in the liver. The classic hepatic lobules presented central vein surrounded by several liver cells. At the portal triad, hepatic vein, hepatic arteries and bile ducts were seen. While the hepatic arteries and veins were lined by endothelium, the bile ducts were lined by simple cuboidal cells. Nerve fibres were also seen in the region of the portal triad. Hepatic sinusoids lined by endothelium were seen in the liver parenchyma between liver lobules. The sinusoids contained macrophages. This report will aid wild life biologist in further inversigative research and Veterinarians in diagnosing the hepatic diseases of the African palm squirrel.

Key Words: palm squirrel, Liver, histology, portal triad

MICROMORPHOLOGIE DU FOIE DE L'ECUREUIL DES PALMIERS AFRICAIN EPIXERUS EBII.

Résumé

L'histologie du foie normal de l'écureuil africain *Epixerus ebii* a été étudiée pour combler le déficit d'information sur sa micromorphologie dans la littérature disponible. Le foie a été recouvert par une capsule de tissu conjonctif dense - la membrane de Glissons. Sous cette capsule se trouve le parenchyme hépatique où les hépatocytes étaient soutenus par des fibres réticulaires. Les hépatocytes dans les lobules étaient de forme hexagonale ou polygonale. Certains hépatocytes étaient binucléées. Des espaces libres dans le parenchyme doivent être des sites de stockage des lipides dans le foie. Les lobules hépatiques classiques présentaient une veine centrale entourée de plusieurs cellules du foie. A la triade portale, une veine hépatique, des artères hépatiques et des voies biliaires ont été observés. Si les artères et les veines hépatiques étaient recouvertes par l'endothélium, les canaux biliaires étaient alignés par des cellules cubiques simples. Des fibres nerveuses ont également été observées dans la région de la triade portale. Des sinusoides hépatiques tapissées par l'endothélium ont été observées dans le parenchyme hépatique entre les lobules du foie. Les sinusoides contenaient des macrophages. Le présent rapport aidera le biologiste de la faune dans ses travaux de recherche analytique et les vétérinaires dans le diagnostic des maladies hépatiques de l'écureuil des palmiers africain.

Mots-clés : Ecureuil des palmiers ; Foie ; Histologie ; tirade portale

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Introduction

The liver is the largest internal organ (Akiyoshi and Inoue, 2004), containing as much as four to five lobes surrounded by capsule of connective tissue fibres referred to as Glissons capsule (Petcoff *et al.*, 2006). The liver parenchyma within the lobules is supported by fine reticular fibres. At the hilus, blood vessels, lymphatics and nerve enter and leave the liver. Within the parenchyma are sinusoids lined by macrophages called Kupfer cells (Lopez *et al.*, 2011; Carollo *et al.*, 2012). These sinusoids are usually in between the hepatic plates (Motta, 1984). The liver cells –hepatocytes are usually polygonal in shape and usually binucleated mammalian adults (Prunescu *et al.*, 2002). The liver has both endocrine (protein secretion) and exocrine (bile secretion) function. It also functions as a storage organ; synthesis of cholesterol, lipid portion of lipoprotein; metabolic activities like glycogenesis, deamination of amino acids, and detoxification of noxious substances (Akiyoshi and Inoue, 2004).

Rodents are the largest order in mammals. They are used as pets, laboratory animals and sources of animal protein (Nzalak *et al.*, 2012). The squirrel being a rodent has wide geographical distribution, but the African palm squirrel is seen mostly in West Africa. From available literature, few studies have been conducted on the squirrel. These include reports on necropsy finding of nematodes on captive African squirrel (Craig *et al.*, 1998), rabies in fox squirrel (Cappucci *et al.*, 1972), hepatocellular carcinoma in Black-tailed prairie dogs- *Cynomys ludovicianus* (Garner *et al.*, 2004), natural infection of the ground squirrel with *Echinococcus granulosus* (Yang *et al.*, 2009). But in the African palm squirrel, there is dearth of information on its basic biology from available literature, hence this microanatomic investigation on its liver, one of the important mammalian organs. The result from this study will fill the knowledge gap. It will help wild life biologist in understanding its adaptive physiology and Veterinarians in managing hepatic diseases of the species.

Materials and Methods

Five adult African palm squirrels of both sexes captured in the wild from Olokoro Umuahia in Abia state, Nigeria from March to November 2012 using metal cage traps were used for the study. Olokoro umuahia is in the rainforest vegetation of southern Nigeria characterized by heavy rains and thick well grown mangrove forest trees. They were immediately transferred to the veterinary anatomy laboratory of Michael Okpara University of Agriculture, Umudike, for acclimatization. During this period, the animals were fed with grasses, oil palm fruit and water *ad libitum*.

The squirrel on the day of sacrifice was sedated with chloroform. The weight of the animal was taken with Mettler balance (Model Ohaus scout PRO-200) with a sensitivity of 0.1gm. Each squirrel was euthanized by chloroform overdose and placed on dorsal recumbency. The animal was cut open through mid ventral incision from the inguinal region to the mandibular symphysis. The liver was dissected out and slices fixed in 10% neutral buffered formalin. The tissues were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1977). The slides were examined and photomicrographs taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

Results

Histologically, the liver was seen covered by a capsule composed of dense regular connective tissue fibres (fig.1). The liver parenchyma contained hepatocytes – the liver cells in a cord like arrangement. These hepatocytes were polygonal in shape (fig. 2). The liver lobule contained a central vein surrounded by hepatocytes (fig.2). Clear spaces were seen in the parenchyma between hepatocytes. The liver portal triad contained a hepatic vein, hepatic artery and bile ducts (fig. 3, 4). The hepatic vein lumen was wide and lined

by simple squamous epithelium. The hepatic artery was also lined by simple squamous epithelium, the smooth muscles of the tunica media were separated by the tunica intima by internal elastic laminae. The bile duct was lined by simple cuboidal cells (fig.4). Some nerve fibres were also at the portal triad (fig.4). Hepatic sinusoids were seen between liver lobules (fig.5). These sinusoids were lined by discontinuous endothelium and macrophages were seen at the sub-sinusoidal space (fig. 5).

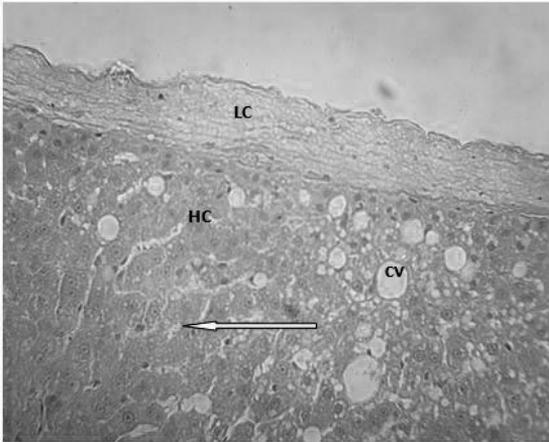


Figure 1: Section of the liver region showing mucus cells MC, liver capsule LC, hepatocytes HC, clear lipid spaces (white arrow), and central vein CV. H&E x400

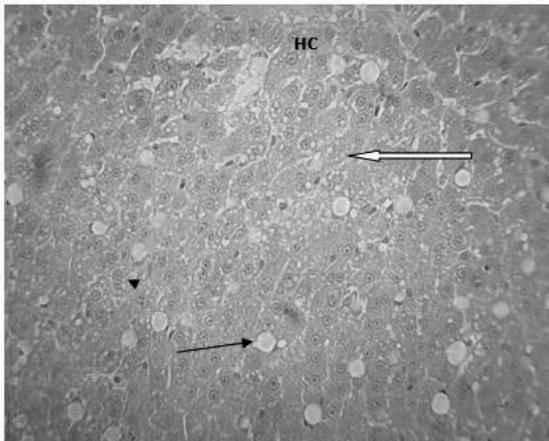


Figure 2: Section of the liver parenchyma showing polygonal hepatocytes HC, central vein (black arrow), and clear lipid spaces (white arrow). Note the binucleated hepatocytes (black arrow head). H&E x400.

