ISSN 0378 – 9721

Volume 65 No 2

June / Juin 2017

African Union
Inter-African Bureau for Animal Resources

Bulletin of

Animal Health and Production

in Africa

Bulletin de la

Santé et de la Production Animales

en Afrique

Union Africaine
Bureau interafricain des Ressources Animales

ISSN 0378 - 9721

INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES
BUREAU INTERAFRICAN DES RESSOURCES ANIMALES
P.O Box 30786, NAIROBI, KENYA

BULLETIN

June
Juin
2017 Volume 65 No. 2

AFRICAN UNION
UNION AFRICAINE

IBAR PUBLICATION
PUBLICATION DU BIRA

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

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

Annual subcription: US$ 100.00

ISSN 0378-9721

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

Abonnement pour un an : 100$

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

VOL. 65 NO. 2 **CONTENTS** **JUNE, 2017**

LOCALIZATION OF OESTROGEN HORMONE RECEPTORS IN THE REPRODUCTIVE TRACT OF GIANT AFRICAN LAND SNAIL (*ARCHACHATINA MARGINATA*) AND POTENTIAL ROLE OF MUCUNA PRURIENS ON LEVEL OF EXPRESSION

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Abstract

This study determines localization of oestrogen hormone receptors in selected parts of the reproductive tract of giant African land snail (*Archachatina marginata*) and potential role of Mucuna pruriens on levels of expression. Thirty (30) snails were used for this experiment, with average weight of 100-150g. The snails were allotted to three dietary treatments which includes concentrate (CON), concentrate + Mucuna pruriens (CON+MSP) and Mucuna pruriens seed powder only (MSP). Each treatment contains 10 Replicate each. After eight weeks, five snails were randomly selected from each dietary treatment and dissected. Organs removed were oviduct, albumen gland, ovo-testis and the spermatheca. RNA and DNA extractions were carried out with commercial kits. Gel electrophoresis on 1% agarose was also carried out to evaluate the expression. Primers sequence used were the forward and reverse β oestrogen primer which was designed to detect the expression of the gene encoding oestrogen receptor in the reproductive tract of the giant African Land Snail (*Archachatina marginata*) were: Forward: 5'-GCT TCG AGC TCA GCC TG-3' Reverse: 5'-AGG ATC ATG GCC TTG ACA CAG A-3'. Result showed that receptors for estrogen were present in oviduct, albumen gland, ovo-testis and spermatheca for both RNA and DNA analysis considering CON and MSP. While oviduct was also moderately expressed for CON and MSP. But combination of concentrate and Mucuna seed powder (CON+MSP) showed no visible expression for estrogen receptor. It was however concluded that combination of concentrate and mucuna seed powder (CON+MSP) down-regulate expression of estrogen receptor gene expression. It was recommended that feed to be combined for snail production must be free from substance(s) that has the potential to block the expression of reproductive hormone function.

LOCALISATION DES RÉCEPTEURS DE L'HORMONE ŒSTROGÈNE DANS LE TRACTUS REPRODUCTEUR DE L'ESCARGOT TERRESTRE AFRICAIN GÉANT (ARCHACHATINA MARGINATA) ET LE RÔLE POTENTIEL DE MUCUNA PRURIENS SUR LE NIVEAU D'EXPRESSION

Résumé

La présente étude a pour objet de déterminer la localisation des récepteurs de l'hormone œstrogène dans certaines parties du tractus reproducteur de l'escargot terrestre africain géant (Archachatina marginata) et le rôle potentiel de Mucuna pruriens sur les niveaux d'expression. Trente (30) escargots d'un poids moyen de 100 à 150 g ont été utilisés dans cette expérience. Les escargots ont été affectés à trois traitements alimentaires : concentré (CON) ; concentré + Mucuna pruriens (CON + MSP) ; poudre de graines de Mucuna Pruriens (MSP) uniquement. Chaque traitement contenait 10 répétitions. Après huit semaines, cinq escargots ont été choisis de manière aléatoire dans chaque traitement diététique et disséqués. Les organes retirés étaient l'oviducte, la glande de l'albumen, la glande hermaphrodite (ovo-testis) et la spermathèque. Les extractions d'ARN et d'ADN ont été réalisées avec des trousses commerciales. Une électrophorèse sur gel à 1% d'agarose a également été effectuée pour évaluer l'expression. La séquence d'amorces utilisée était l'amorce ß oestrogénique directe et inversée conçue pour détecter l'expression du gène codant pour le récepteur d'œstrogène dans le tractus reproducteur de l'escargot terrestre

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africain géant (*Archachatina marginata*) - Directe : 5'-GCT TCG AGC TCA GCC TG -3' ; Inversée : 5'-AGG ATC ATG GCC TTG ACA CAG A-3'. Les résultats ont montré que les récepteurs d'œstrogène étaient présents dans l'oviducte, la glande de l'albumen, l'ovotestis et la spermathèque pour l'analyse de l'ARN et de l'ADN considérant CON et MSP, tandis que l'oviducte était également modérément exprimé pour CON et MSP. Mais la combinaison de concentré et de poudre de graines de Mucuna (CON + MSP) n'a montré aucune expression visible pour le récepteur d'œstrogène. L'étude a toutefois conclu que la combinaison de concentré et de poudre de graines de Mucuna (CON + MSP) régule négativement l'expression du gène du récepteur d'œstrogène. Il a été recommandé que les aliments pour animaux qui seront combinés pour la production d'escargots soient exempts de substance (s) susceptibles de bloquer l'expression de la fonction de l'hormone reproductrice.

Introduction

Production of livestock and mini-livestock to suit the demand of increasing population has led to application of scientific methods to manipulate reproduction. The major targets of manipulation are hormones that play crucial role in reproduction. Oestrogens and androgens are good examples of these hormones. Their activities influence maturation of gonads, development of reproductive tract and promotion of gametogenesis with influence on sexual behaviour in vertebrate (Bolandes, 1994). These sex steroids have also been reported in some invertebrate species, especially in molluscs (LE guellec *et al.,* 1987; D'aniello *et al.,* 1996; Matsumoto *et al.,* 1997). It was also reported that changes in levels of sex steroid correlate with the process of sexual maturation in mollusc which imply that steroid hormones play key role in reproduction in this species (Matsumoto *et al.,* 1997). The use of natural plant to influence reproduction is also a common practice recently. A good example of such plant is Mucuna pruriens. This plant has been reported to enhance fertility in mammals (Ahmad *et al.,* 2008; Shukla *et al.,* 2009; Gupta, *et al.,*2011; Mahajan, *et al.,* 2011; Singh, *et al.,* 2013). The potential role of this plant in fertility may be as a result of its influence on endocrine system as it facilitate or modulate production of steroids needed for this purpose. It may also be possible that hormone receptor expression are also enhanced thereby favouring interaction that could result in possible manipulation of reproduction from exogenous sources of hormones targeted at modulation of reproductive pattern in this animal.

The present study was designed to locate oestrogen hormone receptor in the reproductive tract of giant African land snail (*Archachatina marginata*) and to evaluate possible influence of Mucuna pruriens powder on level of expression of oestrogen hormone receptor.

Materials and Methods

Experimental site

This study work was carried out at the Snail Research Facility of College of Animal Science and Biotechnology Laboratory of the Federal University of Agriculture Abeokuta. The location lies within the rainforest belt of Western Nigeria, latitude 7°N, longitude 3°2' E and altitude 76° m.a.s.l. The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 34.7°C and mean relative humidity of 82 % (Google Earth 2010).

Experimental animals

Thirty snails (*Archachatina marginata*) were purchased from local market. The snails were reared in plastic basket with dimension of 30cm by 40cm by 24cm. Average weight of snails used for this study were 100-150g. Feed and water were provided throughout the period of the experiment. Snails were acclimatized for a period of two weeks before the commencement of the experiment. Three dietary treatments used during the experimental period were:

- Concentrate only
- Concentrate + Mucuna pruriens seed powder (1:1)
- Mucuna pruriens seed powder only

Proper sanitation was carried out to avoid invasion of pathogenic organism throughout the period of the experiment. This experiment was conducted for 8 weeks. Proximate composition of concentrate feed used is shown in Table 1.

Experimental protocols

At the end of eight weeks, snails were dissected and some selected organs of the reproductive tracts were removed. These organs include: Ovo-testis, oviduct, spermotheca and albumen gland. These organs were labelled and stored in the -70 ultra-freezer. Thereafter, RNA from each organ was extracted with the use of the RNA kit. Primer was also designed using a commercial program that could retrieve the sequence of the target DNA. The primers were purchased from integrated DNA technology (IDT) Inc. USA. Both forward and reverse primers used were: Forward primer: 5'-GCT TCG TGG AGC TCA GCC TG-3' and Reverse primer: 5' -AGG ATC ATG GCC TTG ACA CAG A-3'

Sample Preparation and Cell Lysis from animal tissue

Six hundred micro-litres (600 µl) of a Lysis Buffer were added to 5g of each sample and homogenized using a mortar and pestle. An additional 600 µl of Lysis Buffer was also added to the homogenate and vortexed for 15-30 seconds. The samples were then centrifuged for 2 minutes at 14,000 revolutions per minute. After centrifugation, supernatant were collected in fresh eppendoff tube and 600 µl of ethanol was added making a total of 1200 µl in one tube. Thereafter, samples were transferred into a spin column and centrifuged for 1 minute at 10,000 revolutions per minutes. Supernatants were collected and transferred into a fresh set of labelled micro-tubes for further process.

Column loading

600 µl cell lysate was added and vortexed. A spin column was placed into a 2ml collection tube. The mixture was then transferred into spin columns. The samples were then centrifuged at 10,000 revolutions per min for 1 minute. The flow through was then discarded.

Column wash

Four hundred micro-litres (400 µl) of washing solution were added into the extracted RNA that has been previously mixed with ethanol before been transferred into the spin column. The spin column was then centrifuged at 10,000 revolutions per minutes for 1 minute. The flow through was discarded and the spin column was centrifuged again at 10,000 revolutions per minute for 1 minutes to remove residual ethanol. This process took place three times to ensure total removal of

Table 1: Composition of experimental diets (g/100g)

Ingredients	**Quantity (g)**
Maize	50
Wheat offal	27.5
Groundnut cake	12
Soy bean meal	4
Bone meal	3
Oyster shell	3
Premix	0.25
Salt	0.25
Total	**100**

Each 2.5kg of premix contains: Vit.A 10,000,000i.u, Vit D3 2,000,000i.u, Vit. E 20,000i.u, Vit. K 2,250mg, Thiamine B1 1750, Riboflavin B2 5000mg, Pyridozine B6 2750mg, Niacin 27500mg, Pantothenic acid 7500mg, Vit. B12 15mg, Follic acid 7500mg, Biotin H2 50mg, Cholin Chloride 400gr, Cobalt 200mgr, Copper 5g, Iodine 1.2g, Iron 20gr, Manganese 80g, Selenium 200mg, Zinc 50g, Antioxidant 125g. Recommended inclusion is 2.5kg per tonne of feed.

ethanol previously added and to ensure that RNA binds column.

Rna elution

The column was placed into a fresh 1.7 ml elution tube and 50 µl elution solution was then added at centre of the column membrane. The sample was then centrifuged at 2000 revolution per minute for 2 minutes followed by 14,000 revolutions per minute for 1 min to elute the RNA. The sample was later stored in the ultra-freezer.

Agarose gel preparation

A total of 1g of agarose powder was weighed and mixed with 100 ml of TAE buffer. The mixture was placed in the microwave to melt at moderate temperature. Afterwards, the melted mixture was allowed to cool and then mixed with 20 µl of ethidium bromide. The mixture was then poured into an agarose chamber. The agarose chamber was partitioned with a plastic comb. After gel was formed, wells were conspicuous for loading.

Loading of agarose gell and Electrophoresis

The agarose gel was transferred into the electrophoresis tank. The samples were then loaded in the wells after addition of loading dye. The loading dye was allowed to sink properly into the wells. The electrophoresis chamber was then filled with TAE buffer. The electrophoresis chamber was then connected and made to run for 40 minutes.

Polymerase chain reaction

The RNA extracted was mixed with the primers and master mix, which consists of the buffers and other nucleotides. It was thereafter subjected to Reverse Transcriptive Polymerase Chain Reaction (RT-PCR) in the PCR machine for about two hours 30 minutes. The PCR machine was set at an annealing temperature of 94^0c. The PCR products were then subjected to Reverse Transcriptive Polymerase Chain Reaction (RT-PCR) again.

Data analysis

The expressions of estrogen receptor gene in different parts of the reproductive tract were read in bands after gel electrophoresis.

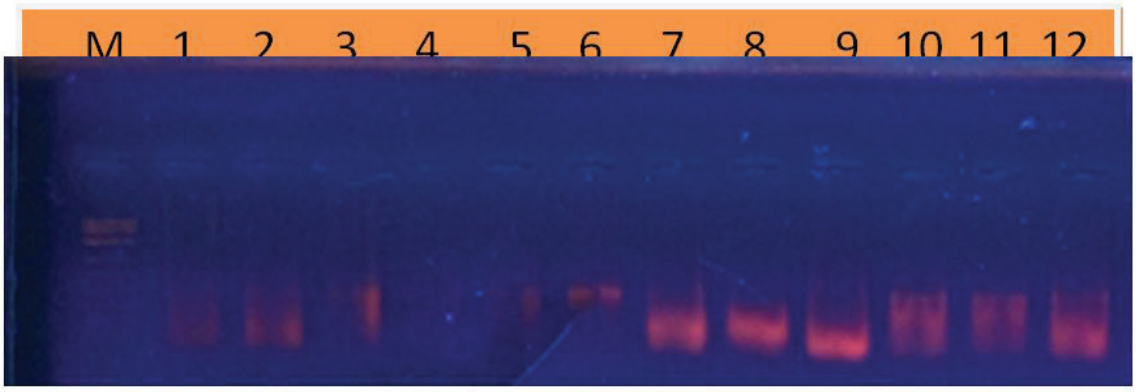
Results

The result of total RNA expressions in the selected part of the reproductive tract of giant African land snail (*Archachatina marginata*) for estrogen receptor gene after exposure to three dietary treatments are shown in table 2 and plate 1. RNA expressions for estrogen receptor were visible for all the selected parts of the reproductive tract (Oviduct, albumin gland, ovo-testis and spermatheca). However, low levels of expression were recorded for oviduct, albumin gland and ovo-testis for both CON and CON+MSP. Spermatheca had moderate level of expression for CON and CON+MSP, while albumin gland followed same trend for MSP. Table 3 and plate 2 showed the

Table 2: Expression of RNA in different reproductive tract of the Giant African Land Snail (*Archachatina marginata*) exposed to three dietary treatments (Concentrate, Concentrate + Mucuna pruriens seed powder, and Mucuna pruriens seed powder).