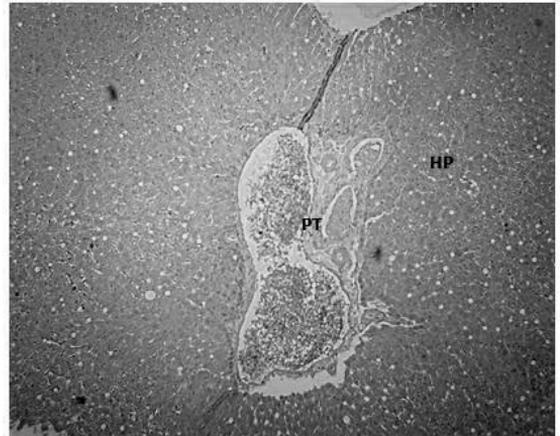


Figure 3: Section of the liver showing hepatic parenchyma PC, portal triad PT, containing hepatic artery and vein, and bile duct. H&E x400

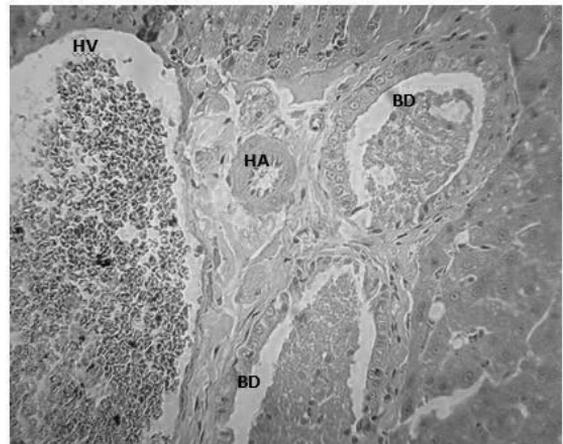


Figure 4: Section of the liver showing hepatic portal triad PT, containing hepatic artery HA, hepatic vein HV, and bile duct BD. H&E x400.

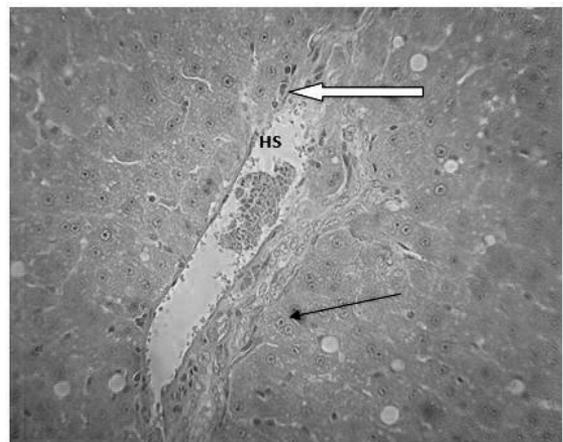


Figure 5: Section of the liver showing hepatic sinusoid HS, subsinusoidal macrophages (white arrow). Note the binucleated hepatocytes (black arrow). H&E x400.

Discussion

The result of this study shows that the Palm squirrel liver histology is similar to most other mammals. The Glissons membrane is for protection of the liver parenchyma. The dense regular connective tissue fibres seen in this liver has been reported in the adult European bison, *Bison bonasus* containing arteries and lymphatics (Prunescu *et al.*, 2002). The hepatocytes of polygonal shape is the liver cell responsible for its physiologic functions (Ayikoshi and Inoue, 2012). The binucleated hepatocytes observed in this study has also been reported (Prunescu *et al.*, 2002). A classic hepatic lobule with a central vein has also been reported (Ayikoshi and Inoue, 2012), and is the livers' basic functional unit. The clear empty spaces seen in the liver parenchyma in between the hepatocytes may be the extracellular lipid storage sites in the liver, but more work should be done to ascertain this claim. The sinusoids present is for free exchange of materials between the hepatocytes and the vascular system. The macrophages present in the subsinusoidal space of Disse is for local defence through phagocytosis of pathogens (Parker and Picut, 2005; Carollo *et al.*, 2012). These macrophages referred to as Kupfer cells have also been reported in other mammals (Carollo *et al.*, 2012). The portal triad of hepatic vein, hepatic artery and bile ducts has been reported in literature (Motta, 1984; Parker and Picut, 2005). The prominent bile duct maybe an adaptation for easy passage of bile salts from the liver to the duodenum since a gall bladder is absent in some rodents as also seen in this study (Voss, 1991;). The absence of lymphatics at the portal area suggest that lymphatics are not regular feature of squirrel portal triad, hence it is not a tetrad as reported in some mammals. The presence of nerve fibres in the portal area is unique as it has not been reported in other rodents from available literature.

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INFLUENCE OF TANNIN IN SELECTED FORAGES ON THE GROWTH PERFORMANCE AND FAECAL EGG COUNT OF SEMI-INTENSIVELY MANAGED SHEEP

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Abstract

The occurrence in economic losses through inadequate nutrition and the increasing prevalence of anthelmintic resistance to nematodes in sheep represent a major constraint in sheep production. A 56 day experiment was carried out to determine the effect of using forages of cassava, *Gliricidia sepium* and *Leucaena leucocephala* containing tannin on the growth performance and faecal egg count (FEC) of semi-intensively managed West African Dwarf (WAD) sheep. Sixteen WAD sheep with an average body weight of 16kg were randomly allocated to four supplementary treatments of forages of cassava forage (CF), *Gliricidia sepium* (GSF), *Leucaena leucocephala* (LLF) and the control (unsupplemented). Data were taken on sheep DM feed intake, body weight changes and faecal egg count (FEC). Results showed that the selected forages containing tannin increased weight gain ($P < 0.05$) and reduced ($P < 0.05$) FEC in sheep. Animals on LLF had the highest ($P < 0.05$) forage intake (707.50g/day) and weight gain (21.87g/day) which ranked the same statistically with sheep on CF diets with the control treatment having the least ($P > 0.05$) weight gain (13.83g/day). Based on the results of the study, it was concluded that tannins in the forages of leucaena and cassava improved growth and is effective in reducing faecal egg count in sheep managed semi intensively. This can be utilized for better weight gain and optimal functioning of animals to get maximum returns.

Keywords: Cassava, *Gliricidia*, *Leucaena*, Tannin, Sheep, growth, Faecal egg count

INFLUENCE DU TANIN DE CERTAINES PLANTES FOURRAGERES SUR LA PERFORMANCE DE CROISSANCE ET CHARGE OVULAIRE FECALE DES OVINS ELEVES EN SYSTEME SEMI-INTENSIF

Résumé

L'apparition de pertes économiques résultant d'une nutrition inadéquate et la prévalence croissante de la résistance aux anthelminthiques des nématodes chez les ovins représentent une contrainte majeure à la production ovine. Une expérience de 56 jours a été réalisée pour déterminer l'effet de l'utilisation de fourrages de manioc, *Gliricidia sepium* et *Leucaena leucocephala* contenant du tanin sur la performance de croissance et la charge ovulaire fécale (FEC) de moutons nains ouest-africains (WAD) élevés en système semi-intensif. Seize moutons WAD d'un poids moyen de 16 kg ont été répartis de manière aléatoire en quatre groupes de traitements supplémentaires aux fourrages : manioc (FC), *Gliricidia sepium* (GSF), *Leucaena leucocephala* (LLF), et groupe témoin (ne recevant aucun complément). Les données ont été recueillies sur la consommation d'aliments MS, les changements de poids corporel et la charge ovulaire fécale (FEC) des moutons. Les résultats ont montré que les fourrages sélectionnés contenant du tanin ont augmenté le gain pondéral ($P < 0,05$) et réduit ($P < 0,05$) la charge ovulaire fécale (FEC) des moutons. Les animaux recevant les fourrages LLF ont montré la plus grande consommation ($P < 0,05$) de fourrage (707.50g/jour) et le gain pondéral le plus élevé (21.87g/jour), ce qui a donné statistiquement le même classement pour les moutons aux régimes de FC, le groupe témoin ayant le plus faible ($P > 0,05$) gain pondéral (13.83g/jour). Sur la base des résultats de l'étude, il a été conclu que le tanin des fourrages de *Leucaena* et de manioc ont amélioré la croissance et est efficace dans la réduction de la charge ovulaire fécale des ovins élevés en système semi-intensif. Il peut donc être utilisé pour un meilleur gain pondéral et le fonctionnement optimal des animaux pour obtenir un rendement maximal.