Reproductive Tract	RNA Estrogen-receptor gene Expression		
	CON	CON+MSP	MSP
Oviduct	+	+	+
Albumin Gland	+	+	++
Ovo-testis	+	+	+
Spermatheca	++	++	+

CON: Concentrate; CON+MSP: Concentrate + Mucuna pruriens seed powder; MSP: Mucuna pruriens seed powder; No visibility : -; Low : +; Moderate : ++



M: RNA ladder (M)
Lane 1: Oviduct [Concentrate]
Lane 2: oviduct [Concentrate + Mucuna pruriens seed powder]
Lane 3: Oviduct [Mucuna pruriens seed powder]
Lane 4: Albumen Gland [Concentrate]
Lane 5: Albumen Gland [Concentrate + Mucuna pruriens seed powder]
Lane 6: Albumen Gland [Mucuna pruriens seed powder]
Lane 7: Ovo-testis [Concentrate]
Lane 8: Ovo-testis [Concentrate + Mucuna pruriens seed powder]
Lane 9: Ovo-testis [Mucuna pruriens seed powder]
Lane 10: Spermatheca [Concentrate]
Lane 11: Spermatheca [Concentrate + Mucuna pruriens seed powder]
Lane 12: Spermatheca [Mucuna pruriens seed powder]

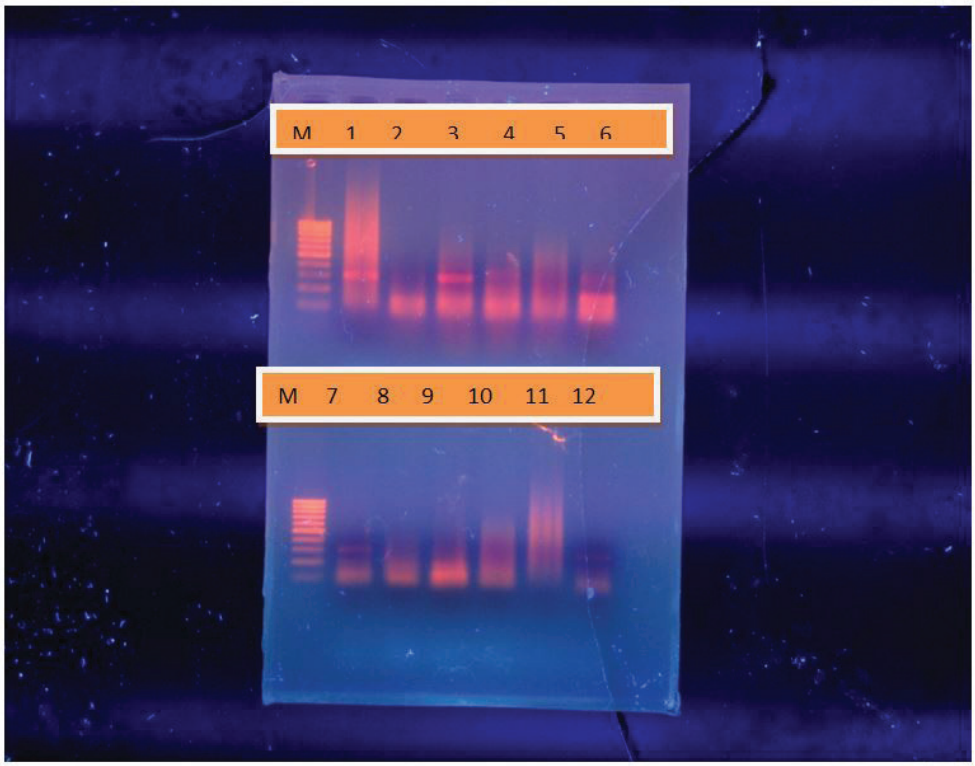


Plate 2: Total DNA extracted from different reproductive tract showing the expression of estrogen receptor gene.

Table 3: Expression of DNA in different reproductive tract of the Giant African Land Snail (*Archachatina marginata*) exposed to three dietary treatments (Concentrate, Concentrate + Mucuna pruriens seed powder, and Mucuna pruriens seed powder).

Reproductive Tract	DNA Estrogen-receptor gene Expression		
	CON	CON+MSP	MSP
Oviduct	++	_	++
Albumen Gland	+	_	+
Ovo-testis	+	_	_
Spermatheca	_	_	+

CON: Concentrate; CON+MSP: Concentrate + Mucuna pruriens seed powder; MSP: Mucuna pruriens seed powder; No visibility : -; Low : +; Moderate : ++

result of DNA expression in the reproductive tract of giant African land snail (A. marginata) for estrogen receptor gene expression. No visible expression was recorded for the entire reproductive tract selected for CON+MSP.

Discussions

Total RNA expression observed is an indication that all the selected parts of the reproductive tract of *Archachatina marginata* have receptors for estrogen. Although the function of vertebrate steroid hormones in molluscs is not yet clear, however, there is increasing evidence that it has influence on reproduction and physiological processes (Dorn, 2000; Lafout and Malhieu, 2007). Moderate level of expression shown for both albumen gland and spermatheca is a reflection of higher number of receptors that are present in these two organs. Spermatheca is known to store external spermatozoa after mating since the animal is hermaphrodite. It may be possible that other substance(s) that contain receptors for estrogens are present in this reproductive apparatus. Although report has shown that estrogen is present in the semen of several species of animal (Adamopoulos et al. 1984; Claus *et al.,* 1992). There is also increasing evidence that germ cells synthesize estrogen and possibly serve as the major source of this steroid in the male reproductive tract (Carreau *et al.,* 2003). The role of albumen gland in deposition of albumen during egg formation is a prominent one. Egg production involves formation of vitellogenin-like proteins which are known to be an egg yolk precursor protein (Matozzo and Marin, 2008). The vertebrate hormone 17β-estradiol (E2) has been reported to increase vitellogenin-like proteins in vertebrate. If this is possible, it is an indication that receptors for estrogen are more prominent in egg production apparatus like albumen gland, thus justifying higher level of expression compared to others except for spermatheca.

In vertebrate and invertebrates, estrogen are known to act via binding to the estrogen receptor (α or β), a transcription factor which consequently lead to up or down-regulation of specific genes. In this study, combination of concentrate and Mucuna seed powder (CON+MSP) is a reflection of down regulatory effect of the combination. It may be possible that substance(s) lead to this effect is formed. Frank et al. (2009) reported down regulatory effect of genistein on juvenile animal exposed to feed containing this substance. Its effect was reported to also lead to decrease estrogen receptors gene expressions (Frank *et al.,* 2009). Oviduct showed moderate expression for CON and MSP while albumin gland showed low expression level in both CON and MSP. Levels of expressions showed in these two organs is a further proof that they contain receptors for estrogen and that both CON and MSP separately does not down-regulate the expressions of estrogen in these two reproductive apparatus. Ovo-testis also followed similar trend for CON while CON+MSP and MSP had no visible expression for ovo-testis. This observation is a

further evidence that substance which down-regulate estrogen expression are present in CON+MSP and MSP alone for ovo-testis. This organ is known to perform dual function of spermatozoa and ova production. It may be possible that genistein that is present in soyabean and some anti-nutritional factors present in mucuna pruriens are the ones responsible for this observation (Frank *et al.,* 2009; Ologhobo *et al.,* 1993; Leon *et al.,* 1991). Low expression level recorded for spermatheca in MSP is further proof of estrogen receptor in this organ without any negative influence on its expression. Lack of visible expression of estrogen receptor gene for spermatheca considering both CON and CON+MSP may also be due to down-regulatory effect of genistein and anti-nutritional factors in Mucuna (Frank *et al.,* 2009; Ologhobo *et al.,* 1993; Leon *et al.,* 1991).

Conclusion

The expression of oestrogen, using β oestrogen primer shows that oestrogen receptors are present in the reproductive tracts of Giant African Land Snail (*Archachatina marginata*) which is a pointer to the fact that synthetic sources may be used to influence reproductive activity in this animal. Furthermore, Combination of CON+MSP was also found to down-regulate expression of estrogen in oviduct, albumen gland, ovo-testis and spermatheca. It can therefore be concluded that feed that tend to down-regulate expression of reproductive hormone should be avoided in the diet of Giant African land snail (*Archachatina marginata*).

Acknowledgment

Authors thank all staff of Biotechnology Centre, Federal University of Agriculture, Abeokuta for their support and contribution during the period of this research work.

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SEROLOGICAL DETECTION OF *BRUCELLA SUIS* ANTIBODIES AMONGST PIGS IN KADUNA STATE, NIGERIA

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Abstract

Increasing reports of poor production associated with swine brucellosis necessitated this serological study. This study was carried out to establish the prevalence of swine brucellosis in Kaduna state, Nigeria. Three hundred (300) sera were randomly collected from porcine species between July – December, 2012 from abattoirs, household pigpens, markets and pig farms in three senatorial zones (Samaru, Kaduna metropolis and Kafanchan). The sera were analyzed at the Department of Veterinary Microbiology Diagnostic Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna state. This sero- assay was carried out using Test-itTM Brucella (Porcine) Lateral Flow Assay Kits in accordance with manufacturer's recommendations. Out of the 300 sera analyzed 29.3% (88) were positive for brucella antibodies. Male pigs (175) and female pigs (125) were tested, with 14.67% of both male and female pigs were positive. Pigs (11%) within the age range 11-15 months showed the highest antibody titers and least within the age group greater than 26 months. Breed distribution revealed 25.0% local and 4.3% exotic breeds respectively were positive. There was significant association ($p<0.05$) between sero-positive reactors and age. No association ($p>0.05$) was established for sex and breed with swine brucella seropositivity. This study showed high prevalence of swine brucellosis in Kaduna state, Nigeria. Hence, the need for effective biosecurity measures during swine breeding, production, slaughtering and marketing to prevent economic losses as well as potential zoonosis during diagnostic handling of suspicious samples.

Keywords: Seroprevalence, *Brucella suis* antibodies, Pigs, Kaduna state, Nigeria.

DETECTION SEROLOGIQUE DES ANTICORPS ANTI BRUCELLA SUIS CHEZ DES PORCS DE L'ETAT DE KADUNA AU NIGERIA

RESUME

L'augmentation de rapports faisant état de la mauvaise production associée à la brucellose porcine a conduit à la conduite de la présente étude sérologique. L'étude a été réalisée dans le but de déterminer la prévalence de la brucellose porcine dans l'État de Kaduna au Nigéria. Trois cent (300) sérums ont été prélevés de manière aléatoire sur des espèces porcines entre juillet et décembre 2012 dans des abattoirs, porcheries de ménages, marchés et fermes porcines dans trois zones sénatoriales (Samaru, la Métropole de Kaduna et Kafanchan). Les sérums ont été analysés au Département du Laboratoire de diagnostic microbiologique vétérinaire de la Faculté de médecine vétérinaire de l'Université Ahmadu Bello Zaria dans l'État de Kaduna. Ce test sérologique a été réalisé à l'aide de trousses d'essai à flux latéral Test-itTM Brucella (Porcine) conformément aux recommandations du fabricant. Des 300 sérums analysés, 29,3% (88) se sont avérés positifs pour les anticorps dirigés contre Brucella. Les porcs mâles (175) et femelles (125) ont été testés, et les résultats ont révélé que 14,67% des porcs mâles et femelles étaient positifs. Les porcs (11%) de la tranche d'âge 11 - 15 mois présentaient les titres d'anticorps les plus élevés, tandis que ceux du groupe d'âge supérieur à 26 mois avaient les plus faibles titres d'anticorps. La répartition des races a révélé que 25,0% des races locales et 4,3% des races exotiques étaient positifs. On a noté une association significative ($p <0,05$) entre les réacteurs séropositifs et l'âge. Aucune association ($p> 0,05$) n'a été établie entre le sexe ou la race et la séropositivité pour la brucellose porcine. Cette étude a montré une forte prévalence de la brucellose porcine dans l'État de Kaduna au Nigeria. Par conséquent, il est nécessaire de mettre en place des mesures efficaces de biosécurité pendant l'élevage, la production, l'abattage et la commercialisation des porcs afin de prévenir les pertes économiques et le risque de zoonose lors de la

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manipulation diagnostique d'échantillons suspects.

Mots-clés : séroprévalence, anticorps contre Brucella suis, porcs, État de Kaduna, Nigeria.

Introduction

Brucellosis is an emerging disease worldwide (Munoza *et al.,* 2012) which affects both animals (pigs, dogs, cattle, sheep and goat) and humans [Nanven *et al.,* 2013]. High prevalence has been reported in South America and South – East Asia (Robson *et al.,* 1993). In Nigeria, Brucellosis is endemic and a major source of zoonosis (Cadmus *et al.,* 2006) causing severe economic losses to livestock farmers (Ocholi *et al.,* 2005). The disease have been documented in different part of the country in various scales especially in ranches, livestock breeding centre, abattoirs and dairy farms (Onunkwo *et al.,* 2011; Bello-Onaghise *et al.,* 2012; Olabode *et al.,* 2012) with the Brucella organisms been isolated from aborted fetuses, hygroma fluid, milk, blood, vaginal swabs and uterine discharges from livestock animals (Ocholi *et al.,* 2005).

Global swine population is estimated at 923M of which 18 million are reared in Africa (FAO, 2002). Pork is a major and popular meat consumed that provides 44% of animal-based meat protein supply (FAO, 2005). However, increasing report of abortion and infertility amongst swine herds with serious economic loss and low income generation by the famers have been documented (Onunkwo *et al.,* 2011). This major constraint that hampers swine production is of reproductive significance of which swine brucellosis has been previously reported (Alton, 1990). Swine brucellosis is caused by *Brucella suis* and is a zoonotic bacterial infection characterized by bacteremia, chronic inflammatory lesions in the reproductive organs of both male and female pigs, with localized lesions in other tissues (Praud *et al.,* 2012). Other clinical signs of swine brucellosis reported include, abortion, orchitis, still birth, birth of weak piglets, epididymitis, hygroma, spondylitis, hind-limb paralysis and arthritis (Megid *et al.,* 2010) sterility and infertility among swine herds (Onunkwo *et al.,* 2011).

Swine brucellosis has been reported in the Southern States of United States of America (USA) and Queensland in Australia (Starnes *et al.,* 2004). In Nigeria, high prevalence of the disease has been reported in the North-Central zones (Ngbede *et al.,* 2013). However, no evidence of the disease was reported in the Western parts of the country (Talabi *et al.,* 2013). The disease is transmitted during ingestion of contaminated feed, copulation and or artificial insemination (Ngbede *et al.,* 2013), handling of infected materials/animals by abattoir and farm workers (Munoza *et al.,* 2012) and also apparently healthy animals have been reported to shed the organisms (CDC, 2005). The internationally prescribed test for trade purposes include the indirect and competitive enzyme-linked immunosorbent assays (ELISAs), Rose Bengal test (RBT), complement fixation test (CFT) and fluorescence polarisation assay (FPA) (CDC, 2005). The allergic skin test and buffered plate agglutination test (BPAT) are also of diagnostic importance (Ngbede *et al.,* 2013). However, there exist limited reports on use of the serological Lateral flow test kits for swine brucellosis in this study area. This test kit is rapid, reliable and sensitive in routine diagnosis of individual pigs which pre-informed the choice the kit for this sero-survey of swine brucella antibodies in three senatorial districts of Kaduna State- Nigeria.

Materials and Methods

Study area

The study was conducted in Kaduna State (Zaria, Kaduna metropolis and Kafanchan), Nigeria. Zaria is located between latitude 11°04 N and longitude 7°42 E, covering an area of 300km^2 and with a population of about 408,198. The vegetation is Northern Guinea Savannah zone, with rainfall ranging from 0.0 to 816.0 mm/month and temperature of 17^{0}C to 33^{0}C (Mortimore, 1970). Kafanchan is a town in Southern Kaduna located between latitude 9^{0}34N and longitude 8^{0}18E, with an estimated population of 83,092 (Archibong, 2006).

Kaduna metropolis is located between latitude $10^0$31'23'N and longitude $7^0$26'25'E, covering an area of 17,781 sq mi (46,053 km^2) and with a population of about 6,066,562 (Anonymous, 2012). Pork and other pork products are major source of meat protein in the southern part of Kaduna State, Nigeria (Adah, 2013) and this zone has the largest porcine population which necessitated the choice of this study area.

Study design

The sampling was carried out from June to August, 2012 in Kafanchan, Zaria and Kaduna metropolis which constitute the nuclei for pig rearing in Kaduna state. The samples were collected based on convenience random sampling method (Castello, 2009).In each farm, abattoir, household, and market visited, pigs were randomly selected and samples collected from 50% of the total herd size to arrive at the sample size. Samples collected from each sampling location were grouped into age, sex, and breed. The total of 300 blood samples was collected from pigs in the three senatorial districts for this serological survey.

Sample collection

Animals were properly restrained and five (5) ml of blood was collected from the anterior vena cava of the heart of each pig, using a 20 ml syringe and 18 G needles. The blood was transferred into clean test tubes without anticoagulant for serum separation and was labeled appropriately. Sera samples were transported to the laboratory in a leak proof container packed with ice (Miller *et al.,* 1990).