Mots-clés : Manioc ; Gliricidia, Leucaena ; Tanin ; Ovins ; Croissance ; Charge ovulaire fécale

Introduction

Sheep are widely distributed throughout the humid savannah zones of Nigeria where they play a significant role in the food chain and overall livelihoods of rural households (Lebbie, 2004; Fasae *et al.*, 2012). In most sheep production areas, inadequate nutrition and infections with gastrointestinal parasites represent a major constraint in sheep husbandry. For many years, the control of these parasites has solely relied on the repeated use of synthetic anthelmintics (Kaplan, 2004). However, the emergence of resistant gastrointestinal parasites populations and the increasing concern of consumers for drug residues in animal products have provided a strong impetus towards the development of alternative strategies to control gastrointestinal parasites (Heckendorn, 2007). In recent times, forages containing tannins have been identified as an alternative non-drug gastro-intestinal parasite control strategies that are practical and realistic for introduction into farm production systems (Waghorn *et al.* 1998) and most of these forages has been evaluated under experimental conditions and some have demonstrated significant benefits for sheep. Tannins can significantly improve productive performance, as well as lessen dependence on anthelmintic drenches (Niezen *et al.*, 1993). However, not all tannins are equal and some may not benefit production at all.

The objective of this study is therefore to investigate into the effect of tannin in the forages of cassava, *Gliricidia sepium* and *Leucaena leucocephala* on the growth performance and faecal egg count of semi-intensively managed West African dwarf sheep.

Materials and Method

Experimental Animals and Management

A total of sixteen (16) West African Dwarf sheep, with average body weight of 16kg were selected from the sheep flock of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, and used for the experiment.

The animals managed semi-intensively were tagged for identification and allowed to graze for six (6) hours daily (8:00am – 2:00pm). The tanniferous forages of cassava, *Gliricidia sepium* and *Leucaena leucocephala* were harvested from established plots within the University farms and wilted for 24 hours before feeding the animals, to reduce the level of anti-nutritional factors as well as other materials that may have negative effect on the animals.

The supplemented forages were given to the animals after grazing at 4% body weight in different feeding troughs in individual pens with 100g of 16% crude protein concentrate diet comprising of 10% maize, 50% maize offals, 25% brewer's dried grain, 17% Soya bean meal, 4% bone meal, 0.5% salt and 0.5% vitamin premix with water provided *ad libitum*. Concentrate were fed to take care of any imbalance in nutrient intake that may arise due to the use of forages.

Initial body weights of the animals were taken using a spring balance at the start and on a weekly basis thereafter during the 8 week experimental period to ascertain the weight changes. Faecal samples of about 2 to 4 grams were obtained directly from the rectum of each animal at the beginning of the experiment and on weekly basis for the faecal egg count determination. The faecal samples were kept refrigerated to keep the parasite eggs from hatching until when required for analysis.

Chemical Analysis

The proximate composition of the selected forages and concentrate diets was determined (AOAC, 1995). The dry matter was determined by oven drying at 650C for 24hours, crude protein by kjeldahl method and fat by soxhlet fat extraction method. The faecal egg count was analyzed using the Modified Mc Master counting technique.

Statistical Analysis

Data collected were subjected to one way analysis of variance in a completely randomized design according to SAS (1999). Significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

Result and discussions

The chemical composition and tannin content of the supplemented forages and concentrate is shown in Table I. *Leucaena leucocephala* foliage (LLF) had the highest crude protein value with cassava foliage (CF) having relatively lower concentrations of crude protein (CP) and ether extract, than LLF and GSF. The crude protein content of cassava foliage was however consistent and similar to earlier reports (Ayodeji 2005; Oni *et al.*, 2011). The CP obtained in LLF and *Gliricidia sepium* forages (GSF) were comparable to values in literature (Yousuf *et al.*, 2007; Fasae *et al.*, 2010). Values reported for NDF and ADF were also similar to values reported elsewhere (Babayemi *et al.*, 2006). The values reported for the present study could be reasonable since the result may largely depend on the age of the forage.

The tannin content of the three supplemented forages was 2.68, 2.50, and 4.12% for LLF, GSF and CF, respectively which falls within the tolerable level for sheep. Tannin levels above 5% have been found to adversely affect feed digestibility in sheep (Diagayette and Huss, 1981). Tannins are powerful anti-metabolites, and in amounts exceeding 5%, which is common in tropical forages, can impair microbial functioning of ruminants (Singh and Bhat, 2003). At high concentrations, the tannins act as anti-feedants because they bind in tight chemical complexes with proteins, as well as microbial enzymes, thereby reducing fermentation and degradation of fibrous tissue in the rumen (Reed, 1995). This results in lowering of the nutritive value of plants containing tannins. Consequently, a huge amount of research in the tropics has been directed towards overcoming these undesirable qualities of tanniferous plants in grazing animals (Norton, 2000). However, the low level observed in forages used in this study could be attributed to the sun drying process employed before being fed to the animals.

Feed intake (%DM) of the supplemented forages in sheep was high and ranked the same ($P < 0.05$) statistically in sheep fed LLF and CF but lower ($P > 0.05$) values were recorded for sheep fed GSF (Table 2). The high CP of the

supplementary diets could have resulted in high intake by the animals as high CP content in diets has been considered an important factor that enables high intake of feed. Oldham and Alderman (1980) established that sometimes ad libitum DM intake by animals is increased by an increased in CP content of the diet. However, the low DM intake in sheep fed GSF could be as a result of the feeding value which has been found to be sometimes lowered, compared to other forages. There is varying opinion about the nutritive value of *Gliricidia sepium*. It is generally agreed, *Gliricidia sepium* is a high quality forage, but of with low palatability when first introduced to animals. Carew (1983) found that *Gliricidia* in the diet of sheep and goats induced diarrhea and depressed consumption of dried leaves during the first 3 weeks of feeding, thereby making the level of feed intake was initially low. Similar observations were made by Robertson (1988) and Fasae *et al.*, (2010) in goats fed varying levels of GLS. The odour of the leaves has been implicated in this initial reluctance of animals to eat *Gliricidia* (Brewbaker, 1986). However, once adapted, there appears to be no long-term detrimental effects on sheep (Chadhokar, 1982).

The growth pattern of semi intensively managed sheep supplemented with forage containing tannin as depicted in Figure I showed an increase in weight gain of sheep across the treatment groups. However, differences in body weight gain among the treatment groups were significant ($P < 0.05$) with the sheep on LLF and CF growing faster than those supplemented with GSF and the control treatment.

The trend observed in the values for the weight gain of the animals among supplementary forage treatments in this study was similar to that reported for goats but with higher values of 27.86, 26.43 and 18.12 g/day were reported for *Leucaena*, cassava and *Gliricidia* leaf meal, respectively (Yousuf *et al.*, 2007). The slight variation maybe attributed to animal, management type, variety type and duration of experiment. The low weight gain observed in sheep fed GSF could be attributed to its low intake by the animals.

However, the three supplementary treatments gave an increased in weight gain ($P < 0.05$) of sheep relative to the control. This

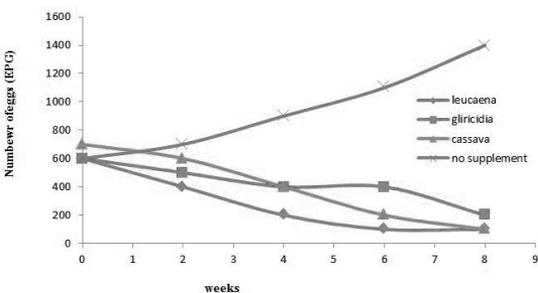
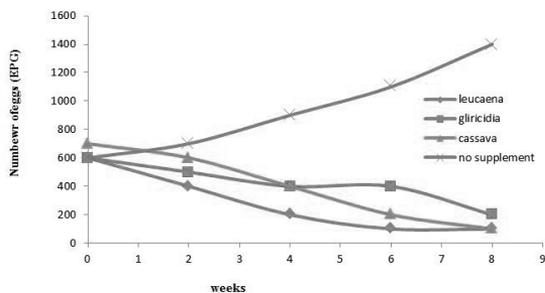
Table 1: Chemical composition and tannin content (%) of the forages fed to sheep.

Parameters	Leucaena	Gliricidia	Cassava	Concentrate
Dry matter	85.20	81.09	87.00	84.24
Crude protein	29.68	26.30	24.40	16.72
Ether Extract	4.07	3.96	2.09	18.12
Ash	8.16	7.02	6.92	9.45
Neutral detergent fibre	38.96	47.11	59.81	28.10
Acid detergent fibre	28.11	29.74	38.25	46.04
Acid detergent lignin	11.54	8.67	7.34	3.01
Tannin	2.68	2.50	4.12	-

Table 2: Performance of semi-intensively managed West African Dwarf Sheep supplemented with forages containing tannins.

Parameters	Cassava	Gliricidia	Leucaena	Control	SEM
Total feed intake(g/day)	695.00 ^a	657.50 ^b	707.50 ^a	-	28.37
Concentrate intake(g/day)	84.24	84.24	84.24	84.24	-
Forage intake(g/day)	610.76 ^a	573.26 ^b	623.26 ^a	-	27.78
Initial weight(kg)	16.75	17.00	16.50	16.75	1.30
Final weight(kg)	28.25 ^a	26.25 ^b	28.75 ^a	24.50 ^c	1.30
Weight gain(kg)	11.50 ^a	9.25 ^b	12.25 ^a	7.75 ^c	0.87
Weight gain(g/day)	20.53 ^a	16.51 ^b	21.87 ^a	13.83 ^c	5.09

^{a, b, c} means with same superscript within the rows are not significantly different ($P < 0.05$)



could be as a result of the higher crude protein content in these foliageages which are higher than 7% needed to give positive utilization of feed (Gatenby, 2002) and were within the range of appropriate requirement for optimal functioning of the rumen microbes. This suggests the benefits of these foliageages in improving the growth performance of sheep compared to the animals on the control treatment that were exposed only to natural pasture.

The effect of tannin in supplementary forages on the faecal eggs count (FEC) of sheep is shown in Figure 2. Sheep on the supplemented treatments had a reduction ($P < 0.05$) in FEC compared to the unsupplemented treatment and this supports earlier reports of the beneficial effects of condensed tannins in reducing nematode infestations in small ruminants. The vast majority of studies investigating the effects of tannins on gastro intestinal nematode parasites, either in experimental or in grazing conditions, have been conducted using sheep (Niezen *et al.*, 1996; Athanasiadou *et al.*, 2001). Feeding forages containing condensed tannins, significantly increased the growth rate of parasitized lambs compared with that of lambs

fed legumes not containing tannins, and the rate of establishment of the parasites was lower in lambs (Robertson *et al.*, 1995).

Conclusion

In conclusion, this study supports that tannin in forages of cassava, *Gliricidia sepium* and *Leucaena leucocephala* have the potential to help to increase growth and decrease faecal egg counts in sheep managed semi-intensively. However, supplementing sheep with *Leucaena leucocephala* and cassava forage resulted in better growth rate with a reduction in the FEC of sheep. It is therefore recommended that since these plants are naturalized to the tropical conditions and represent a rich, renewable, and unexplored source of tannins for grazing sheep, the possibility of using feeds containing these plant materials which contain tannins need to be explored for effective sheep production.

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ANTIBODIES TO TOXOPLASMA GONDII IN BACKYARD AND ROAMING PIGS: IMPLICATIONS FOR PORK CONSUMPTION.

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Abstract

Toxoplasma gondii, the etiologic agent of Toxoplasmosis, can be transmitted to pigs through the ingestion of oocysts, and to humans through consumption of pork containing viable cysts causing neonatal deaths and abortion in animals, and opportunistic infections in immunocompromised humans. The objective of this study was to investigate the occurrence of antibodies to *T. gondii* in backyard and Roaming pigs slaughtered for human consumption in Ibadan, southwestern Nigeria. Serum samples were collected from 100 pigs and tested for the presence of *T. gondii*-specific IgG antibodies using a commercially available ELISA kit.

The overall frequency of IgG antibodies to *T. gondii* was 25%. More antibodies were detected in exotic breeds (31.5%) than the local breed (20%), and in pigs raised under the free-range (35.7%) than backyard (20.8%) management systems.

The result of this study showed that pigs raised either as backyard or free-range stock are exposed to *T. gondii* infection, and suggests that the consumption of pork from both sources may be a risk factor for human infection with *T. gondii*.

Keywords: Toxoplasmosis; *Toxoplasma gondii*; Roaming Pigs; Backyard Pigs; Pork.

ANTICORPS DE TOXOPLASMA GONDII CHEZ LES PORCS DE BASSE-COUR ET LES PORCS EN LIBERTE : IMPLICATIONS POUR LA CONSOMMATION DE PORCS

Resume

L'agent étiologique de la toxoplasmose, *Toxoplasma gondii*, peut être transmis aux porcs par l'ingestion d'oocystes et à l'homme par la consommation de viande de porc contenant des kystes viables, et causer des mortalités néonatales et des avortements chez les animaux et des infections opportunistes chez les personnes immunodéprimées. L'objectif de cette étude était de se pencher sur l'apparition d'anticorps de *T. gondii* chez les porcs de basse-cour et ceux en liberté, abattus pour la consommation humaine à Ibadan, dans le sud-ouest du Nigeria. Des échantillons de sérum ont été prélevés sur 100 porcs et testés pour rechercher la présence des anticorps IgG spécifiques à *T. gondii* à l'aide d'un kit ELISA disponible dans le commerce.

La fréquence globale des anticorps IgG contre *T. gondii* était de 25%. Plus d'anticorps ont été détectés chez les races exotiques (31,5%) par rapport à la race locale (20%), et chez les porcs élevés en liberté (35,7%) par rapport à ceux élevés en système de basse-cour (20,8%).

Le résultat de cette étude a montré que les porcs élevés en basse-cour ou en liberté sont exposés à l'infection par *T. gondii*, et laisse entendre que la consommation de viande de porc provenant des deux sources peut être un facteur de risque d'infection de l'homme par *T. gondii*.

Mots-clés : Toxoplasmose ; *Toxoplasma gondii* ; Porcs en liberté ; Porcs de basse-cour ; Viande de porc

Introduction

Toxoplasma gondii, an obligate intracellular apicomplexan protozoan parasite is the etiology of Toxoplasmosis, a disease that is widely prevalent in animals and humans worldwide (Dubey, 2010). Toxoplasmosis is an important cause of neonatal deaths and abortion in animals (Dubey and Urban, 1990) and it is now increasingly implicated as an opportunistic infection in immunocompromised humans e.g. HIV, pregnant women and cancer patients (Tenter *et al.*, 2000).

T. gondii is mainly transmitted to animals and humans through the consumption of food or drink contaminated with oocyst excreted in the faeces of cats, the definitive host (Dubey, 2009). However, there is increasing evidence showing that meat containing tissue cysts (especially undercooked pork) is an important source of *T. gondii* infection in humans in several countries (Torda, 2001; Dubey, 2009; Tao *et al.*, 2011). Pigs and other warm-blooded animals act as intermediate hosts and are infected by the ingestion of oocysts present in the environment or by the consumption of cysts present in tissues of infected mammals, commonly small rodents (Kijlstra *et al.*, 2008).

The practice of rearing pigs outdoors in many countries around the world is likely to increase seroprevalence of *T. gondii* in pigs. Roaming pigs, because of their unique feeding habits are used as indicators to determine the extent of soil contamination with the *T. gondii* oocyst (Kijlstra *et al.*, 2008).

The inability of beef from cattle to meet the demand of the population for animal protein supply has made the consumption of pork to be on the increase. For example, a report from China showed that pork contributes about 65% of total meat consumption, an estimate of about 39kg pork consumed per person per year (Wang *et al.*, 2012). It is then not a surprise that recent reports revealed that the seroprevalence of antibodies to *T. gondii* in pigs in Northeast China is 12% (Liu *et al.*, 2012), 18.03% in East China (Wang *et al.*, 2012) and 30.6% in Chongquin, China (Wu *et al.*, 2011). Pigs are important in the epidemiology of Toxoplasmosis, especially now that the consumption of pork is on the increase.

In Nigeria, there is paucity of information on the seroprevalence of *T. gondii* in pigs in spite of the increase in pork consumption, especially in the Southwestern and Eastern part of the country where there is no religious restriction on it. Furthermore, there are also very few modern intensive pig farming facilities in Nigeria. Pigs slaughtered for human consumption are mostly from outdoor rearing systems where they are allowed to scavenge for food, thereby increasing the risk of *T. gondii* infection to the pigs and consequently pork eaters.

This study is therefore aimed at investigating the occurrence of antibodies to *Toxoplasma gondii* in backyard and Roaming pigs slaughtered for human consumption in Ibadan, Southwestern Nigeria.

Materials and Methods

Animals

One hundred blood samples were collected randomly from pigs brought for slaughter at a municipal abattoir in Ibadan, Nigeria. Information on the breed, age, sex and management system were obtained before the animals were taken to the slaughter slab.

Sample collection and storage

Blood samples collected into sterile tubes at slaughter were left at room temperature for about 2 hours after which the sera was harvested and stored at -20°C until analyzed.