Serological procedures

Test-itTM Brucella (porcine) Lateral Flow Assay Kits obtained from Life Assay Diagnostics (pty) Ltd, South Africa were used for serological assay. Serum sample was placed in the sample port and running fluid was added to solubilise the detection reagent as well as, carry the sample and detection reagent through the porous membrane in the test zone. The results were interpreted as described by the manufacturers. A positive result was indicated by the presence of a line at the test zone (T) and a line at the control zone (C), while a negative result was indicated by absence of a line at the test zone (T) and presence of a line at the control zone (C).

Data analysis

All data obtained were expressed as frequency and percentage and were further subjected to Chi square statistical analysis.

Results

Out of the 300 swine sera analyzed, 29.3% (88) were sero-positive and Two hundred and twelve (212) were negative as shown in Table 1. The distribution of swine Brucella antibodies according to sex revealed that 14.67% of both sex were sero-positive as shown in Table II. The age distribution of swine Brucella antibodies revealed that pigs within the age range 11-15 months had the highest 11% occurrence while the pigs greater than 26 months had least (0.3%). However, the age group 6-10 months had 9.3% occurrence as indicated in Table III.

Swine Brucella antibody distribution according to breeds showed that 25% local breeds and 4.3% exotic breeds were sero-positive. There was no statistical significant association ($p>0.05$) between positivity of reactor pigs and their sex or breed. However, a statistically significant association ($p<0.05$) between sero-positive reactor pigs and age was observed.

Table 1: Occurrence of swine brucella antibodies in Kaduna state - Nigeria

Porcine species	Frequency	Percentage (%)
Positive	88	29.3
Negative	212	70.7
Total	**300**	**100**

Table II: Distribution of swine brucella antibodies according to sex

Sex	Brucella Lateral Flow Assay		Total
	Positive	Negative	
Male	44 (14.66%)	131 (43.7%)	175 (58.3%)
Female	44 (14.66%)	81 (27%)	125 (41.7%)
Total	**88 (29.3%)**	**212 (70.7%)**	**300 (100%)**

df = 1; X2 = 0.059

Table III: Distribution of swine brucella antibodies according to Age

Age (months)	Brucella Lateral Flow Assay		Total
	Positive	Negative	
0 – 5	11 (3.7%)	48(16%)	59(19.7%)
6 – 10	28 (9.3%)	44(14.7%)	72(24%)
11 – 15	33 (11%)	102(34%)	135(45%)
16 – 20	13 (4.3%)	14(4.7%)	27(9%)
21 – 25	2 (0.7%)	3(1%)	5(1.7%)
26+	1 (0.3%)	1(0.3%)	2(0.6%)
Total	**88 (29.3%)**	**212 (70.7%)**	**300 (100%)**

df = 5; X2 = 0.021

Table IV: Distribution of swine brucella antibodies according to Breed

Breed	Brucella Lateral Flow Assay		Total
	Positive	Negative	
Local	75 (25%)	193 (61.4%)	268 (89.3%)
Exotic	13 (4.3%)	19 (6.3%)	32 (10.7%)
Total	**88 (29.3%)**	**212 (70.7%)**	**300 (100%)**

df = 1; X2 = 0.138

Discussion

The overall prevalence of 29.3% obtained in this study is lower than previous reports (Abdoel *et al.,* 2008) and (Ngbede *et al.,* 2013) in Portugal and Makurdi, North-Central Nigeria respectively. This finding could be attributed to the variation in study areas and volume of porcine production and marketing activities. However, this study prevalence was higher than previous reports (Cadmus *et al.,* 2006), (Talabi *et al.,* 2013), (Onunkwo *et al.,* 2011) in Oyo, Ogun states in Western and South Eastern Nigeria respectively. This finding suggests that cases of swine brucellosis is on the rise in the swine population in Kaduna state, Nigeria which is in line with the report (Ngbede *et al.,* 2013) that states brucellosis is endemic among swine and other livestock population in Nigeria.

Age distribution of brucella antibodies in this study, reveals statistical significant association as younger pigs (0 – 15 months) were more exposed to the brucella antigens, probably due to their increased ability to scavenge, indiscriminate copulation and or cross reaction between maternal antibodies and brucella antigens which enhances predisposition of younger pigs. This is in line with previous report (Ngbede *et al.,* 2013). However, contrast other report (Megid *et al.,* 2010) which states increased occurrence amongst adult pigs (>16 months). The sex distribution of swine brucella antibodies indicates both boar and sow are equally susceptible to the disease. In spite of this observed association of

Brucella antibodies with age and the absence of statistical association of sex and occurrence of swine brucellosis in this study. Pigs of both sex and all ages are equally susceptible, signifying sex and ages of pigs are potential risk factors in disease occurrence.

Swine brucellla antibodies distribution according to breed indicates that local pigs in comparison to the exotic breeds were more exposed to the antigen. This could probably be associated with herd management practice in the study area where the local pigs were reared on semi-intensive and the exotic breeds are kept on intensive system (Tewe and Adesehinwa, 1995), (Ngbede *et al.,* 2013). The brucella infected pigs within this study area are therefore potential carriers as documented by Lucero *et al.,* (2005); OIE, (2009); Godfroid *et al.,* (2010). Thus, they may serve as a potential source of infection to other susceptible pigs, livestock and humans.

Conclusion

This study has shown a baseline report of high prevalence (29.3%) of brucellosis in the study area especially amongst male and female, young, local breeds of pigs. Hence, the need for effective biosecurity measures during porcine breeding, production, slaughtering and marketing to prevent economic losses as well as any potential zoonosis during diagnostic handling of suspicious samples. Swine farmers and public health awareness programme is also advocated.

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ECONOMIC IMPACTS ASSESSMENT OF PLEUROPNEUMONIA BURDEN AND CONTROL IN PASTORAL CATTLE HERDS OF NORTH-CENTRAL NIGERIA

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Abstract

Contagious bovine pleuropneumonia (CBPP) is a trans-boundary infectious and contagious respiratory disease of cattle caused by Mycoplasma mycoides subsp. mycoides. It is a disease of high economic importance because of its ability to compromise food security. Information on its economic burden in pastoral cattle herds of Niger State, North-central Nigeria is not readily available. This study was aimed at investigating the economic impacts of CBPP to pastoralists in Niger State, North-central Nigeria, by determining its burden, returns to investments in its control, and cost-effectiveness of the control interventions ex-post evaluation, to provide baseline estimates that will assist animal health authorities and international donors in making investment decisions on its control in Nigeria. A questionnaire-based cross sectional study was conducted in 125 pastoral cattle herds. Economic analyses were conducted using total economic cost, benefit-cost analysis and cost-effectiveness analysis models. The values of mortality and morbidity losses to the herders were 219,038.5 USD and 35,598.8 USD, respectively. The total economic cost of CBPP to pastoralists was estimated to be 294,800.3 USD. Return on investment in CBPP control by vaccination and treatment was positive, with a benefit-cost ratio of 6.4. The Average cost-effectiveness ratio value for treatment intervention was 13.7 USD per life cattle saved and for vaccination option was 0.6 USD per death/cull averted. The estimated economic costs due to CBPP have shown that the disease was of high economic importance and must be controlled.

Keywords: Benefit-cost analysis, CBPP, cost-effectiveness analysis, economic impact, pastoralist, Nigeria.

ÉVALUATION DES IMPACTS ÉCONOMIQUES DU FARDEAU ET DU CONTROLE DE LA PLEUROPNEUMONIE CHEZ LES TROUPEAUX BOVINS EN MILIEU PASTORAL DANS LE CENTRE-NORD DU NIGERIA

Résumé

La pleuropneumonie contagieuse bovine (PPCB) est une maladie respiratoire transfrontalière infectieuse et contagieuse des bovins, causée par Mycoplasma mycoides - sous-espèce mycoides. C'est une maladie d'une grande importance économique en raison de sa capacité à compromettre la sécurité alimentaire. Les informations sur son fardeau économique dans les troupeaux bovins pastoraux de l'État du Niger dans le nord-centre du Nigeria ne sont pas facilement disponibles. La présente étude avait pour objectif d'analyser les impacts économiques de la PPCB sur les pasteurs de l'État du Niger dans le nord-centre du Nigéria, en déterminant son fardeau, le rendement des investissements dans son contrôle et l'évaluation ex-post du rapport coût/efficacité des interventions de contrôle, dans le but de fournir des estimations de référence qui aideront les autorités de la santé animale et les donateurs internationaux à prendre des décisions d'investissement sur son contrôle au Nigeria. Une étude transversale fondée sur un questionnaire a été menée dans 125 troupeaux bovins pastoraux. Des analyses économiques ont été effectuées en utilisant des modèles d'analyse du coût économique total, du rapport coûts-avantages et du rapport coût-efficacité. Les valeurs des pertes subies par les éleveurs en raison de la mortalité et de la morbidité étaient respectivement de 219 038,5 USD et 35 598,8 USD. Le coût économique total de la

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PPCB pour les pasteurs était estimé à 294 800,3 USD. Le rendement des investissements dans le contrôle de la PPCB par la vaccination et le traitement était positif, avec un rapport bénéfice-coût de 6,4. La valeur du rapport coût-efficacité moyen pour l'intervention de traitement était de 13,7 USD par vie bovine sauvée et l'option de vaccination était de 0,6 USD par mortalité / abattage évité. L'estimation des coûts économiques liés à la PPCB a montré que la maladie était d'une grande importance économique, et qu'elle doit être contrôlée.

Mots-clés : analyse coûts/bénéfices, PPCB, analyse du rapport coût-efficacité, impact économique, pasteur, Nigeria.

Introduction

Investing in livestock sub-sector development has increasingly been recognized as an effective way to contribute to broad-based poverty reduction for macro- and micro-economic reasons. At the macro-economic level, the livestock sub-sector is the single largest contributor to agricultural Gross Domestic Product (GDP) in developed countries accounting for over 50% of agricultural value-added and about 30% in developing countries. At the micro-economic level, a large share of the rural poor depends on livestock for their livelihoods (FAO, 2009). An important factor which militates against livestock productivity in developing countries is the prevalence of animal diseases (OAU/IBAR/PARC, 1998). Most governments of sub-Saharan Africa are unable to maintain effective surveillance and control programs against these diseases due to inadequate budgetary provisions to the animal health sub-sector (Tambi *et al.,* 1999), and depend on international donors when faced with challenges of epidemics or pandemics. One of the major disease challenges to cattle production in Nigeria is contagious bovine pleuropneumonia (CBPP) (Aliyu *et al.,* 2003; Adamu and Aliyu, 2006; Alhaji and Babalobi, 2016a).

CBPP impacts animal health and poverty of livestock-dependent people through decreased animal productivity, reduced food supply, and the cost of control measures. It is an OIE listed disease, and the Pan African programme for the Control of Epizootics (PACE) had identified CBPP as the second most important transboundary cattle disease in Africa after rinderpest (Tambi *et al.,* 2006), and a barrier to trade in many African countries as it reduces the value of livestock and the income of many value chain stakeholders (Danbirni *et al.,* 2010; Jores *et al.,* 2013). It is a disease of high economic importance and serious threat to livestock production and development in Sub-Saharan Africa (Tambi *et al.,* 2006). About 24.4 million cattle herders in 19 sub-Saharan African countries are at risk of CBPP and 30–50% of them are living below poverty levels (Thomson, 2005). Once introduced to a new area, initial losses can be very high and it requires major expenditure for control.

There are four essential tools in CBPP control: movement control, stamping out, vaccination, and treatment. Each control measure acts by reducing the effective reproductive number of the agent in the population (Tambi *et al.,* 2006). There are difficulties with movement control implementation in pastoral production systems due to their transhumance and socio-cultural practices (Masiga *et al.,* 1998; Alhaji and Babalobi, 2015; Alhaji and Babalobi, 2016b), as well as with stamping out policy because of the reluctance of owners to slaughter their animals and of governments to pay compensation (Mullins *et al.,* 2000; Thomson, 2005). It is, therefore, intuitively probable that the best approach to the control of CBPP would be to regularly vaccinate cattle in endemically infected areas and to treat clinical disease. In this way the benefits of both vaccination (creation of high levels of herd immunity) and treatment (enabling animals that would otherwise die or be seriously debilitated to recover) would hopefully act synergistically to reduce losses (Tambi *et al.,* 2006).

Economic information on CBPP in pastoral cattle herds of Niger State are not readily available and this is required for economic impact assessment necessary for development

of surveillance and control strategies specific to this herding system. This study was aimed at assessing the economic impacts of CBPP burden, returns to investments in its control programmes, and to the cost-effectiveness of the control interventions against the disease by vaccination and treatment interventions in pastoral cattle herds in Niger State, North-central Nigeria. These were carried out as ex-post costs evaluations of CBPP effects in single year to provide baseline estimates that will assist the state, national animal health authorities and the international donors in making investment decisions regarding costs of CBPP control in the state and Nigeria as a whole. The last mass CBPP vaccination campaign in the state was carried out in November 2011. Therefore, pastoralists had resorted to use of private veterinarians for vaccination and treatment of their herds with T1/44 vaccine and antibiotics. Even the previous campaigns before the 2011 exercise were irregular due to logistic problems.

Materials and Methods

Study area

Niger State is located in the North-central geopolitical zone, at the Southern Guinea Savanna ecological zone of Nigeria, between latitude 8°20' N and 11°30' N, and longitude 3°30'E and 7°20'E. The State is one of the 36 states of Nigeria, a gateway between Northern and South-western and South-southern parts of the country, and provides transit routes for pastoral nomads on seasonal migrations from the northern parts of the country to the southern parts and back. It shares a common international boundary with the Republic of Benin in its western border and has three Agro-ecological zones: A (southern zone with eight local government areas, LGAs), B (western zone with nine LGAs) and C (northern zone with eight LGAs) with variable climatic conditions. According to the Nigerian Livestock Resources Survey, Niger State has an estimated cattle population of about 1.165 million in 1991 (RIM, 1992; Bourn *et al.,* 1994) and about 2.4 million in 2012 (MLFD, 2014). These cattle are kept by nomadic and sedentary pastoralists.

Study design and population

A cross sectional interview-based questionnaire study was conducted in nomadic and sedentary cattle production systems in the state, to evaluate a one year operational CBPP economic burdens, benefit-cost and cost-effectiveness of the two independent control interventions preceding the time of the survey, which began in January 2014.

Target populations were the nomadic and sedentary households domiciled in the state during survey. Each household derived its socio-economic livelihoods mainly from herding cattle. Study eligibility was based on a participant being a household head or spouse. Participants had to be 30 years of age and above. They were expected at these ages to possess existing veterinary knowledge and traditional oral history on livestock health and production management (Meriner and Paskin, 2000).

For the purpose of this research, a nomadic pastoral household was defined as a household that kept mainly cattle, usually a large herd of one hundred cattle and above and took part in year-round long movements over large ranges for grazing without a permanent homestead. A sedentary (agro-pastoral) pastoral household was defined as a semi-settled household with less than one hundred cattle in herd, cultivating few crops, and having limited movements on low-range grazing within their environments.

Sample size determination and selection of participants

The simple random sampling method (Thrusfield, 2007) was used to determine the sample size for the pastoralist participants, with expected CBPP prevalence of 8.7% (Alhaji, 2011), at 95% confidence level and 5% margin of error. In mathematical notation: $n = Z2 \times Pexp(1 - Pexp) / d2$. Where: n, is the required sample size; Z2, is standard deviation at 95% confidence level (1.96); Pexp, is expected prevalence; and d is desired absolute precision. There were 125 herd owners who are the

primary sampling units that participated in the survey.

A two-stage sampling method was conducted to select pastoral herd owners. In the first stage, the state was stratified into the existing three agro-ecological zones A, B and C where LGAs in each zone were considered. In the second stage, 40 pastoral herds were selected in each of the Agro-ecological zones A and C (with eight LGAs each), and 45 in Agro-ecological zone B (with nine LGAs). In total, in all the zones, 125 pastoral cattle herds were randomly selected. The lists of herds from each of the LGAs in each zone were obtained from the Zonal Animal Health Officers. Herds visited were randomly selected from the lists and questionnaires were administered on heads (owners) of the selected herds.