Serological assay

Serum samples were tested for the presence of *Toxoplasma gondii*-specific IgG antibodies using a commercially available ELISA kit (ID Screen® Indirect Multi-species, IDVET, Montpellier, France). The test was carried out according to the manufacturer's protocol and the microplates read at 450nm. The test was validated and results obtained were interpreted using the specified equations in the instruction manual.

Statistical analysis

Variables were analyzed by Fisher exact test using the GRAPHPAD PRISM

5.01 (GraphPad Software, La Jolla, CA, USA). Association among variables and occurrence of seropositives were estimated from values obtained by the odds ratio (OR). Values were considered significant at 95% confidence interval ($P < 0.05$).

Results

The overall frequency of IgG antibodies to *T. gondii* was 25% (25/100). The distribution among breeds was Large White, 31.4% (16/51); Durock, 25.9% (7/27) and local breeds, 20% (2/20) while 25% (11/49) and 27.5% (14/51) were male and female respectively. Antibodies to *T. gondii* found in pigs aged less than 1 year was 26.1% (6/23), 1 year was 28.6% (10/35), 1.5 to 2 years was 20% (8/40) and those greater than 2 years was 50% (1/2). The distribution based on management system was 20.8% (15/72) and 35.7% (10/28) for backyard and free-range pigs respectively (Table 1). The odds ratio analysis among variables showed that the female and the young (≤ 1 year old) had the odds of getting infected than the male and adult (> 1 year old) respectively, while the exotic breeds of pigs and those raised under free-range management system had about twice the odds of getting infected than the local breeds and those raised in the backyard management system respectively (Table 2).

Although there were numerical differences between the variables, these differences were not statistically significant ($p > 0.05$).

Discussions

Toxoplasma gondii is an ubiquitous parasite that has been reported in mammals worldwide (Dubey, 2010). An overall frequency of 25% obtained in this study is comparable to the report from China where there is increase in the demand for pork as source of protein for the growing population (Wu *et al.*, 2011; Liu *et al.*, 2012; Wang *et al.*, 2012). The findings of our study suggest that the environment where the sampled pigs were reared might have been contaminated with *T. gondii* oocysts. Pig reared in both backyard and free-range management systems could get infected with the parasite through the ingestion of feed contaminated with *T. gondii* oocysts from (resident or roaming) cats or the consumption of infected rodents.

The findings of this study showed that the free-range pigs were more positive and had the odds of getting infected with *T. gondii* infection than the backyard-raised pigs. The differences in type of food and feeding habits might account for this observation. Backyard pigs are raised in restricted environment where they are mainly fed with formulated

Table 1: Demographic characteristics of the seroprevalence of *Toxoplasma gondii* antibody among sampled pigs in Ibadan, Nigeria

Variables	Group	No. Tested	No. Positive	Percentage (%)
BREED	Large White (LW)	51	16	31.4
	Durock (D)	27	7	25.9
	LW x D	12	-	0
	Local	10	2	20
SEX	Male	49	11	25
	Female	51	14	27.5
AGE (Years)	<1	23	6	26.1
	1	35	10	28.6
	1.5 – 2	40	8	20
	>2	2	1	50
SOURCE	Backyard	72	15	20.8
	Free Range	28	10	35.7
Total	Sample	100	25	25

Table 2: Crude odds ratios (OR) with 95% confidence intervals (CI) for various variables associated with seropositivity of *Toxoplasma gondii* antibodies among sampled pigs in Ibadan, Nigeria

Variables	Group	Percentage Positivity (%)	Odd Ratio (95% CI)
BREED	Exotic (LW & D)	31.5	1.673 (0.330-8.491)*
	Local	20	0.598 (0.118-3.035)
SEX	Male	25	0.765 (0.308-1.901)*
	Female	27.5	1.307 (0.526-3.249)
AGE (Years)	Young (≤ 1)	27.6	1.397 (0.548-3.560)*
	Adult (> 1)	21.4	0.765 (0.308-1.901)
SOURCE	Backyard	20.8	0.4737 (0.182-1.237)*
	Free Range	35.7	2.111 (0.808-5.513)

*No significance difference between the variables ($P > 0.05$)

feed and given water in troughs while the free-range pigs roam around scavenging for food in their surroundings and are thus more exposed to *T. gondii* oocysts from faeces of infected stray cats. There are reports to show that the prevalence of *T. gondii* in pigs is influenced by management systems (Dubey, 2009).

In this study, while the detection of higher levels of antibodies to *T. gondii* infection in young female pigs than in the adult male pigs supports a previous report that higher seroprevalence in sows has been associated with access to cats (Dubey, 2009), the reason for detecting higher antibodies in the exotic than in the local breed of pigs is not known. Although innate properties could account for increased susceptibility of the exotic breeds, the relatively small number of local pigs found during sampling could also contribute to the difference. Rearing of local breed of pigs is no more a common practice in the study area. There is therefore the possibility of increase in seroprevalence of toxoplasmosis in pigs in developing countries if the exotic breeds are more susceptible to *T. gondii* infection, since they are preferred because of their greater feed conversion ability and profitability after slaughter. There is a need to investigate the role of different breeds of pigs in the transmission of *T. gondii* infection to humans.

Conclusion

The finding of this survey suggests that the consumption of pork from both backyard

and Roaming pigs may be a risk factor for human infection with *T. gondii* in the study area. It is therefore recommended that measures to prevent Toxoplasmosis should be put in place where pigs are raised for human consumption under the backyard and free-range management systems. Pork should also be properly cooked or processed before eaten.

Acknowledgement

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UTILIZATION OF WHEAT OFFAL-CARRIED PINEAPPLE WASTE IN THE DIET OF WEST AFRICAN DWARF (WAD) GOATS

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Abstract

This study investigated the processing of wheat offal-carried pineapple waste meal (WCPW) and its utilization in the diet of West African Dwarf (WAD) goats with a view to ascertaining the inclusion level for optimal performance. The WCPW was obtained by evaluating six different combinations of wheat offal (WO) and pineapple waste (PW). The WO and PW mixing ratio 1:2 (w/w) was found to be optimal as feedstuff for WAD goats using keeping quality and nutrient content as criteria. Four dietary treatments (control diet (0%), 20%, 30% and 40% WCPW) were fed to growing WAD goats of both sexes in a completely randomized design. The proximate, mineral and vitamin composition of all treatment diets were determined. Performance variables such as feed intake, average daily gain, feed efficiency, nitrogen balance and retention, apparent digestibility coefficient and blood counts of the goats were evaluated. Chemical composition shows that the mineral, vitamin and crude protein (CP) contents of WCPW containing diets were more than sufficient than that required by goats for maintenance. The total feed intake (462.13 g/day), feed efficiency (9.90%), average daily weight gain (45.54%), the apparent digestibility coefficient of crude protein, nitrogen balance (2.63 g/day) and retention (53.46%) of goats fed 20% WCPW were significantly higher ($p < 0.05$) than the values obtained for goats fed other experimental diets. This indicates that animals fed 20% WCPW had the best performance characteristics. Although, the analysis of blood cells (red blood cell, white blood and packed cell volume counts) were significantly different ($p < 0.05$) among the goats fed experimental diets, the counts fell within the normal physiological range for goats. The study demonstrated that WCPW can be included in the diet of WAD goats without any adverse effect and dietary inclusion of WCPW up to 20% was optimal for growth performance of WAD goats.