Questionnaire development, pre-testing and data collection

A structured questionnaire was designed containing closed and open-ended questions to gather information on epidemiological and economic parameters. The questionnaire contained questions on the herd population, number of sick cattle (morbidity) in a year, estimated annual costs of treatment, estimated annual costs of vaccination, estimated annual costs of production losses, number of cattle lost (mortality) in a year, and market values of healthy and sick cattle.

The questionnaire was pre-tested prior to the study based on the recommendation that pre-testing should be performed in the same population in which the actual study is to be carried out (Thrusfield, 2007). Questionnaires were administered by the researchers on the selected pastoralists whose cattle herds were presumed to be single and independent of others. During the survey, each pastoral herder who attested CBPP clinical manifestations to be cough, rapid and difficult breathing, nasal discharge, hyperthermia, and sneezing in the questionnaire response were considered as appropriate understanding of clinical manifestations of the disease (Alhaji and Babalobi, 2016b). Understanding of this case definition by the respondents helped in getting good responses to other economic attribute questions.

Data were collected using semi-structured, interviewer-administered, paper-based questionnaires on pastoralists' demographics, knowledge about and attitudes towards CBPP control and socio-economic characteristics, such as estimates of costs of the control measures, losses, among others. Further information was concurrently obtained through interviews of the pastoralists on the composition of herds, husbandry management system and production losses attributable to the disease. Before commencement of each questionnaire administering and semi-structured interview, informed consent was verbally obtained from the respondents who were assured of voluntary participation, confidentiality of their responses and the opportunity to withdraw at any time without prejudice in line with the Helsinki Declaration (WMADH, 2001).

Costs and benefits

Only avoidable production losses due to morbidity and mortality from the disease were considered. Appropriate vaccination and treatment eliminates or reduces morbidity and mortality losses and the death/culls averted were considered as benefits, and the livestock values were measured in terms of their replacement costs. A CBPP-infected animal experiences a loss in productivity due to poor condition, lowered milk production, and a reduction in ability to work for draught animals. Elimination of the disease permits the animal to achieve its potential productivity.

The benefit–cost and cost-effectiveness analyses were limited to these costs as well as benefits that arose from the morbidity and mortality losses avoided. They only assumed a 'with control' program for CBPP.

Economic analyses

The generated economic data were analyzed using three economic models: total economic cost, benefit-cost analysis, and cost-effectiveness analysis. Total economic cost estimates the economic impact (burden) of the disease to pastoralists; benefit–cost analysis evaluates returns to investments in

CBPP control; and cost-effectiveness analysis evaluates the most cost effective intervention options against the disease. Financial costs tend to be most thoroughly used in these analyses due to relatively abundant livestock market prices information.

Economic burden

The economic burden was estimated in terms of total economic cost, which is the relationship between the value of production output lost in the presence of the disease and costs of disease controls applied. The total economic cost (C) of a livestock disease is the sum of the value of both direct and indirect production losses (L), and the control expenditures (E) (Rushton *et al.,* 1999; Tambi *et al.,* 2006). This is presented as:

$$C = L + E \quad \text{.....(1)}$$

Where: C, is the total economic cost; L, is the value of production losses (direct and indirect); and E, is the costs of control measures.

In this survey, only direct production losses were considered, and were estimated from mortalities (values of cattle that died or culled due to CBPP) and morbidities (estimated losses from dropped in sales of milk of lactating cows with CBPP signs, sales of emaciated cattle with CBPP signs, and decrease value of draught power in cattle with the disease). The definition of indirect losses varies from author to author; however, these are usually associated with reduced fertility, loss of market opportunities through trade bans, quarantine costs and delayed marketing (Alhaji and Babalobi, 2016b). However, indirect losses were not considered in this study because of difficulty in accessing and quantifying their variables since they are mostly intangible. Therefore, only direct costs were used.

CBPP control costs in this research included costs of vaccination, obtained from estimated costs of CBPP vaccines purchased and payments for vaccinating the animals by professionals, in both the nomadic and sedentary cattle herds, as well as costs of treatment of all sick cattle with antibiotics, obtained from estimated cost of antibiotics purchased and payments for administering the drugs by professionals. All production losses were valued using the approximated current market prices of cattle products and values of sick animals due to the disease in the respective localities during the period of survey.

Benefit-cost analysis

The benefit-cost analysis was presented in a benefit-cost ratio, which involves aggregating all costs associated with the control interventions and comparing these costs with the total value of benefits generated attributable to the interventions. Since costs and benefits occur over a period of time, these values were also appropriately discounted to account for the time value of money (Putt *et al.,* 1988; Rushton *et al.,* 1999; Tambi *et al.,* 2006; Rushton, 2009) from year 0 when the interventions were instituted to year 1.

Benefit-cost ratio (BCR) was computed by dividing the sum of the present value of benefits by the sum of the present value of costs as follows:

$$BCR = [\sum Bt / (1 + r)t] / [\sum Ct /(1 + r)t] \text{.....(2)}$$

Where: BCR, is the benefit–cost ratio; B, is the benefit accruing from the control intervention; C, is the cost of disease control; r, is the discount rate; and t, is the number of years in the future (0 to n years). A benefit–cost ratio greater than one (BCR>1) indicates that contagious bovine pleuropneumonia control was economically viable while a value below one (BCR<1) suggests that it was not beneficial.

Cost-effectiveness analysis

Cost-effectiveness analysis (CEA) was used to evaluate and compare the most cost-effective CBPP control strategy among treatment and vaccination options that were used by pastoral cattle herders. However, health outcomes in terms of number of morbidity and mortality averted with treatment or vaccination were considered. It relates cost to specific intervention effectiveness. CEA

model was presented in a cost-effectiveness ratio (CER), which is expressed by dividing total costs discounted by units of effectiveness (Briggs *et al.,* 1994; Fenwick *et al.,* 2006).

That is:

CER =Total cost/ Units of effectiveness…..….(3)

Unit of effectiveness (benefits or number of dropouts averted) was considered as a measure of quantifiable outcomes central to the control's objectives, which is the number of cattle deaths/culls averted with application of treatment or vaccination per Nigerian naira (NGN) value. Those cattle saved from dying/culling were considered as assumed benefits. They are actual benefits because they were really presumed to be prevented by control interventions of treatment or vaccination.

Average cost-effectiveness ratio (ACER), one of the analytical tools of cost-effectiveness analysis, was used. It is defined as total implementation costs divided by the total benefits. It deals with interventions that are independent of each other; a single intervention at a time and evaluates that intervention against its baseline option (common practice) (Fenwick *et al.,* 2006).

The ACER is expressed thus:

ACER = PVC / Units of effectiveness…...….(4)

Where: PVC, is the present value of costs discounted; and Unit of effectiveness, is the number of death/cull averted or live cattle saved.

Key field data obtained from pastoralists

Key estimates of epidemiological variables and parameters obtained from the field survey were used for the economic analyses. These include:

a. Total number of herds surveyed: 125 herds or households [nomadic - 81 (64.8%) and sedentary – 44(35.2%)];
b. Total cattle population in the herds: 9,979 cattle [nomadic – 8,634 (86.6%) and sedentary – 1,338 (13.4%);
c. Total number of sick animals due to CBPP treated with antibiotics in the two husbandry management systems: 2,320 heads;
d. Total annual cost of treating all sick cattle with antibiotics: 5,464,330.0 NGN;
e. Total number of cattle apparently healthy and vaccinated in the herds: 7,659 heads [nomadic - 6,634(86.6%) and sedentary - 1,025(13.4%)];
f. Total annual cost of vaccinating these herds: 769,820.0 NGN;
g. Estimated costs of decreased production (dropped in milk, weight loss, etc) due to CBPP in surveyed herds in one year: 9,110,000.0 NGN (nomadic - 5,280,000.0 NGN and sedentary - 3,830,000.0 NGN);
h. Number of cattle loss (deaths/culls) in the surveyed herds in one year: 289 heads [(nomadic – 224 (77.5%) and sedentary – 65 (22.5%)]
i. Social discount rate of 10% was used (Putt *et al.,* 1988).
j. Number of years in the future: one year for the health management interventions (treatment with antibiotics is a routine practice, while CBPP vaccination campaign is often done annual in Nigeria).

Discounting

To put all relevant costs and benefits on a common temporal footing for benefit-cost and cost-effectiveness analyses of the two interventions, we used time value of money, by converting the future expected streams of costs and benefits in one year time into present value amount in year zero using 10% discount rate. The discounting process is presented thus:

$$PV=FV/(1+r)t$$

$$PV = FV * (1 + r)\text{-}t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

Where: PV, is the present value; FV, is the value of amount of money in years ahead; r, is the social discount rate; and t, is the number of

years from the present date. When all benefits and costs are converted to present values, comparison is possible especially on decision-making criteria for cost-effectiveness ratio and benefit-cost ratio.

Sensitivity analysis

Responses from the participants were based on their existing veterinary knowledge, which sometimes may not be accurate (estimates). Because BCA and CEA must rely on estimates (assumptions) that are sometimes best guesses, it is critical that they contain an explicit sensitivity analysis that discusses key assumptions in the standard base case and varies those assumptions to see how a change affects the analysis (Fenwick *et al.,* 2006). Discount rate parameter, which determined certain level of variability with potentials of affecting the study results, was used. This was subjected to one-way deterministic sensitivity analysis, an approach that varies only one assumption or one parameter at a time, holding all else constant to assess its impact and determine the robustness of the results. The values were recalculated using 5% lower limit (best case scenario) and 15% upper limit (the worst case scenario) discount rates for a year for both costs and benefits of benefit-cost ratio and costs of average cost-effectiveness ratio.

Exchange rates

Central Bank of Nigeria (CBN) exchange rates of One Hundred and Fifty Six Nigerian naira (156 NGN) to One American dollar (1 USD) and 212 NGN to 1 EUR were used (CBN, 2014).

Results

Demographic information

A total of 125 pastoralists participated in the survey, with mean age of 52.1±10.9 SD, and the majorities (30.4%) were in the age group of 41-50 years. All respondents were married and majority (97.6%) was male. Majority (64.8%) was of Fulani tribe and most (66.4%) were nomadic pastoralists. Other tribes that participated in the survey were Nupe (7.2%), Hausa (12.8%) and other tribes (15.2%), who were sedentary pastoralists. Over two-thirds (69.9%) never had formal education, whilst in all 31.1% had formal education, however 8.0% stopped at primary level, 10.4% went to get secondary education, and 12.8% progressed further to obtain tertiary education.

Total economic cost

The total value of output loss incurred by pastoralists engaged in the two production systems was estimated to be 254,837.3 USD, giving an average of 2,038.7 USD per herd (Table 1). The value of losses due to morbidity (production losses) accounted for 14% of the total value of losses while mortality losses accounted for 86%. The physical losses are the results of morbidity and mortality associated with the disease. Morbidity losses in this survey had two components. The first was the declining productivity that led to losses in milk production, meat production (or live weight), and draught power. The second was the loss of output as a result of animals' dead or culled.

The total annual economic impact of CBPP in both nomadic and sedentary pastoral cattle herds in Niger State, Nigeria was estimated at 294,800.3 USD. The average annual economic cost per herd was 2,358.4 USD (Table 2).

Benefit-cost returns to investments

The avoided losses were also presented as benefits together with the costs of control and net benefits. The estimates showed that investment of 39,963.0 USD in CBPP control yielded a gross return of 254,837.3 USD and a net benefit of 214,875.0 USD. This was equivalent to a net benefit of 1,719.0 USD for a herd in any of the production systems. The annual benefit-cost ratio from the control of CBPP by both vaccination and treatment in the pastoral cattle herds was 6.4 (Table 3). The value was positive and therefore the two control methods were economically viable.

Table 1: Estimated value of annual losses (in USD) in cattle (deaths/culls) and cattle production losses due to contagious bovine pleuropneumonia in 125 pastoral cattle herds of Niger State, Nigeria

Production system	Value of cattle culled/death	Value of production losses	Total losses
Nomadic	144,038.5	32,485.3	176,523.8
Sedentary	75,000.0	3,313.5	78,313.5
Total	219,038.5	35,598.8	254,837.3
Mean/herd	1,752.3	284.8	2,038.7

Table 2: Estimated annual economic cost (in USD) of contagious bovine pleuropneumonia in 125 pastoral cattle herds of Niger State, Nigeria

Production system	Total value of losses	CBPP control cost by vaccination	CBPP control cost by treatment	Total economic cost
Nomadic	176,523.8	4,502.6	31,198.1	212,224.5
Sedentary	78,313.5	432.1	3,830.2	82,575.8
Total	254,837.3	4,934.7	35,028.3	294,800.3
Mean/herd	2,038.7	39.5	280.2	2,358.4

Table 3: Estimated annual benefits and costs (in USD) of contagious bovine pleuropneumonia control by both treatment and vaccination in 125 pastoral cattle herds of Niger State, Nigeria

Production system	Undiscounted benefits	Discounted benefits	Undiscounted costs	Discounted costs	Net benefits	Benefit-cost ratio
Nomadic	176,523.8	160,476.3	35,700.4	32,455.2	140,823.4	4.9
Sedentary	78,313.5	71,194.3	4,262.2	3,874.4	74,051.3	18.4
Total	254,837.3	231,670.6	39,962.5	36,329.6	214,875.0	6.4
Mean/herd	2,038.7	1,853.4	319.7	290.6	1,719.0	

Table 4: Estimated annual cost-effectiveness (in USD/live cattle saved) of contagious bovine pleuropneumonia interventions in 125 pastoral cattle herds of Niger State, Nigeria

Intervention	Undiscounted cost	Discounted cost	Benefit (deaths/culls averted)	ACER (USD/life saved)
Treatment	35,027.8	31,843.4	2,320	13.7
Vaccination	4,934.8	4,486.1	7,659	0.6

Cost-effectiveness of CBPP interventions

The ACER outcome value for treatment was 13.7 USD per life saved) and that of vaccination that was 0.6 USD per death/cull averted (Table IV). There is an indication that there was a cost saving with vaccination option compared with treatment option, meaning that vaccination was less costly and with simple dominance and could be more effective than treatment at the perspective of the pastoralists. One intervention is more cost effective if it is less costly with equal or better outcome or, it is more costly with better outcomes, and the added benefit is worth the added cost.

Partial deterministic sensitivity analysis

This exercise re-estimates the benefit-cost ratio and average cost-effectiveness ratio for the treatment and vaccination by adopting lower and upper limits (5% and 15%) for discount

rate parameter. The outcomes of benefit-cost ratio with both scenarios (discount rates) did not apparently vary, as BCR remained 6.4, but relatively varied in average cost-effectiveness ratio, in which 5% discount for treatment was 14.4 USD/life saved and vaccination was 0.6 USD/life saved; and 15% discount treatment was 13.1 USD/cattle saved and vaccination was 0.6 USD/life saved).

Discussion

Increasing pastoralists' awareness about costs of CBPP mitigation and benefits accruing from interventions has been a major issue of concern. This cross-sectional study has provided a useful means for collecting economic information about the disease, assessment of its economic burden and return to investments in control in the pastoral cattle herds of Niger State, Nigeria.

The total annual economic impact of CBPP due to production losses and costs of control measures from treatment and vaccination to pastoralists in the state was estimated to be 294,800.3 USD (216,928.2 EUR) (CBN, 2014). This is very high for Niger State, which is a unit state out of 36 states in Nigeria, when compared with the Tambi et al. reported total estimated economic cost of 45 million EUR for CBPP in twelve countries with average economic cost of 3.7 million EUR per country (Tambi *et al.,* 2006). Some authors have reported variable economic burdens of the disease. Osyemi (1981) reported economic losses due to CBPP to be 3.6 million USD in Nigeria, while Egwu et al. (1996) estimated the direct economic cost of CBPP in the northern part of Nigeria to be 1.5 million USD. Furthermore, Townsend et al. (1998) estimated the total cost to the Botswana economy to be 350 million USD due to decline in beef and other products, and Masiga et al. (1998) estimated the annual losses directly or indirectly attributable to CBPP in Africa to be around 1.2 billion USD. Further, Jiuqing et al. (Jiuqing *et al.,* 2011) also reported that between 1949 and 1989, China lost an estimated 178,570 cattle due to CBPP estimated at 33.5 million USD. All these overviews indicate that CBPP is a disease of very high economic significance and every possible effort must be made to control it.