Keywords: Pineapple waste, WAD goats, Wheat offal

UTILISATION D'UN MELANGE D'ISSUES DE BLE ET DE DECHETS D'ANANAS DANS LE REGIME DE CHEVRES NAINES D'AFRIQUE DE L'OUEST (WAD)

Résumé

Cette étude s'est penché sur le traitement d'un repas à base d'un mélange d'issues de blé et de déchets d'ananas (WCPW) et son utilisation dans l'alimentation de chèvres naines (WAD) d'Afrique de l'Ouest, en vue de déterminer le taux d'inclusion susceptible de produire une performance optimale. Le WCPW a été obtenu en évaluant six combinaisons différentes d'issues de blé (WO) et de déchets d'ananas (PW). Le ratio de mélange WO / PW 1:2 (w / w) a été jugé optimal comme aliment pour les chèvres WAD sur base des critères de qualité et de teneur en éléments nutritifs. Quatre traitements diététiques (régime témoin (0%) ; 20% ; 30% et 40% de WCPW) ont été administrés à des chèvres WAD des deux sexes selon un schéma complètement aléatoire. La composition en macronutriments et la composition minérale et vitaminique de tous les traitements diététiques ont été déterminées. Les variables de performance telles que la consommation alimentaire, le gain pondéral quotidien moyen, l'efficacité alimentaire, le bilan azoté et la rétention de l'azote, le coefficient de digestibilité apparente et les taux sanguins des chèvres ont été évalués. La composition chimique montre que la teneur en minéraux, en vitamines et en protéines brutes (CP) des régimes contenant WCPW était plus que suffisante par rapport à celle requise par les chèvres pour la maintenance. La consommation alimentaire totale (462.13 g / jour), l'efficacité alimentaire (9,90%), le gain pondéral moyen quotidien (45,54%), le coefficient de digestibilité apparente des protéines brutes, le bilan azoté (2,63 g / jour) et la rétention de l'azote (53,46%) des chèvres nourris avec 20% de WCPW étaient significativement plus élevés ($p < 0,05$) que les valeurs obtenues pour les chèvres nourries avec d'autres régimes expérimentaux. Ceci montre que les animaux nourris avec 20% de WCPW avaient

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les meilleures caractéristiques de performance. Bien que l'analyse des cellules sanguines (numération de globules rouges, numérations de globules blancs et valeurs de l'hématocrite) ait été significativement différente ($p < 0,05$) entre les chèvres nourries avec les régimes expérimentaux, les taux se situaient dans la fourchette physiologique normale pour les chèvres. L'étude a démontré que WCPW peut être inclus dans l'alimentation des chèvres WAD sans causer aucun effet indésirable, et l'inclusion alimentaire de WCPW jusqu'à 20% était optimale pour la performance de croissance des chèvres WAD.

Mots-clés : Déchets d'ananas ; Chèvres WAD ; déchets de blé

Introduction

Feed accounts for between 50 and 80% of the total cost of production depending on the type of livestock (Odeyinka and Ajayi, 2004). Olomu (1984) estimated the proportion of feed cost of the total cost of production of a ruminant under intensive management as 55%. One of the major problems confronting the small ruminant production is the non-availability of feed all year round to meet the maintenance and productive requirements of the animals. Babayemi (2007) stated that in the tropics, ruminants are raised mainly on grasses, which are poor in nutrients and digestibility coupled with scarcity during the dry season. Poor productivity and high mortality of stock, which characterize this industry result mostly from not feeding the right quantity and quality of feeds to the various livestock species (Odeyinka *et al.*, 2003). The unprecedented increase in the cost of conventional ingredients (e.g. maize) used in compounding livestock feed has necessitated intensive investigations into the use of agricultural and agro-industrial by-products (Hamzat and Babatunde, 2001). Some of the agro-industrial wastes include the following: citrus molasses, cull onions, citrus pulp, coffee-husk and pulp, cassava waste, sugar cane molasses shrimp waste meal, pineapple waste (Babatunde, 1998).

Food and Agricultural Organization (2004) ranked Nigeria among the leading pineapple producing countries with about 800, 000 metric tonnes since year 2001. Therefore, efforts at finding better use for the pineapple waste generated from such huge quantities may be important in terms of preventing environmental pollution and waste of potential animal feed resource. Lamidi *et al.* (2008) reported that broiler chickens could tolerate up to 10% pineapple waste (PW) in their diets

without any deleterious effect. Taiwo *et al.* (2011) concluded that PW could be included in the diet of sheep up to 45% without adverse effect. A possible loss of essential nutrients from seepage during the drying of wet PW was observed in the study reported by Taiwo *et al.* (2011), where drying took about 14 days. Therefore, a more efficient processing method seems desirable. One strategy is to use dry feed materials to absorb the exudates from PW. Such dry feed materials previously utilized as effective absorbents are maize offal/bran, wheat offal/bran, brewers' dried grains and dewatered rumen contents (Makinde and Sonaiya, 2007); and the most effective absorbent was wheat offal (WO). Consequently, mixing PW and wheat offal may provide a more efficient alternative to existing methods in terms of reducing nutrient loss and quick drying. Makinde *et al.* (2011) has investigated this procedure and confirmed its effectiveness resulting in an optimal combination of WO and PW (referred to as wheat offal-carried pineapple waste – WCPW) at ratio 1:2 (w/w) after sun drying for 4 h. This WCPW had approximately 88% DM, 16% CP and 10% CF thus can serve as supplement feed source for ruminant animals (Makinde *et al.* 2011). Therefore, the general objective of this study was to evaluate the nutritive value of WCPW as animal feed and specifically the performance of West African Dwarf (WAD) goats on diets with graded levels of WCPW.

Materials and Methods

Experimental station and period

The experiment was carried out at the Sheep and Goat Unit of Obafemi Awolowo University Teaching and Research Farm Ile-Ife, Osun State, Nigeria at altitude of 240 m above sea level, 7° 28'N and 4° 23' E. Ile Ife ecologically typifies the hot and humid tropical forest zone.

The experiment lasted 26 weeks between mid February and August 2011.

Processing of wheat offal-carried pineapple waste meal (WCPW)

The wheat offal-carried pineapple waste meal was processed as reported by Makinde et al. (2011). Briefly, wheat offal (WO) collected from Eagle Flour mills, Ibadan, Nigeria was thoroughly hand-mixed with fresh wet pineapple waste (PW; skins, peelings and the pulp peelings) collected in polyethylene woven sacks from the Lafia Canning Factory of Fumman Agricultural Products Nigeria Ltd, Moor Plantation, Ibadan, Nigeria. Six sets of the WO and PW mixture (1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5) were evaluated based on capacity to dry to $\leq 10 - 12\%$ moisture content in 4 h (Makinde and Sonaiya, 2007 and 2010). The decision criterion was to select the mix with the highest PW content that dried to $\leq 10 - 12\%$ moisture content in 4 h. Moisture content $> 12\%$ had been reported not desirable for long term preservation (Rozis, 1997). The mixtures were sun-dried by spreading thinly on black polythene sheets (0.7 mm thickness) in two replicates each on the concrete roof (20.5 m high) of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. These were ground with a plate/burr mill after drying and the proximate composition determined according to the methods of AOAC (2000). Based on the selection criterion, the mix-ratio 1:2 (WO: PW) of the wheat offal-carried pineapple waste meal (WCPW) was found to be the optimum combination (Table 1).

Animals, feeding and management

The experiment utilized twenty WAD weaner goats of both sexes in a feeding trial for 16 weeks. The animals were between 5 and 7 months old, weighed between 4.0 and 10.5 kg and randomly allotted to four treatments in a completely randomized design. Four concentrate diets were compounded comprising 0, 20, 30 and 40% levels of inclusion of the 1:2 (WO: PW) wheat offal-carried pineapple waste meal (WCPW) (Table 2). The diets were fed to West African Dwarf (WAD) weaner goats as supplements to a basal ration of guinea grass (*Panicum maximum*). The

goats were fed based on 3% of their body weights. Each animal was weighed before the commencement of the study and subsequently weekly throughout the experimental period. The goats were confined in slated floor pens in an open-sided house constructed from wood and wire gauze, with asbestos roof and concrete floor. The goats were fed ad libitum with free access to water and routine management and vaccination schedules carried out. Feed intake, feed efficiency, weight gain, nutrient digestibility, nutrient utilization and blood parameters were performance evaluation variables.

Measurement of digestibility

Two digestibility trials were carried out between 8 - 9th and 14 - 15th weeks during which the goats were housed individually in metabolism cages designed for the separate collection of faeces and urine, and fresh feed and water provided daily. Faeces were collected each morning before the feed was served. A 10% sample of faeces voided per day was dried in a forced-draught oven at 70°C for 24 hours. Faecal samples stored daily were bulked, thoroughly mixed, ground and sub-samples taken for chemical analysis. A 5 ml sample of the urine collected was preserved in 5% (v/v) glacial acetic acid and stored at 4°C in a deep freezer for chemical analysis. Apparent nutrient digestibility was determined according to the following:

$$\text{Apparent digestibility (\%)} = \frac{\text{Nutrient in feed} - \text{Nutrient in faeces} \times 100}{\text{Nutrient in feed}}$$

Chemical composition and blood analysis

The proximate composition, mineral and vitamin content of WCPW was determined at the Animal Sciences Department, Soil Science and Land Management Department and Central Science Laboratory, Obafemi Awolowo University, Ile-Ife according to procedure of AOAC (2000). Fecal and urine samples were analyzed for proximate and nitrogen content, respectively. Blood collected from the goats at the jugular vein into EDTA bottles were analyzed for packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts (Baker and Silvertown, 1976).