It is important to note that in this survey about 86.4% of the economic cost of CBPP was due to morbidity and mortality losses while the remaining 13.6% was due to the cost of disease control. The total cost of disease control was estimated to be 39,963.0 USD with the cost of treatment accounting for 87.7% and cost of vaccination accounting for 12.3%. These estimates suggested that by spending 39,963.0 USD (29,406.4 EUR) annually to control CBPP in the state, a net loss of about 254,837.3 USD (187,521.8 EUR) would be averted, translating to average annual averted loss of a 2,038.7 USD (1,509.4 EUR) in each herd if CBPP were to be controlled using only treatment and vaccination.

CBPP was found to be a disease of high economic importance because of the observed considerable losses in productivity that translated to heavy financial losses. This observation was in consonant with the reports of Aliyu and Kyari (2005) on the heavy financial losses due to this disease in northern Nigeria. In 2003, the Nigeria Animal Diseases Information System under the auspices of the Pan-African Programme for the Control of Epizootics (PACE) classified Nigeria as CBPP infected zone based on her CBPP status (PACE, 2003).

Estimates of annual benefit–cost ratios of CBPP control by treatment and vaccination were found to be beneficiary economically to pastoralists in the state. The avoided losses were presented as benefits together with control costs and net benefits. The estimates showed that an investment of 39,963.0 USD on CBPP control in Niger State yielded a gross return of 254,837.3 USD and a net benefit of 214,875.0 USD. This was equivalent to a net benefit of 1,719.0 USD per herd in the state. The return to investment was positive in both nomadic and sedentary production systems with an overall benefit–cost ratio of 6.4. The obtained BCR value in this survey was higher than the values obtained in other African countries: Burkina Faso 1.91, Chad 1.61, Côte d'Ivoire

1.59, Ethiopia 2.19, Ghana 1.66, Guinea 1.98, Kenya 2.56, Mali 1.85, Mauritania 1.95 Niger 1.95, Tanzania 2.24, and Uganda 1.80 (Tambi *et al.,* 2006). The survey economic estimates revealed that CBPP control by treatment with antibiotics and vaccination was economically beneficial to pastoralists. Nevertheless, the estimated benefits greatly underestimated the actual benefits that would be attained if movement control, stamping out, quarantine and surveillance were to be instituted in the state. Tambi et al. reported CBPP control by stamping out followed by strict movement control to be the most effective and beneficial control and eradication strategy (Tambi *et al.,* 2006). However, because of the cost involved and the fact that Niger State government lacks the financial resources to compensate pastoralists, these options are currently not feasible, not only in the state but in Nigeria at large. And even if total cost is not put into consideration by the state, instituting effective movement control would still be difficult since the state's borders stretch for hundreds of kilometers with other states and the Republic of Benin.

In comparing the ACER outcomes, ACER value for treatment intervention was found to be 13.7 USD per death/cull averted or life saved, while the value for vaccination option was 0.6 USD per deaths and/or culls averted or life saved. There was an indication of cost saving for vaccination option over treatment option, meaning that vaccination was less costly and effective in protecting (saving) cattle lives at individual pastoral herd level.

Cost effective interventions and benefit-cost return on CBPP control investment were subjected to the best and worst scenarios sensitivity analysis using only discount rate parameter in both cases. The outcomes of sensitivity analysis of benefit-cost ratio with both scenarios did not change, BCR remains 6.38. However, these relatively varied with average cost-effectiveness ratio, in which 5% discount for treatment was 14.4 USD/life saved and vaccination was 0.6 USD/life saved; while with 15% discount treatment was 13.1 USD/cattle saved and vaccination was 0.6 USD/life saved). This indicates that variation in discount parameter in BCR did not significantly influence overall results but did in ACER. In the later model it provided estimate threshold values above and below, of which the highest discount would be preferred because it gave a lower cost for the intervention with same output effectiveness.

It is safe to say that vaccination was attained at a lower cost than treatment, making it a more cost effective and beneficial control investment. The reduced cost-effectiveness of treatment may make it to be excluded so that it does not consume limited resources in possession of the poor pastoralists. Treatment should be excluded because it cannot provide good outcomes in terms of elimination of sequestrated lesions in chronic cases (Provost *et al.,* 1987), and therefore whatever added benefit from its application may not be worth the added cost. This view has the support of observations made by Gekonge (1990) and Oyaya and Rifkin (2003), who considered that non dominant intervention may be excluded to save limited available resources. Further, vaccination may have been more cost-effective but not without its own short-comings, as the conventional annual mass vaccination campaigns are often associated with complex logistics and result in irregular coverage of pastoral cattle in subpopulations. In the absence of comprehensive vaccination coverage, these pastoral foci are often left to the treatment option with antibiotics.

Arguments can be made for and against the variables used for burden, benefit-cost and cost-effectiveness assessments, yet obtained data are still the best in the circumstance of the economic evaluation approaches in veterinary practice at the local rural settlements because most of these herds are nomadic in nature. What was obtained in this study was a good epidemiological survey. Tambi et al. (2004) observed that disease intelligence information from a good epidemiological surveillance system is essential for developing a cost-effective animal disease control program from selective low cost interventions as opposed to a blind intervention using mass vaccination.

Both benefit-cost and cost-effectiveness tools are user-friendly economic models, as they use benefit and effectiveness of measures that reflect interests of people involved in the processes and that can be estimated and used for real-time decision-making.

In assessments of animal health and production, benefit-cost analysis can be used at both ex-ante appraisals and ex-post evaluations, where costs and benefits are estimated in monetary terms, such as programs dealing with livestock diseases (Goldbach and Alban, 2006). But when benefits include welfare changes for humans and/or animals that are complex to measure in monetary terms, CEA is the best alternative tool that can capture more appropriately the desired outcomes from a change such as programs that target control of zoonoses and interventions in diseases of high economic importance, with an impact on animal welfare, human health or on the environment (Lyons *et al.,* 2012; Babo Martins and Rushton, 2014).

The values presented in this study are mainly estimations of the economic impacts of the disease and the figures may grossly underestimate its real economic impact considering that only two control options were considered, which underscore the economic interest in controlling CBPP in the state. In view of the limitations of this study, there may be need for a more detailed study that will consider other control methods such as movement control, stamping out and epidemio-surveillance to derive reliable data on the economic impact of CBPP with backup sensitivity analysis of the different parameters to take care of uncertainties or assumptions where necessary. The limitations notwithstanding, we believe our findings represent an alarming paucity of evidence and reflect the concerns of others in the field that there is a gap in economic assessment research on CBPP interventions in developing countries. This cost-effectiveness study is the first of its kind on CBPP control interventions as no report of this nature, to our knowledge, has been documented in the recent past. Since no study has been documented on the cost-effectiveness of the disease control methods, it was not possible to make comparisons.

Conclusion

CBPP is a disease that causes high morbidity and mortality losses to pastoral cattle herds in Nigeria. This economic assessment was undertaken to estimate the economic cost of CBPP and the benefits of its control in pastoral cattle herds of Niger State, Nigeria. Following the growing interest of policymakers and animal health stakeholders in improving the access and quality of cattle production in sub-Saharan Africa, this empirical research conducted on the total economic cost, benefit-cost and cost-effectiveness of CBPP controls by treatment and vaccination in pastoral cattle herds is required and timely. However, much can be learned about an intervention in creating a framework to consider benefits and costs: simply attempting to identify them, measure them, and value them can provide important information for the decision maker. Adding a sensitivity analysis and a clear explanation of each assumption (estimate) and estimate, CEA and CBA can be extremely effective tools in CBPP and other trans-boundary animal diseases control interventions evaluation. The financial and economic losses CBPP causes to pastoral cattle herders in the state have been shown to be so enormous that the disease cannot be left uncontrolled. Control of this disease is therefore important as a way to salvage the losses and increase the incomes of cattle pastoralists, not only in Niger State but also in Nigeria as a whole.

Acknowledgments

The authors are grateful to the Area Animal Health Officers of the Niger State Ministry of Livestock and Fisheries Development, for their assistance during samples collection.

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THE RESPONSES OF THREE NIGERIAN INDIGENOUS GOAT BREEDS TO PRIMARY AND SECONDARY EXPERIMENTAL CHALLENGES WITH *HAEMONCHUS CONTORTUS*

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Abstract

A study was designed to investigate if an initial infection is sufficiently protective to prevent the development of subsequent re-infection with *H. contortus*, and to determine whether or not the removal of primary infection could enhance stronger acquired immunity which is protective enough to prevent the establishment of a secondary infection. Responses of three Nigerian breeds of goat; Red Sokoto (RS), West African Dwarf (WAD) and Sahel White (SW) were investigated following primary and secondary experimental infections with *Haemonchus contortus*. Forty five (45) goats, (n=15) of each breed type were used in the experiment. During the primary challenge, (n=11) goats each of the three breeds were infected with 100 (L3) larvae of *H. contortus* by gavage weekly for 7 weeks, while four (4) goats each per breed served as control. On day 42 post infection, (n=12) goats, (4 per breed) of the infected animals were humanely euthanized and worm count determined. The second phase was carried out on the remaining twenty one (21) infected goats. They received the weekly infective dose of *H. contortus* by gavage up to week 10, when they were divided into 2 groups. The first group of 12 (4 SW, 4 WAD and 4 RS) animals were untreated, while the second group of 9 (3 per breed) animals were treated. The 2 groups were further reinfected with 2000 L3 of *H.contortus* each for a period of 7 consecutive weeks after a rest period of ten days. Clinical signs, worm establishment rate, faecal egg count (FEC), packed cell volume (PCV), eosinophil count, total serum protein and C3 complement were determined. The PCV, C3 complement and total serum protein of infected animals declined significantly ($p<0.05$) when compared to the control, after both primary and secondary challenges, irrespective of breed. Conversely, eosinophil count increased significantly ($p<0.05$). WAD showed improved parameters than other breeds, indicating better adaptability or greater resistance than the other breeds.

Keywords: Haemonchosis, Haematology, Nigerian goats, Primary and secondary infections, Resistance, Responses.

LA RÉPONSE DE TROIS RACES DE CHÈVRES INDIGÈNES NIGÉRIANES AUX INFECTIONS EXPÉRIMENTALES PRIMAIRES ET SECONDAIRES AVEC HAEMONCHUS CONTORTUS

Résumé

Une étude a été conçue dans le but de déterminer si une infection initiale était suffisamment protectrice pour prévenir le développement d'une réinfection ultérieure par H. contortus, et si l'élimination de l'infection primaire pourrait ou non améliorer la forte immunité acquise qui est suffisamment plus protectrice pour empêcher le maintien d'une infection secondaire. Les réactions de trois races nigérianes de chèvre – chèvre rouge de Sokoto (RS : Red Sokoto), chèvre naine d'Afrique de l'Ouest (WAD : West

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African Dwarf) et chèvre blanche du Sahel (SW : Sahel White) - ont été étudiées après des infections expérimentales primaires et secondaires avec Haemonchus contortus. Quarante-cinq (45) chèvres, (n = 15) de chaque type de race ont été utilisées dans l'expérience. Au cours de la première infection expérimentale les chèvres (n = 11) de chacune des trois races ont été infectées avec 100 (L3) larves de H. contortus par gavage chaque semaine pendant 7 semaines, tandis que quatre (4) chèvres par race ont servi de témoins. Au jour 42 post-infection, les chèvres (n = 12) - 4 par race - des animaux infectés ont été euthanasiées sans cruauté et le nombre de vers a été déterminé. La deuxième phase a été réalisée sur les vingt-et-une (21) autres chèvres infectées. Elles ont reçu la dose infectieuse hebdomadaire de H. contortus par gavage jusqu'à la semaine 10, et à cette période elles ont été divisées en 2 groupes. Le premier groupe de 12 animaux (4 SW, 4 WAD et 4 RS) n'a pas été traité, tandis que le deuxième groupe de 9 animaux (3 par race) a été traité. Les 2 groupes ont été encore une fois réinfectés avec 2000 L3 de *H.contortus* pendant une période de 7 semaines consécutives après une période de repos de dix jours. Les signes cliniques, le taux de maintien des vers, le nombre d'œufs fécaux (FEC), l'hématocrite (PCV), le nombre d'éosinophiles, la protéine sérique totale et le complément C3 ont été déterminés. L'hématocrite, le complément C3 et la protéine sérique totale des animaux infectés ont diminué de manière significative ($p < 0,05$) par rapport à ceux du groupe témoin après les infections primaires et secondaires, quelle que soit leur race. À l'inverse, le nombre d'éosinophiles a augmenté de façon significative ($p < 0,05$). Les WAD ont montré des paramètres améliorés par rapport aux autres races, ce qui porte à croire qu'elles ont une meilleure adaptabilité ou une plus grande résistance par rapport aux autres races.

Mots-clés : haémonchose, hématologie, chèvres nigérianes, infections primaires et secondaires, résistance, réactions.

Introduction

Caprine parasitic gastro-intestinal infection is worldwide in distribution (Chiejina *et al.,* 2002; Chiezey *et al.,* 2008), most importantly in Africa and other tropical and sub tropical regions. In Nigeria, parasitic gastroenteritis is mainly caused by several genera of nematode, with the genus Haemonchus been the most prevalent (Ikeme, 1997).

The responses of different breeds of goat have been previously studied under experimental condition (Fakae *et al.,* 1999; Bambou *et al.,* 2008; Idika *et al.,* 2012; Bambou *et al.,* 2013), with varied results from such studies. Most of such studies in Nigeria have been mainly on WAD, as other breeds have not been fully investigated. Similarly, acquired immunity was previously shown to be enhanced following routine truncated primary infection in goats and sheep (Emery, 1996; Stankiewiez *et al.,* 1996; Hooda *et al.,* 1999). This method of inducing immunity to haemonchosis was found to be very effective in the East African goat breed and the crosses between the East African and Tuggenberg (Hooda *et al.,* 1999). In contrast, it was found to be ineffective in the stimulation of acquired immunity to challenge infections in goats and sheep on the same pasture (Pomroy *et al.,* 1986). In addition, a significant immunity was reported in WAD goats following serial experimental challenges with *H. contortus* when compared to other Nigerian indigenous breeds (Sonibare *et al.,* 2011). Furthermore, the relative resistance of Red Sokoto goat compared to Sahel goat, when exposed to trickle infection with *Haemonchus contortus* has been reported by Makun et al. (2008). These findings thus indicate that serial infection may be a viable option for selecting resistant goats. The current study was therefore designed to investigate if an initial infection is sufficiently protective to prevent the development of subsequent re-infection with *H. contortus*, and to determine whether or not the removal of primary infection could enhance stronger acquired immunity which is protective enough to prevent the establishment of a secondary infection. Also, breed variations in response to infection was studied by monitoring clinical, haematological, parasitological and biochemical parameters.

Materials and Methods

Study Location

The study location is Abeokuta, Ogun State-Nigeria, located in the rain forest vegetation zone of South-western Nigeria on Latitude 70 13' 49.46" N, Longitude 30 26' 11.98" E and at an altitude of 76 m above the sea level (Google earth, 2014).

Experimental Animals

Forty five goats aged 5-6 months and weighing between 4 and 6.5 kg and (15 per breed) consisting of WAD, RS and SW were used for the experiment. Breed wise, WAD weighed 4-4.5 kg, RS 5-6 kg, while SW 6-6.5 kg. Identification of the breeds was as previously described (Porter and Mason, 2002).

Acclimatization and Pre-experimental Screening

Experimental animals were allowed thirty days to acclimatize before the commencement of experiment, during which they were screened for gastrointestinal, ecto- and haemoparasites using standard protocol (Urquhart *et al.,* 1996), while faecal egg culture was also carried out for the specific identification of helminth infective third stage larvae (L3) as described by Zajac and Garya (2006). Blood samples were collected in order to screen for haemoparasites. Goats with parasitisms were individually treated with Ivermectin given at 0.2 mg/kg once, Trimethoprim (200 mg) + Sulphadiazine (100 mg) orally for three days, Diaminazene aceturate (Berenil®) at 3.5 mg/kg and Cypermethrine. They were similarly vaccinated with homologous Peste des petits ruminants (PPR) vaccine (National Veterinary Research Institute, Vom, Jos, Nigeria). Tsetse fly traps were strategically located around the pen houses to trap tsetse flies in the environment.

Housing and Feeding

Experimental animals according to breed type were housed in wooden pens with a slatted floor which ensured that animals did not have access to the faeces. Their respective controls were in confined pens based on breed type. All pens were netted with fly-proof nets and fly-proof aluminium netting placed underneath the slatted floor for total recovery of faeces from each pen.

The animals were initially fed with groundnut haulms during the period of acclimatization, which was gradually withdrawn and replaced with dried Guinea and Elephant grasses previously harvested from fields that had not been grazed by livestock, washed and sun dried. This was supplemented by Concentrate feed. Salt lick and water were provided *adlibitum* throughout the period of the experiment.

Source of Parasite

The L3 larvae of *H. contortus* were obtained as previously described (Sonibare *et al.,* 2012). Briefly, active gravid females were isolated from the abomasums of *H.contortus* infected goats at the Abeokuta abattoir and identified based on vulval morphology as described by (Urquhart *et al.,* 1996). They were subsequently inoculated directly into abomasa of worm free lamb following right side laparatomy as described previously (Fubini and Ducharme, 2004).

Faecal Culture and L3 Harvest

Faeces harvested from the lamb were coprocultured to obtain pure third stage infective larvae according to MAFF (1986). The infective larvae were maintained as described by Hubert and Kerboeuf (1992). Estimation of the number of infective larvae as well as infectivity and dosages were determined according to MAFF (1986).

Experimental Design

The protocol for this study was approved by the Ethical Review Committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria and was carried out according to International guiding principles for biochemical research involving animals (CIOMS 1985). In the first phase (primary challenge), the forty five goats were randomly divided into three groups of fifteen animals each, based on breed type. Eleven (11) goats of each breed type were

individually infected with 100 *H. contortus* L3 larvae given by gavage weekly for 7 consecutive weeks (days 0, 7, 14, 21, 28, 35, 42), while the remaining four (4) animals of each breed were designated as controls for the respective breeds. On day 42 of the primary challenge, 12 goats (4 per breed) with obvious signs of infection were humanely euthanized, necropsied and worm count determined as described by Cock and Halliwell (2002). The remaining 21 infected goats continued to receive 100 *H. contortus* L3 on days 49, 56 and 63. In the second phase of the experiment (secondary challenge), the 21 infected animals (From the primary challenge phase) were divided into two groups; the first group consisted of 12 (4 SW, 4 WAD and 4 RS) goats and were left untreated, while the remaining nine (9) (3 per breed) were treated with ivermectin (0.2 mg/kg) on day 66 of the primary challenge. Both groups were then rested for 10 days and further re-infected (secondary challenge) with a higher dose of 2000 L3 larvae by gavage weekly for 7 consecutive weeks.

Clinical Observations and Sample Collection

Experimental animals were observed for signs of haemonchosis after both primary and secondary infections.

Faecal sample collection and examination

Faecal materials were collected directly from the rectum on days (21, 22, 23, 24, 25, 26 and 28) and thereafter weekly up to day 42 post infection, for faecal egg count determination using floatation technique according to MAFF (1986).

Blood Collection and Processing for Haematological Parameters

Five mililtres of blood was collected from the jugular vein of both infected and control goats into EDTA bottles weekly for 7 weeks each during both primary and secondary challenges. PCV was determined on days (0, 7, 14, 21, 28, 35, and 42), while eosinophil was determined on days (0, 7, 14 and 28) of both primary and secondary challenges. Both parameters were determined according to standard procedure of Dacie and Lewis (1995).

Blood Collection for Serum biochemical Parameters

Five milliliters of blood was collected into plain sterile sample bottles. The blood samples were allowed to clot at room temperature for three hours, after which blood clots were removed and serum centrifuged at 900 x g for 5 minutes. Sera samples were preserved at -20°C until use.

Complement C3 was determined as described by Hudson and Hay (1980) immediately after separation of serum. TSP was determined as described by Toro and Ackermann (1975). Both parameters were measured on days 0, 7, 14 and 28 post-primary and secondary challenges

Humane Sacrifice and Determination of % Worm Established

A total of twelve (12) and Nine (9) infected goats were respectively sacrificed humanely during the primary and secondary challenges for the determination of worm establishment rate.

Statistical Analysis

Generated data were analysed using the statistical analysis system (SAS, 2003) package version 8.0 developed in 2003. The data were tested for significant differences between group means using analysis of variance (ANOVA) and means were separated using Duncan multiple range test.

Results

Clinical observations

No clinical signs attributable to haemonchosis were observed in the first 14 days of primary infection among the breeds. By day 28 post infection, 54% (6/11), 45% (5/11) and 18% (2/11) of infected SW, RS and WAD goats respectively showed signs typical of haemonchosis. Similarly, by day 42 of the primary challenge, one SW goat had complete loss of appetite with progressive depression, weakness and pallor of visible mucous membranes, while one RS goat exhibited

Table 1: Mean (±SEM) Faecal egg count of three Nigerian goat breeds following primary challenge with *Haemonchus contortus*

Breed (n=11) Primary challenge	Days Post-Infection								
	21	22	23	24	25	26	28	35	42
WAD	200±100^{a}	733±284^{a}	2066±451^{ab}	2933±338^{ab}	3250±534^{ab}	3350±571^{ab}	5833±781^{b}	4483±909^{b}	7000±2578^{bc}
RS	1767±525^{a}	2633± 695^{ab}	2783±75^{ab}	2700±711^{ab}	4033±806^{ab}	4567±906^{ab}	6066±1743^{b}	7800±2184^{b}	9000±3775^{bc}
SW	917±436^{a}	1700±725^{ab}	2067±451^{ab}	2933±338^{ab}	3250±534^{ab}	4350±571^{ab}	6516±1022^{b}	7750±2167^{b}	9500±4900^{bc}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW. Different superscripts in rows or columns differed significantly (P□0.05)

Table 2: Mean (±SEM) Faecal egg count of three Nigerian indigenous goat breeds following secondary challenge with *Haemonchus contortus*

Breeds	Days Post-Infection						
Secondary Challenge & treated (n=3)	**0**	**7**	**14**	**21**	**28**	**35**	**42**
WAD	6500±436^{a}	9000±725^{ab}	9250±451^{ab}	7560±209^{ab}	5456±1022^{abc}	5690±106^{abc}	5100±801^{abc}
RS	6000±390^{a}	7600± 300^{ab}	9500±39^{ab}	6800±501^{abc}	6066±1003^{abc}	6600±1680^{abc}	5900±101^{abc}
SW	8200±300^{a}	9800±414^{ab}	9500±349^{ab}	8600±650^{ab}	6833±1200^{abc}	6483±909^{abc}	6010±903^{abc}
Challenge & untreated:							
WAD (n=4)	7080±1436^{b}	8900±1650^{b}	7900±2250^{b}	6530±1750^{b}	4700±2050^{bc}	3980±1900^{bc}	3500±741^{bc}
RS (n=4)	5800±1750^{b}	6504±2050^{b}	7500±2450^{b}	7290±2650^{b}	6980±2250^{b}	4900±2430^{bc}	3857±899^{bc}
SW (n=4)	6300±1500^{b}	7900±2800^{b}	7360±2950^{b}	7600±2050^{b}	7290±2780^{b}	6790±2850^{b}	6000±1500^{b}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW,
Different superscripts in rows or columns differed significantly (P□0.05)

Table 3: Mean (±SD) Worm Establishment rate at necropsy by day 42 post-infection of three Nigerian goat breeds following primary and secondary challenges with *Haemonchus contortus*

Infection	Breeds			
	WAD	RS	SW	Mean Total
Primary Challenge:	342.00±9.45[b]	350.00±50.00[b]	413.00±20.81[a]	368.00±43.48[b]
Secondary Challenge & treated:	338.33±18.39[b]	440.00±20.00[b]	666.67±15.77[b]	481.67±146.24[b]
Secondary Challenge & untreated:	250.00±33.67[c]	312.50±20.60[c]	352.00±30.40[b]	305.00±42.60[b]

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW,

Different superscripts in rows or columns differed significantly (P<0.05)

opisthonous with intense grunting few hours before it was humanely sacrificed. Intermittent anorexia, slight depression, slightly pale mucous membrane of the eyes with rough coats were also observed among two WAD goats by day 42 post infection.

Following secondary challenge with or without treatment of the primary infection, severity of infection was breed dependent as intense clinical presentation was observed in SW, less intense initial infection that later became severe in RS, while most WAD goats remained agile and active without any obvious signs of haemonchosis until they were sacrificed.

Faecal Egg Count and Worm Establishment Rate

The results of the faecal egg count of three indigenous (SW, RS and WAD) goat breeds following primary and secondary challenges with *Haemonchus contortus* are presented in Tables 1 and 2. Following primary challenge, the faecal egg count increased across the days in all the infected animals, with higher counts in SW and RS than WAD. Similarly, the same pattern of change was observed following secondary infection and treatment. A significant ($p<0.05$) higher egg count was observed in SW and RS, when compared to WAD (Table 1). Among infected animals that survived primary challenge and remained untreated, there was progressive increase in the faecal egg count in the first 14 days irrespective of breed. This was subsequently followed by decrease across the days up to day 42 post infection.

The results of the Haemonchus worm establishment rate are presented in Table 3. The worm establishment rate was significantly ($p<0.05$) low among untreated animals that survived primary infection when compared to treated animals and animals that have undergone primary challenge only. This pattern was observed for all the breeds. Furthermore, establishment rate following primary challenge was significantly ($p<0.05$) higher in SW than RS and WAD. Also, following secondary challenge with or without treatment, significantly ($p<0.05$) higher establishment rate was found among animals of SW and RS breeds when compared to WAD.

Packed Cell Volume

Irrespective of breed, infected animals did not show any significant variation ($p\square 0.05$) up to day 21 post infection, following primary challenge (Table 4). However, there was a statistically ($p\square 0.05$) significant decline on day 28 and thereafter to the end of the primary challenge. Animals that were secondarily challenged and treated showed similar pattern with the primary challenge (Table 4).

Most of the statistically significant ($p\square 0.05$) changes observed among untreated secondarily infected animals of all breeds were on days 28, 35 and 42 (Table 4).

C3 Complement, Eosinophil and Total Serum Protein

The results of C3 complement, eosinophil and total serum protein of three indigenous goat breeds following primary and

Table 4: Mean (±SEM) Packed cell volume (%) of three Nigerian indigenous goat breeds following primary and secondary challenges with *Haemonchus contortus* and their respective controls

Breeds	Days Post-Infection						
	0	7	14	21	28	35	42
Primary challenge							
WAD (n=11)	32.20±3.82^{a}	32.23±3.54^{a}	26.80±2.42^{a}	25.00±1.79^{a}	22.20±2.11^{b}	19.70±4.23^{b}	19.00±1.85^{bc}
Contr. (n=4)	30.22±2.30^{a}	31.70±2.75^{a}	30.10±3.05^{a}	29.28±2.00^{a}	28.40±2.00^{a}	30.28±3.14^{a}	30.00±1.38^{a}
RS (n=11)	32.00±2.56^{a}	33.33±3.54^{a}	31.38±2.45^{a}	25.66±2.59^{a}	20.00±2.11^{b}	17.40±1.22^{b}	18.00±3.11
Contr. (n=4)	33.00±3.09^{a}	32.25±1.48^{a}	32.00±2.34^{a}	31.00±3.18^{a}	32.25±1.00^{a}	31.00±1.98^{a}	29.50±1.27^{a}
SW (n=11)	30.50±1.82^{a}	32.23±3.54^{a}	31.33±2.42^{a}	29.67±1.79^{a}	26.00±2.21^{a}	21.50±2.23^{bc}	15.40±3.85^{b}
Contr. (n=4)	31.00±3.09^{a}	32.25±1.48^{a}	29.40±2.24^{a}	28.00±1.18^{a}	27.25±1.00^{a}	30.00±1.28^{a}	29.50±1.67
Secondary Challenge & treated:							
WAD (n=3)	26.70±1.00^{b}	25.00±1.00^{b}	24.20±1.00^{b}	23.50±1.00^{b}	22.80±0.70^{ab}	22.00±0.70^{a}	19.00±0.69^{a}
Contr. (n=4)	28.30±1.00^{b}	27.00±1.00^{b}	28.30±1.00^{b}	29.30±1.00^{b}	28.50±1.00^{b}	29.20±1.40^{b}	28.50±1.20^{b}
RS (n=3)	25.00±1.00^{b}	24.50±1.00^{b}	23.90±1.00^{b}	21.70±1.00^{b}	19.40±0.50^{a}	19.20±2.10^{a}	17.00±1.00^{a}
Contr. (n=4)	27.50±1.00^{b}	28.00±2.00^{b}	27.50±1.00^{b}	27.00±1.00^{b}	27.00±1.00^{b}	28.00±1.10^{b}	28.00±1.58^{b}
SW (n=3)	26.50±1.10^{b}	25.10±1.10^{b}	23.30±4.10^{b}	21.10±2.10^{b}	20.50±1.00^{a}	18.00±1.00^{a}	15.00±0.95^{a}
Contr. (n=4)	29.50±1.50^{b}	28.90±1.50^{b}	28.90±1.50^{b}	29.00±0.50^{b}	29.50±0.50^{b}	29.00±0.92^{b}	28.00±1.10^{b}
Secondary Challenge & untreated:							
WAD (n=4)	19.20±3.82^{a}	18.23±3.54^{a}	16.80±2.42^{a}	15.00±1.79^{a}	18.20±2.11^{b}	17.70±4.23^{b}	17.00±1.29^{b}
Contr. (n=4)	30.22±2.30^{a}	31.70±2.75^{a}	30.10±3.05^{a}	29.28±2.00^{a}	28.40±2.00^{a}	30.28±3.14^{a}	29.20±1.04^{a}
RS (n=4)	18.00±2.56^{b}	14.33±3.54^{b}	13.38±2.45^{b}	14.66±2.59^{b}	16.00±2.11^{b}	15.40±1.22^{b}	16.30±0.96^{b}
Contr. (n=4)	33.00±3.09^{a}	32.25±1.48^{a}	32.00±2.34^{a}	31.00±3.18^{a}	32.25±1.00^{a}	32.00±1.98^{a}	31.10±1.40^{a}
SW (n=4)	17.50±1.82^{a}	13.23±3.54^{a}	14.33±2.42^{a}	13.67±1.79^{a}	12.00±2.20^{b}	12.50±2.23^{bc}	13.02±2.38^{b}
Contr. (n=4)	31.00±3.09^{a}	32.25±1.48^{a}	29.40±2.24^{a}	28.00±1.18^{a}	27.25±1.00^{a}	30.00±1.28^{a}	30.28±3.14^{a}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW, Contr= Control

Different superscripts in rows or columns differed significantly ($P<0.05$)

Table 5: Mean (±SEM) Eosinophil of three Nigerian indigenous goat breeds following primary and secondary challenges with *Haemonchus contortus* and their respective controls