Table 1: The proximate composition of different blends of wheat offal and pineapple waste

Parameter	Feed ratio (Wheat Offal: Pineapple Waste)					
	1:1	1:1.5	1:2.0	1:2.5	1:3.0	1:3.5
Dry matter	91.70	90.80	88.30	85.30	85.00	84.70
Crude protein	15.55	15.40	16.20	15.70	16.00	16.20
Crude fibre	7.61	7.96	9.96	9.97	9.74	10.00
Ether extract	9.54	8.15	10.10	10.06	11.50	9.46
Ash	4.22	5.70	5.89	6.27	6.34	6.74
NFE	63.00	62.70	57.50	57.30	56.30	57.80

NFE; Nitrogen Free Extractives

Source: Makinde et al, (2011).

Table 2: Gross and chemical composition of the experimental diets

Parameter (%)	0%WCPW	20%WCPW	30%WCPW	40%WCPW
Gross composition of diets				
Corn bran	40.00	20.00	10.00	-
WCPW	-	20.00	30.00	40.00
Palm Kernel Cake	53.00	53.00	53.00	53.00
Groundnut Cake	4.50	4.50	4.50	4.50
Bone Meal	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50
Chemical composition of diets				
Dry Matter	92.78	92.95	92.78	93.23
Organic Matter	92.99	92.73	92.53	92.03
Crude Protein	16.38	18.20	17.75	17.75
Crude fibre	10.77	9.12	9.62	11.44
Ether Extract	11.87	12.59	10.05	9.88
Ash	7.01	7.27	7.47	7.97
Nitrogen free extract	46.75	45.77	47.89	46.19

WCPW:Wheat offal carried pineapple waste

Statistical analysis

Data were analyzed as completely randomized block design using the General Linear Models procedure of SAS® (2008) for analysis of variance (ANOVA). The main effects were the diets and replicates. The replicates per treatment were considered as blocks in order to increase the precision of the experiment. Differences between means were resolved by Duncan's multiple range test of the SAS® statistical package. Statistical significance was established when probability was less than 5% level of significance.

Results

Table 2 shows gross and chemical composition of the experimental diets. The gross composition of diets indicates the inclusion levels (0, 20, 30 and 40%) of WCPW to replace corn bran in the experimental diets. The crude protein content of diets containing WCPW was higher than that of the control diet while the values of other proximate components were similar across all the experimental diets.

Table 3 shows the mineral and vitamin

composition of WCPW as compared to NRC (1975, 2007) values for sheep. Macro and micro mineral content of WCPW were comparable and exceeded NRC values. Similarly, vitamins A and E content of WCPW were higher than the NRC values.

Table 4 shows the performance of the WAD goats on WCPW diets. The total feed intake was significantly different ($p < 0.05$) for goats on different diets. Goats fed 20%WCPW had the highest feed intake overall and for Panicum followed by goats on 0%WCPW, which was higher than goats on 30 and 40%WCPW. The final live weight for goats on 20%WCPW

was significantly higher ($p < 0.05$) than goats on 0%WCPW followed by 30 and 40%WCPW, in that order. Similar trend was followed for total weight and average daily weight gained. Goats fed 20%WCPW were superior to goats on other diets in all performance variables.

Table 5 shows the apparent nutrient digestibility coefficients. Digestibility coefficients of dry matter and organic matter were highest ($p < 0.05$) for 0%WCPW and least for 30%WCPW and 20%WCPW, respectively. For NFE, digestibility was highest ($p < 0.05$) for 30%WCPW and least for 0%WCPW. Utilisation of ether extract was highest ($p < 0.05$)

Table 3: Mineral and Vitamin composition of wheat offal-carried pineapple waste (WCPW)

Mineral	WCPW	NRC (1975)
Macro mineral (%)		
Calcium	0.48	0.21 – 0.52
Phosphorus	0.55	0.16 – 0.37
Magnesium	0.38	0.04 – 0.26
Sodium	0.16	0.04 – 0.01
Sulphur	0.16	0.14 – 0.26
Micro mineral (ppm)		
Iron	65.60	30 - 50
Copper	11.68	5.00
Manganese	140.00	20 - 40
Zinc	282.50	N/A
Vitamin (I.U)		
A	8000	5000
E	2300	1400

N/A: Not available

Table 4: Performance characteristics of experimental goats over a sixteen-week period

Parameter	0%WCPW	20%WCPW	30%WCPW	40%WCPW	SEM	P value
ADFC (g/day)						
Concentrate	117.98 ^a	117.20 ^b	109.44 ^c	112.10 ^b	0.66	<0.0001
Panicum	303.53 ^b	344.93 ^a	292.66 ^c	287.80 ^c	2.07	<0.0001
Total Intake	421.51 ^b	462.13 ^a	402.10 ^c	399.90 ^c	2.11	<0.0001
AILW (kg)	6.68	6.61	6.43	6.18	0.02	<0.0001
AFLW (kg)	10.51 ^b	11.06 ^a	9.85 ^c	9.63 ^d	0.02	<0.0001
TWG (kg)	3.83 ^b	4.45 ^a	3.42 ^c	3.45 ^c	0.02	<0.0001
ADG (g/day)	39.21 ^b	45.54 ^a	35.00 ^c	35.24 ^c	0.10	<0.0001
FE (%)	9.66 ^b	9.90 ^a	8.60 ^d	8.82 ^c	0.66	<0.0001

^{a,b,c,d} means on the same row with different superscripts are significantly different ($p < 0.05$), WCPW:Wheat offal carried pineapple waste, ADFC: Average daily feed consumption, AILW: Average initial live weight, AFLW: average final live weight, TWG: Total weight gain, ADG: Average daily weight gain, FE: Feed efficiency.

Table 5: Apparent digestibility coefficient of experimental goats

Parameter	0%WCPW	20%WCPW	30%WCPW	40%WCPW	SEM	P value
Dry Matter	61.77 ^a	60.74 ^b	57.73 ^d	60.20 ^c	0.38	<0.0001
Organic Matter	87.37 ^a	83.34 ^d	85.29 ^c	85.98 ^b	0.57	<0.0001
Crude Protein	51.27 ^c	53.92 ^a	52.70 ^b	50.23 ^d	0.28	<0.0001
Crude Fibre	60.97 ^c	65.13 ^a	63.21 ^b	52.52 ^d	0.30	<0.0001
Ether Extract	64.81 ^d	68.32 ^c	70.11 ^b	72.36 ^a	0.46	<0.0001
Ash	44.50 ^d	56.36 ^a	49.22 ^b	43.15 ^c	0.27	<0.0001
NFE	83.64 ^d	90.41 ^b	91.42 ^a	86.04 ^c	0.68	<0.0001

a, b, c, d: Means within each row with different superscript are significantly different (p<0.05)

WCPW:Wheat offal carried pineapple waste

Table 6: Mean nitrogen utilization of goats fed experimental diets

Parameter	0%WCPW	20%WCPW	30%WCPW	40%WCPW	SEM	P value
Nitrogen Intake (g/day)	4.64 ^c	4.93 ^a	4.85 ^b	4.85 ^b	0.01	<0.0001
Nitrogen excretion faecal	1.33 ^c	1.41 ^b	1.41 ^b	1.47 ^a	0.03	<0.0001
Urinary	0.93 ^b	0.89 ^c	1.02 ^a	0.96 ^b	0.01	<0.0001
Total	2.26 ^b	2.30 ^b	2.43 ^a	2.43 ^a	0.02	<0.0001
Nitrogen loss (%N intake)						
Faecal	28.73 ^b	28.46 ^d	28.87 ^b	30.30 ^a	0.02	<0.0001
Urinary	20.10 ^b	18.09 ^d	21.04 ^a	19.80 ^c	0.02	<0.0001
Total	48.83 ^b	46.55 ^c	49.91 ^a	50.10 ^a	0.05	<0.0001
Nitrogen balance (g/day)	2.38 ^c	2.63 ^a	2.42 ^b	2.42 ^b	0.01	<0.0001
Nitrogen retention (%)	51.19 ^b	53.46 ^a	50.17 ^c	49.88 ^c	0.08	<0.0001

a, b, c, d means on the same row with different superscripts are significantly different (p<0.05)

WCPW:Wheat offal carried pineapple waste

Table 7: Blood analysis of experimental goats

Parameter	0%WCPW	20%WCPW	30%WCPW	40%WCPW	SEM	P value
PCV (%)	35.60 ^a	32.80 ^b	32.00 ^b	28.80 ^c	1.23	<0.0001
RBC (106)	11.00 ^b	12.00 ^a	10.20 ^c	9.70 ^d	0.29	<0.0001
WBC (103)	9.27 ^c	11.66 ^a	7.88 ^d	10.74 ^b	0.26	<0.0001

a, b, c, d Means within each row with different superscripts are significantly different (p< 0.05)

WCPW:Wheat offal carried pineapple waste; PCV: Packed cell volume; RBC: Red blood cell; WBC:White blood cell.

for 40%WCPW and least for 0%WCPW. Comparatively, 20%WCPW had the highest digestibility score overall when digestibility scores for all the nutrients are considered.