Breeds	Days Post-Infection			
	0	14	28	42
Primary challenge:				
WAD (n=11)	1.00±0.70^{a}	1.80±0.40^{a}	2.70±0.50^{a}	4.70±1.60^{bb}
Contr. (n=4)	1.30±0.60^{a}	1.40±0.50^{a}	1.00±0.50^{a}	1.30±0.40^{a}
RS (n=11)	1.10±0.50^{a}	1.70±0.50^{a}	2.10±0.80^{a}	3.20±2.00^{b}
Contr. (n=4)	1.00±0.50^{a}	1.30±0.30^{a}	1.20±0.30^{a}	1.00±0.60^{a}
SW (n=11)	1.00±0.50^{a}	1.40±0.30^{a}	2.00±1.00^{a}	2.00±1.00^{a}
Contr. (n=4)	1.20±0.30^{a}	1.00±0.40^{a}	1.10±0.40^{a}	0.80±0.30^{a}
Secondary Challenge & treated:				
WAD (n=3)	1.10±0.80^{a}	2.30±1.10^{b}	3.00± 2.00^{c}	3.50±1.00^{bc}
Contr. (n=4)	1.20±0.40^{b}	1.30±2.00^{b}	1.50±2.00^{ab}	1.40±0.60^{a}
RS (n=3)	1.20±0.70^{a}	2.10±1.00^{b}	2.00± 1.00^{b}	2.70±1.90^{b}
Contr. (n=4)	1.40±1.20^{b}	1.20 ±1.90^{c}	1.50±1.20^{b}	1.20±1.00^{b}
SW (n=3)	1.00±1.00^{b}	1.70±1.00^{b}	1.50± 1.10^{a}	2.85±1.50^{b}
Contr. (n=4)	1.30±1.00^{b}	1.30±1.50^{b}	1.40± 0.80^{b}	1.00±0.80^{b}
Secondary Challenge & untreated:				
WAD (n=4)	4.40±1.20^{b}	5.30±1.00^{b}	4.10±7.30^{b}	2.00±1.70^{b}
Contr. (n=4)	1.10±1.10^{ab}	4.30±1.30^{b}	4.40±1.00^{b}	1.40±0.60^{a}
RS (n=4)	3.00±1.30^{ab}	3.90±1.00^{b}	2.00±1.00^{ab}	0.90±0.60^{ab}
Contr. (n=4)	1.30±1.00^{ab}	3.70±2.30^{b}	3.40±2.20^{ab}	1.20±1.00^{b}
SW (n=4)	2.50±1.00^{ab}	2.90±1.03^{ab}	2.30±1.20^{ab}	0.70±0.60^{ab}
Contr. (n=4)	1.00±1.00^{ab}	3.40±1.00^{ab}	3.50±2.00^{ab}	1.00±0.80^{b}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW, Contr= Control

Different superscripts in rows or columns differed significantly ($P<0.05$)

Table 6: Mean (±SEM) Complement C3 of three Nigerian indigenous goat breeds following primary and secondary challenges with *Haemonchus contortus* and their respective controls

Breeds	Days Post-Infection			
	0	14	28	42
Primary challenge:				
WAD (n=11)	132.00±18.50^{a}	105.00±23.50^{a}	120.00±17.50^{a}	59.40±20.10^{ab}
Contr. (n=4)	148.70±20.20^{a}	144.30±23.50^{a}	140.80±10.70	138.00±39.60^{b}
RS (n=11)	85.70±11.20^{a}	80.30±17.20^{a}	45.80±14.90^{a}	21.79±10.30^{a}
Contr. (n=4)	80.60±30.40^{a}	80.60±23.80^{a}	80.70±21.70^{a}	84.00±30.50^{c}
SW (n=11)	78.90±00.50^{a}	45.70±10.30^{a}	49.10±15.30^{a}	18.30±07.90^{a}
Contr. (n=4)	74.80±20.50^{a}	78.70±15.30^{a}	72.80±17.25^{a}	75.50±20.40^{c}

Breeds	Days Post-Infection			
	0	14	28	42
Secondary Challenge & treated:				
WAD (n=3)	134.00±30.20^{ab}	120.00±20.20^{ab}	72.00± 25.20^{b}	59.30±1.30^{ab}
Contr. (n=4)	145.00±25.30^{ab}	139.00±25.30^{ab}	140.00± 21.40^{b}	140.00±30.20^{b}
RS (n=3)	70.20±26.20^{b}	65.60±22.50^{b}	41.10± 10.28^{ab}	28.20±2.60^{a}
Contr. (n=4)	74.30±32.50	73.50±20.80^{b}	74.00± 15. 30^{b}	78.20±30.10^{c}
SW (n=3)	72.40±30.20^{b}	60.40±10.90^{b}	53.69±12.60^{bb}	38.40±1.80^{a}
Contr. (n=4)	75.10±28.35^{b}	70.30±21.40^{b}	76.30±10.30^{b}	74.20±20.10^{c}
Secondary Challenge & untreated:				
WAD (n=4)	58.40±07.30^{ab}	70.20±18.00^{ab}	50.00±11.00^{ab}	48.40±6.03^{ab}
Contr. (n=4)	138.00±10.50^{b}	150.00±20.50^{b}	152.00±10.30^{b}	140.00±30.20^{b}
RS (n=4)	30.10±9.00^{ab}	45.00±10.00^{ab}	34.00±8.20^{ab}	24.50±2.60^{a}
Contr. (n=4)	78.25±11.50^{ab}	80.00±17.50^{ab}	79.00±9.30^{c}	78.20±30.10^{c}
SW (n=4)	27.30±14.80^{ab}	39.00±13.90^{ab}	20.00±17.00^{ab}	14.90±3.20^{a}
Contr. (n=4)	82.30±07.30^{ab}	77.00±12.40^{ab}	73.10±11.30^{ab}	74.20±20.10^{c}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW, Contr= Control

Different superscripts in rows or columns differed significantly ($P<0.05$)

Table 7: Mean (±SEM) Total serum protein (TSP) of three Nigerian indigenous goat breeds following primary and secondary challenges with *Haemonchus contortus* and their respective controls

Breeds	Days Post-Infection			
	0	14	28	42
Primary challenge:				
WAD (n=11)	4.60±1.60^{a}	4.20±1.91^{a}	4.20±1.90^{a}	2.70±1.60^{ab}
Contr. (n=4)	4.70±1.94^{a}	4.40±1.00^{a}	4.80±0.70^{a}	5.10±2.40^{a}
RS (n=11)	4.90±1.60^{a}	3.25±1.91^{a}	2.30±0.90^{ab}	1.50±1.30^{ab}
Contr. (n=4)	4.30±0.94^{a}	4.50±2.00^{a}	4.30±1.80^{a}	4.40±1.30^{a}
W (n=11)	4.30±1.11^{a}	4.25±2.91^{a}	4.20±1.10^{a}	2.10±1.60^{ab}
Contr. (n=4)	4.10±2.91^{a}	4.20±1.61^{a}	4.20±1.20^{a}	4.60±2.10^{a}
Secondary Challenge & untreated:				
WAD (n=3)	3.60±2.20^{a}	3.40±2.10^{a}	2.90± 1.10^{b}	3.00±2.00^{a}
Contr. (n=4)	4.70±3.20^{a}	4.70±1.10^{a}	4.40± 2.0^{b}	4.70±3.60^{a}
RS (n=3)	2.80±3.20^{ab}	2.60±1.10^{b}	2.00± 2.20^{a}	1.80±1.00^{ab}
Contr. (n=4)	3.20±2.20^{ab}	3.10±1.00^{b}	3.40± 2.00^{b}	3.60±2.80^{a}
SW (n=3)	2.80±3.10^{ab}	2.20±1.10^{b}	1.50± 1.70^{a}	1.70±2.20^{ab}
Contr. (n=4)	3.40±2.20^{ab}	3.50±2.30^{a}	3.45± 1.80^{b}	3.80±2.20^{a}

Breeds	Days Post-Infection			
	0	14	28	42
Secondary Challenge & untreated:				
WAD (n=4)	2.50±2.30^{ab}	2.00±1.40^{ab}	1.40±1.00^{ab}	1.20±1.00^{ab}
Contr. (n=4)	4.30±2.00^{a}	4.60±2.30^{a}	4.50±2.00^{a}	4.70±3.60^{a}
RS (n=4)	2.00±4.10^{ab}	1.80±1.30^{ab}	1.30±1.00^{ab}	1.00±0.90^{ab}
Contr. (n=4)	3.50±2.60^{a}	3.50±2.30^{a}	3.40±1.30^{a}	3.60±2.80^{a}
SW (n=4)	1.80±1.30^{ab}	2.00±2.00^{ab}	1.00±1.00^{ab}	0.90±1.40^{ab}
Contr. (n=4)	3.60±2.00^{a}	3.60±1.50^{a}	3.20±1.30^{a}	3.80±2.20^{a}

Keys: WAD= West African dwarf, RS=Red Sokoto, Sahel White=SW, Contr= Control

Different superscripts in rows or columns differed significantly (P<0.05)

secondary challenges are presented in Tables 5, 6 and 7. Following primary challenge, the C3 complement and the total serum protein decreased significantly ($p<0.05$) across the days irrespective of breed, with more pronounced decline in RS than SW and WAD. Contrarily, eosinophil count increased significantly ($p\square 0.05$) during the same period. The pattern of change observed in secondarily infected and treated animals of all breeds was similar to the findings among infected animals following primary challenge.

In the untreated secondarily infected animals, irrespective of breed, C3 complement increased on day 7 post infection. This later declined ($p\square 0.05$) significantly on day 42 post-infection. Similarly, the total serum protein declined significantly ($p\square 0.05$) across the days irrespective of breed, while eosinophil count showed an irregular pattern throughout the experimental period.

Discussion

The current study evaluated the responses of three indigenous goat breeds to primary infection with *Haemonchus contortus*, and the subsequent exposure to heavier challenge with or without the removal of the initial infection. This scenario represents what small scale rural farmers' encounter. The removal of the primary infection with an anthelmintic simulates the natural field condition in the tropics, where goats are regularly treated with anthelmintic to mitigate the pathogenic effects of helminthes, especially *H. contortus* by reducing pasture contamination most especially during the post parturient periods (Michel, 1976; Schillhorn van Veen, 1978).

The clinical signs of haemonchosis observed in this study irrespective of breed are consistent with previous observations of Chiezey and Oyedipe (2009) in Yankasa sheep and Ameen *et al.,* (2006) among WAD kids experimentally infected with *Haemonchus contortus*. More remarkable signs including diarrhea were however reported by Ameen *et al.,* (2006), probably due to age of the kids.

The pattern of change for both FEC and worm establishment rate was observed to be breed dependent as SW and RS had higher counts than WAD. Higher FEC among RS and SW was previously reported by Makun *et al.,* (2008), while haemonchotolerance by WAD goats was extensively elucidated in a review by Chiejina *et al.,* (2015). The consistent pattern of reduced FEC in the WAD following both primary and secondary challenge with or without removing initial challenge is an important feature of the host parasite relationship. The reduced FEC of WAD compared to RS and SW at all levels of infection is likely to be strongly influenced by the genetic make-up as previously shown (Chiejina *et al.,* 2009; Chiejina *et al.,* 2010).

Packed cell volume (PCV) of infected animals during primary and secondary challenges with or without treatment showed

similar pattern of change. The previous report by Idika et al. (2012) where significant changes were observed by day 35 post-infection among WAD goats infected with *Haemonchus contortus* has also been observed among WAD and SW in this study during the primary challenge. However earlier decline (day 28) was observed among RS. The relative stability of the PCV during the secondary challenge phase especially without treatment is a reflection of improved immunological response by the animals to the presence of the infecting parasite.

The C3 complement and the total serum protein were observed to have declined significantly across the days of experiment irrespective of whether the primary infection was treated or not. This decline was also observed to be breed-dependent. The trend of change in the complement C3 from the primary to secondary infections might reflect the superior immunological responses of the WAD goat over their RS and SW counterparts. In goats, the complement C3 is basically associated with anaphylaxis and leukocycte chemotaxis which are both suggestive of an inflammatory response, they might also be potential markers of immunological resistance to infections in goats. They similarly, sustained increase in eosinophil count for all breeds, irrespective of whether primary infection was treated or not, was observed. This observation is supported by previous findings among Creole kids experimentally infected with *Haemonchus contortus* (Bambou *et al.,* 2008), where transient eosinophilia was maintained even among resistant kids.

The clinical and haematological values were significantly higher after treatment of primary infections than their corresponding values before treatment in all the breeds. The goats whose primary infections were terminated with an anthelmintic treatment failed to develop appreciable resistance against subsequent challenge infections (Altaif *et al.,* 1978). However, those goats that were secondarily infected without treatment showed improved immunological responses, probably due to continued existence of the parasites in the infected goats.

Conclusively, this study has demonstrated that, WAD breed of goat is likely to survive and thrive better than RS and SW breeds in *H. contortus*-endemic area. Also, goats infected secondarily with *H. contortus* without removal of primary infection showed improved immunological responses compared to those treated for primary challenge. Thus, animals in endemic areas without treatment are better adapted to survive *Haemonchus* challenge.

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FERTILITY, HATCHABILITY AND GROWTH PERFORMANCE OF NATIVE AND CROSSBRED CHICKENS IN A TROPICAL CLIMATE

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Abstract

Fertility and hatchability serve as measures of genetic and reproductive fitness of individual bird. These two along with growth performance are important yardsticks in evaluating the economic efficiency of parent stocks. There is no previous study on the evaluation of Goliath and Sussex chickens for hatchability, growth and crossbreeding potentials in Nigeria. The present study was conducted to evaluate fertility, hatchability and early growth performance of Yoruba ecotype, Marshall, Sussex, Goliath and their crossbred chickens in Nigeria. A total of 895 eggs were used to evaluate fertility and hatchability of eggs. 583 chicks were used to evaluate early growth performance in the 10 genetic groups. Mean %Fertility of egg, %Hatchability of egg set and %Hatchability of fertile eggs were 74.44%, 61.50% and 82.09%, respectively. Crossbreeding (reciprocal) between Yoruba ecotype and either Marshall or Goliath chicken gave better fertility and hatchability than the cross between Yoruba ecotype and Sussex chicken. Reciprocal effects on fertility and hatchability and liveability generally favour the use of Yoruba ecotype cocks on exotic hens. Marshall chicks were significantly higher ($p<0.05$) in weekly body weights than all the other nine genotypes from the 5th to the 8th week of age. Crossbred chicks produced by Marshall cocks and Yoruba ecotype hens were significantly higher ($p<0.05$) in weekly body weights than other crossbred chicks from the 5th to the 8th week of age. Male chicks were significantly higher ($p<0.05$) in weekly body weights than female chicks. Interaction between genotypes and sex was significant ($p<0.05$) on the weekly body weights. It was concluded that the highest genetic gain in body weight and Feed efficiency through crossbreeding would be achieved by crossing Marshall cocks with local Yoruba ecotype hens. However, higher fertility and hatchability of eggs and survivability of chicks may be achieved by mating Yoruba ecotype cocks with any of the female exotic hens.