Table 6 shows that there was no significant difference (p>0.05) in nitrogen intake of goats fed 30%WCPW and 40%WCPW but higher (p<0.05) than for goats on 20%WCPW and 0%WCPW, which were equal. However,

the % total nitrogen loss (nitrogen in faeces and urine) of goats fed 30 and 40%WCPW were significantly higher (p<0.05) than goats fed other diets and 20%WCPW had the least loss. The nitrogen retention percentage was significantly highest (p<0.05) in 20%WCPW than other diets.

The analysis of blood components packed cell volume (PCV), red blood cell (RBC)

and white blood cell (WBC) counts (Table 7) showed that there were significant differences ($p < 0.05$) in each of these parameters among the animals fed experimental diets. Goats fed 20% WCPW scored most satisfactorily in a combination of the blood parameters. Packed cell volume was highest in 0%WCPW followed by 20%WCPW and lowest in the 40%WCPW diet. Further, goats on 20%WCPW had the highest RBC scores ($p < 0.05$) followed by 0%WCPW, 30%WCPW and 40%WCPW, in that order. Similarly, goats fed 20% WCPW had highest scores ($p < 0.05$) for WBC but were followed by those fed 40%WCPW, 0%WCPW and 30%WCPW, in that order.

Discussion

The main objective of this study was to evaluate the nutritive value of WCPW as animal feed and especially the effect on the growth performance of West African Dwarf (WAD) goats on diets with graded levels of WCPW. Results indicate a good potential for WCPW as animal feed and for inclusion in concentrate diets for growing small ruminants.

The gross and chemical composition of the experimental diets in Table 2 signifies comparable potential feeding value of diets with WCPW against the control diet (0%WCPW). However, the crude protein content of diets containing WCPW was higher than that of control diet (0%WCPW) while other proximate components were similar except crude fiber and ether extract (Table 2). Crude fiber decreased then increased as the WCPW content increased, apparently due to increasing fiber content contribution by WCPW. In contrast, ether extract decreased as the content of WCPW increased. Nevertheless, all diets met the minimum nutrient requirements for growing WAD goats (NRC, 1985). This suggests suitability of WCPW in the diets of small ruminants.

Further, the mineral and vitamins A and E content of WCPW (Table 3) met and even more than exceeded that recommended by NRC (1975, 2007) for sheep. In practice, mineral and vitamin requirements for goats are usually approximated from sheep values because of lack of data on goats (NRC, 1985).

This result further underscores the feeding value of WCPW.

It appears that inclusion of WCPW beyond 20% depresses feed intake (Table 4). However, feed intake of goats in this study was higher than that reported by Olosunde (2010) for goats fed diets with sun-dried pineapple waste. This could be due to the mixing of wheat offal with pineapple waste, which probably resulted in improved palatability of the diets. In addition, Butterworth and Mossi (1985) and Akinlade et al. (2005) observed that the use of concentrate as a supplement for ruminants can improve dry matter feed intake. This result probably indicates that there is no adverse effect on intake when WCPW replaces corn bran in concentrates for WAD goats. Goats on 20%WCPW were superior in growth probably due to superior feed intake and feed efficiency compared to goats on the other diets. Masafu (2006) describes feed intake as a measure of diet appreciation, selection and consumption by an animal. Nevertheless, all animals on all diets were in a positive weight balance, suggesting nutritional adequacy of diets. The average daily weight gain range (35.00 – 45.51 g/day) in this study was higher than that reported for WAD goats fed conventional protein supplements (palm kernel cake, soybean meal, brewers' dried grains and cotton seed cake) by Arigbede (2007) but similar to the results of studies by Alikwe (2011) who fed soybean meal and dried poultry waste as supplements. Arigbede (2007) recorded 19.83 – 33.36 g/day and Alikwe (2011) 22.10 – 54.30 g/day. Similarly, the feed efficiency values obtained in this study were higher than those obtained for WAD goats by Arigbede (2007) and Alikwe (2011). These results indicate the potential of WCPW as a good substitute for conventional protein supplements in WAD goat diets.

Goats fed 20%WCPW were superior to others in overall utilisation of nutrients (Table 5), which was reflected in better growth performance. This was probably especially due to highest feed intake (Table 4) and digestibility scores in crude protein and crude fibre. It is possible that inclusion of WCPW at 20% favoured increased activities of fibre degrading bacteria in the rumen. Feed intake and digestibility are regarded as major factors that determine

potential animal performance (Beever, 1993). The superior scores for digestibility and intake by goats on 20%WCPW indicates better acceptability, consumption and utilization than other diets. Although, neutral detergent fiber (NDF) and lignin were not determined, the depression in dry matter digestibility for goats on 20%WCPW compared to 0%WCPW may be due to increase in neutral detergent fiber and lignin because of higher feed intake. Bakshi and Wadhwa (2004) observed that voluntary dry matter intake and dry matter digestibility are dependent on fiber, especially neutral detergent fiber and lignin. Norton (1994) also reported increased digestibility with reducing NDF values. Although, the digestibility coefficients for all the diets were lower than what was obtained by Olosunde (2010) who fed sun-dried pineapple waste to sheep, goats in this study had higher growth rates (Table 4). These results further underline the potential of WCPW as feedstuff for WAD goats.

Nitrogen balance has been described as a good indicator of the protein value of a diet when the amino acid supply is balanced with the energy supply (Babayemi and Bamikole, 2006). A positive nitrogen balance indicates that the protein requirement for maintenance in experimental animals was adequately met by the dietary treatments. All the diets had positive nitrogen balances, which indicates adequacy in protein requirement for maintenance. Animals fed 20%WCPW diet had the highest ($p < 0.05$) N balance (2.63) and N retention percentage (53.46%). This indicates that the optimum level of inclusion of WCPW was at 20% and that at higher inclusion levels efficiency of protein utilization decreased. All the nitrogen balance values obtained in this study (2.37 – 2.63 g/day) are similar to 2.23 – 3.30 g/day reported by Ogunmoye (1995) for WAD goats fed soybean-based diets. Nitrogen retention as a percentage of nitrogen intakes ranged between 49.88 (40%WCPW) and 53.46% (20%WCPW). However, these were lower than values obtained by Arigbede (2007) and Alikwe (2011) probably because they fed conventional protein sources. Nevertheless, all the values were however similar to the range (32.6 to 58.3%) reported by Ndemanisho *et al.*, (1998) and Babayemi and Bamikole (2006) for

WAD goats fed concentrate diets.

The PCV values, red blood cell (RBC) and white blood cell (WBC) counts obtained in this study were within normal physiological ranges (27.0 – 45.0%, 9.0 - 15.0 $\times 10^6$ and 4.0 – 12.0 $\times 10^3$, respectively) reported by Jain (1993). This probably shows that feeding WAD goats diets with WCPW up to 40% inclusion did not adversely affect their physiological well-being.

The study demonstrated that dietary inclusion of WCPW up to 20% was optimal for growth performance of WAD goats. Further studies that could enhance the utilization of pineapple waste in WAD goat diets will contribute to the pool of alternative feed ingredients and reduction in environmental pollution from pineapple waste by pineapple processing industries.

Impact

Combination of wheat offal and pineapple waste using simple techniques for quick recycling resulted in a potential animal feedstuff. This feedstuff was satisfactorily utilized by WAD goats, which are commonly found and reared by smallholder farmers and householders in most rural communities in Nigeria and many other developing countries in sub-Saharan Africa. This may be important as a supplemental feed source for ruminant animals during the dry season when forages are in short supply. Recycling pineapple waste would reduce the extra cost incurred by pineapple canning industries in disposing the waste and ultimately reduce environmental pollution from it.

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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 Inter-African 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 of 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:

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- 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
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- Socio economics and economics of animal resources development

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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.
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