Key words: Crossbreeding, Feed efficiency, Hatchability, Reciprocal effects, Yoruba ecotype

FERTILITÉ, POTENTIEL D'ECLOSION ET PERFORMANCE DE CROISSANCE DES POULETS LOCAUX ET CROISES EN CLIMAT TROPICAL

Resume

La fécondité et le taux d'éclosion sont utilisés comme mesures de l'aptitude génétique et reproductive des oiseaux individuels. Ces deux facteurs, ainsi que les performances de croissance, sont des éléments de référence importants dans l'évaluation de l'efficacité économique des stocks parentaux. Aucune étude n'avait été faite sur l'évaluation des poulets Goliath et Sussex concernant le potentiel d'éclosion, de croissance et de croisement au Nigeria. La présente étude a été réalisée dans le but d'évaluer la fertilité, le potentiel d'éclosion et la performance de croissance précoce des poulets de l'écotype Yoruba, Marshall, Sussex, Goliath et leurs croisements au Nigeria. Au total, 895 œufs ont été utilisés pour évaluer la fertilité et le potentiel d'éclosion des œufs. 583 poussins ont été utilisés pour évaluer la performance au cours des premières phases de leur croissance dans les 10 groupes génétiques. Le taux moyen de fertilité des œufs, le taux d'éclosion des couvées et le taux d'éclosion des œufs fertiles étaient respectivement de 74,44%, 61,50% et 82,09%. Le croisement (réciproque) entre l'écotype Yoruba et le poulet Marshall ou le poulet Goliath a donné de meilleurs taux de fertilité et d'éclosion par rapport au croisement entre l'écotype Yoruba et le poulet Sussex. Les effets réciproques sur les taux de fertilité et d'éclosion et de viabilité favorisent généralement l'utilisation des coqs de l'écotype Yoruba sur les poules exotiques. Les poussins Marshall avaient des poids corporels hebdomadaires significativement plus élevés ($p <0,05$) que tous les neuf

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autres génotypes depuis la 5ème jusqu'à la 8ème semaine d'âge. Les poussins croisés produits par les coqs Marshall et les poulets de l'écotype Yoruba avaient des poids corporels hebdomadaires significativement plus élevés ($p < 0,05$) que les autres poussins croisés de la 5ème à la 8ème semaine d'âge. Les poussins mâles avaient des poids corporels hebdomadaires significativement ($p < 0,05$) plus élevés que les poussins femelles. L'interaction entre les génotypes et le sexe était significative ($p < 0,05$) sur les poids corporels hebdomadaires. On a conclu que le gain génétique le plus élevé au niveau du poids corporel et l'efficacité des aliments par croisement serait réalisé en procédant au croisement des coqs Marshall avec des poules de l'écotype Yoruba. Cependant, un taux plus élevé de fertilité et d'éclosion des œufs et la capacité de survie des poussins peuvent être obtenues en procédant au croisement des coqs de l'écotype Yoruba avec l'une quelconque des poules exotiques.

Mots-clés : croisement, efficacité des aliments, taux d'éclosion, effets réciproques, écotype Yoruba

Introduction

The fertility and Hatchability estimates encompass the union of the ova and spermatozoa which leads to the development and hatching of a viable chick at the end of incubation process. Fertility in poultry refers to the percentage of incubated eggs that are fertile while hatchability is the percentage of fertile eggs that hatch (Ahmedin and Mangistu, 2016). The estimate of fertility of eggs is important because it affects the percentage hatchability of the total number of chicks to be hatched. Both fertility and hatchability serve as measures of the genetic and reproductive fitness of individual bird and important yardsticks in evaluating the economic efficiency of parent stocks. Hatchability of eggs is influence by a number of non-genetic factors such as age of flock (Zita et al, 2009), storage duration and conditions (Heier and Jarp, 2001, Demirel and Kirikci, 2009) and management conditions (such as temperature) during incubation and hatching (Lourens *et al.,* 2007). These environmental factors are controllable and can be optimized for maximum economic returns.

In Nigeria, local chicken constitute 80% of the 120 million poultry type raised in the rural areas (Ajayi., 2010) these contribute majorly to the animal protein in human diet. They are mostly found in the rural areas, they are good scavengers as well as foragers. They exhibit higher fertility and hatchability under natural incubation and good maternal qualities. They are harder when compared to the exotic breeds and have high survival rates with minimal care and attention (Salako and Ige, 2006). However, local chicken have been adjudged to be genetically poor producer of meat and eggs (Gueye, 1998; Tadelle *et al.,* 2000). Growth in chicken relates to increase in body cells and volume. It is a complex process, which is controlled by both genetic and non-genetic factors (Kor *et al.,* 2006). Growth rate along with feed conversion and degree of muscling are among the most important criteria in genetic improvement or selection programmes.

Adaptation is the ability of an animal to survive and reproduce within a define environment (Prayaga and Henshall, 2005). The exotic chickens have been developed to produce higher number of eggs and meat, but high temperature, disease and shortage of feed pose great challenges to the performance of these exotic chickens in tropical climes (Islam and Nishibori, 2009). Previous attempt was aimed at improving the productivity of the indigenous chickens by mating them with the improved exotic cocks. However, Permin (2008) observed that such schemes has failed due to problem of adaptation of the introduce breed to hot tropical climate. It is therefore important to evaluate the exotic chickens for their adaptation and crossbreeding potentials in tropical environments. This could lead to the production of hybrid that is resistant to harsh tropical conditions and at the same time produces a reasonable amount of egg and meat (Mekki *et al.,* 2005).

Marshall, Goliath and Sussex are some of the exotic trains of chickens that can be found in the country. There is no previous

study on the evaluation of Goliath and Sussex chickens for growth and crossbreeding potentials in Nigeria. The aim of the present study is to evaluate fertility, hatchability and early growth performance of Yoruba ecotype, Marshall, Sussex and Goliath chickens and their crossbreds in Nigeria.

Materials and Methods

A total of 895 eggs collected from Yoruba ecotype, Sussex and Goliath and six crossbred chickens were used to evaluate fertility and hatchability of eggs (Table 1). Fertile eggs of Yoruba ecotype chicken were obtained from local farmers in Oyo states. The fertile eggs of exotic and crossbred chickens were obtained from flocks kept at a poultry unit in Oyan, Osun state. The eggs collected were set into commercial incubator for 21-days. Eggs were candled to determine the number of fertile eggs. Hatched chicks were collected, counted, weighed and wing-banded according to genotypes.

A total of 483 chicks obtained from hatchability experiments and 100 day old purebred Marshall Chicks obtained from a commercial hatchery were used for the evaluation of early growth performance of the 10 ten chicken genotypes. Birds were raised in deep litter floored pens at a poultry facility located at Oyan (Osun state, South-West). Oyan is located on latitude 8.05 and longitude 4.77 and at an elevation of 422 meters above sea level. Birds were supplied with feed and water ad libitum. All birds were given unrestricted access to water, and were fed the same diet ad-libitum (Table 2). Routine vaccination was carried out to prevent endemic diseases such as Newcastle and Gumboro.

Fertility and hatchability of total eggs and hatchability of fertile eggs were estimated as follows:

$$\text{Fertility \%} = \frac{\text{Number of fertile eggs}}{\text{Total number of egg set}} \times 100$$

$$\text{Hatchability\% (TES)} = \frac{\text{Number of chicks hatched}}{\text{Number of egg set}} \times 100$$

$$\text{Hatchability (FES)} = \frac{\text{Number of chicks hatched}}{\text{No of fertile eggs}} \times 100$$

Records of bodyweight of chicks (in grammes) were taken for a period of 8 weeks. Feed intake was recorded as the difference between the quantity of feed supplied and left-over for the week. Mortality was recorded as the number of birds dead per genotype per week. Data on fertility and hatchability of eggs were subjected to descriptive statistics

Table 1: Total egg set, fertile eggs and hatched chicks in YEC, Goliath and Sussex and crossbred Chickens

Strains	No of eggs set	Fertile eggs	Total hatched
Purebred groups			
YEC x YEC	363	206	148
GO x GO	100	75	61
SS x SS	119	90	79
Crossbred groups			
GO x YEC	61	50	40
YEC x GO	58	39	35
SS x YEC	64	40	30
YEC x SS	60	42	35
ML x YEC	30	25	20
YEC x ML	40	39	35
Total	**895**	**606**	**483**

YEC, GO, SS, ML represent:Yoruba Ecotype Chicken, Goliath, Sussex, and Marshall Broiler, respectively

Table 2: Nutrient composition of chicks mash fed to pure and crossbred chicks

Ingredients	% composition
Calcium %	1.70
Available phosphorus%	0.60
Na%	0.38
Cl % (min)	0.55
Methionine digest%(min)	0.64
Cysteine digest% (min)	0.38
Lysine digest%(min)	1.43
Threonine digest%(min)	0.89
Crude fibre%(max)	4.46
Crude fat%(min)	1.13
Crude Protein %	27.80
ME Kcal/kg	1968.40

Table 3: Percentage fertility and hatchability of eggs of Yoruba ecotype, Goliath and Sussex and crossbred Chickens

Strains	%Fertility of egg	%Hatchability of egg set	%Hatchability of fertile egg
Purebred groups			
YEC x YEC	56.75	40.77	71.85
GO x GO	75.00	61.00	81.33
SS x SS	75.63	66.39	87.78
Crossbred groups			
GO x YEC	81.97	65.57	80.00
YEC x GO	67.24	60.35	89.74
SS x YEC	62.50	46.89	75.00
YEC x SS	70.00	58.33	83.33
ML x YEC	83.33	66.70	80.00
YEC x ML	97.50	87.50	89.74
Total	**74.44**	**61.50**	**82.09**

YEC, GO, SS, ML represent:Yoruba Ecotype Chicken, Goliath, Sussex, and Marshall Broiler, respectively

(percentages of total). Feed efficiency was calculated as the ratio of feed intake to weight gain for the week. Data on weekly bodyweight was subjected to a two-way ANOVA such that sex and genotypes of chicks were taken as fixed effects. Where significant difference exists among genotypes, the mean genotypic values were separated using Duncan multiple range test.

Results

Table 3 shows the result of fertility and hatchability of eggs of pure and crossbred chickens. Mean %Fertility of egg, %Hatchability of egg set and %Hatchability of fertile egg for the 9 genotypes were 74.44%, 61.50% and 82.09%, respectively. Sussex and Goliath hens used in pure-breeding produced eggs that were higher in fertility and hatchability than Yoruba ecotype. Generally, crossbreeding improved

the %Fertility of egg, %Hatchability of egg set and %Hatchability of fertile egg. Crossbreeding (reciprocal) between Yoruba ecotype and either Marshall or Goliath chicken gave better fertility and hatchability than the cross between Yoruba ecotype and Sussex chicken. Reciprocal effects on fertility and hatchability generally favour the use of male Yoruba ecotype and female exotic chicken.

Table 4 shows the effects of genotype and sex on 8 weeks bodyweight of local, exotic and crossbred chickens. The three exotic chickens were significantly higher ($p<0.05$) in weekly body weights than the local Yoruba ecotype chicken. Marshall chicks had significantly heavier ($p<0.05$) weekly body weights than other genotypes from the 5th to the 8th week of age. Reciprocal crossbred chicks were significantly higher ($p<0.05$) in weekly body weights than the local Yoruba ecotype chicken. Generally, the body weights of crossbred chicks lie between the body weights of purebred exotic and local chickens. Crossbred chicks from Marshall male and Yoruba ecotype female were significantly heavier ($p<0.05$) in weekly body weights than other crossbred chicks from the 5th to the 8th week of age. Male chicks were significantly higher ($p<0.05$) in weekly body weights than female chicks. Interaction between genotypes and sex on early body weights of chicks was significant ($p<0.05$).

Table 5 shows the interaction between genotypes and sex on body weight of chicks from hatch to 8 weeks of age. Interaction between genotypes and sex was significant ($p<0.05$) on weekly body weight from hatch to 8 weeks of age. Purebred male Goliath and Sussex chicks were significantly higher ($p<0.05$) in hatch weight compare with other genotypes while the best hatch weight among the female chicks was obtained in Purebred Marshall. MLxYEC male chicks was significantly higher ($p<0.05$) in 8 weeks body weight compare with other genotypes while purebred Marshall had the best 8-week body weight among the female chicks.

Table 6 shows the feed conversion efficiency of local, exotic and crossbred

Table 4: Effects of genotype and sex on early bodyweights of local, exotic and crossbred chickens

	Weeks								
Genotype	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
YEC x YEC	23.61±0.22^{e}	45.82±0.68^{e}	74.13±1.41^{f}	100.11±2.97^{h}	129.85±3.26^{f}	170.62±3.87^{f}	234.77±4.56^{f}	317.48±5.33^{h}	382.26±5.73^{h}
GO x GO	32.66±0.31^{a}	79.40±0.96^{a}	135.90±1.98^{b}	218.46±3.48^{b}	322.82±4.59^{a}	437.69±5.45^{b}	570.83±6.41^{b}	702.47±7.49^{c}	804.95±8.06^{c}
SS x SS	32.58±0.29^{a}	77.79±0.87^{a}	124.73±1.80^{c}	194.99±3.16^{c}	286.52±4.18^{b}	384.06±4.95^{c}	465.10±5.83^{c}	568.93±6.82^{d}	674.96±7.33^{d}
ML x ML	31.61±0.31^{b}	60.07±0.96^{c}	108.29±2.90^{e}	166.38±3.47^{ef}	270.91±4.59^{c}	515.63±5.44^{a}	704.67±6.40^{a}	908.03±7.49^{a}	1025.41±8.06^{a}
GO xYEC	30.52±0.46^{c}	72.21±1.27^{b}	155.46±2.62^{a}	254.38±4.61^{a}	328.85±6.08^{a}	394.73±7.21^{c}	475.48±8.49^{c}	544.67±9.93^{e}	627.13±10.68^{e}
YEC x GO	24.76±0.42^{d}	52.86±1.28^{d}	93.96±2.64^{e}	137.84±4.64^{g}	187.86±6.13^{e}	252.47±7.27^{e}	316.30±8.55^{e}	384.87±10.00^{g}	442.90±10.76^{g}
SS xYEC	24.76±0.42^{d}	60.43±1.41^{c}	101.38±2.9^{e}	157.90±5.10^{f}	224.15±6.74^{d}	293.92±7.99^{d}	366.01±9.39^{d}	435.63±10.99^{f}	533.34±11.83^{f}
YEC x SS	22.88±0.43^{ef}	60.40±1.32^{c}	121.82±2.72^{c}	179.98±4.78^{d}	235.13±6.31^{d}	287.49±7.48^{d}	346.46±8.79^{d}	396.84±10.29^{g}	435.47±11.07^{g}
ML xYEC	22.88±0.43^{ef}	63.34±1.76^{c}	136.38±3.66^{b}	182.14±6.42^{cd}	257.49±8.48^{c}	424.33±10.06^{b}	570.75±11.83^{b}	737.57±13.84^{b}	913.21±14.88^{b}
YEC x ML	22.88±0.43^{ef}	52.87±1.36^{d}	110.64±2.80^{d}	176.41±4.93d^{e}	234.36±6.52^{d}	293.65±7.73^{d}	342.56±9.09^{d}	394.44±10.64^{g}	437.79±11.44^{g}

Genotype	Weeks 0	1	2	3	4	5	6	7	8
Sex effect									
Male	30.46±0.19[a]	69.48±0.59[a]	128.66±1.22[a]	200.55±2.15[a]	285.61±2.84[a]	399.68±3.36[a]	507.71±3.96[a]	626.46±4.63[a]	726.36±4.98[a]
Female	26.06±0.16[b]	55.56±0.50[b]	103.88±1.03[b]	153.17±1.81[b]	209.98±2.39[b]	291.84±2.84[b]	370.87±3.34[b]	451.73±3.91[b]	529.13±4.21[b]
Interaction (BxS)	*	*	*	*	*	*	*	*	*

*YEC, GO, SS, ML represent:Yoruba Ecotype Chicken, Goliath, Sussex, and Marshall Broiler, respectively. a,b,c,d,e,f,g Means with different superscripts for the same week are significantly different,(p< 0.05), * indicates significant interaction*

Table 5: Details of interaction between genotype and sex on weekly body weights in Yoruba ecotype, Goliath, Sussex, Marshal and their crossbred chicks.

Weeks	Sex	GENOTYPES YECxYEC	GOxGO	SSxSS	MMxML	GOxYEC	YECxGO	SSxYEC	YECxSS	MLxYEC	YECxML
0	M	24.11±0.31[h]	36.32±0.47[a]	36.46±0.44[a]	33.05±0.54[b]	34.08±0.68[b]	25.77±0.65[fg]	32.91±0.71[b]	24.55±0.71[gh]	32.20±0.74[b]	25.18±0.71[ghi]
	F	23.11±0.32[i]	29.00±0.42[d]	28.70±0.36[d]	30.17±0.32[c]	26.96±0.48[ef]	23.75±0.53[hi]	29.06±0.59[cd]	21.22±0.49[j]	28.71±0.89[cd]	19.95±0.54[j]
1	M	47.43±0.96[i]	92.08±1.44[a]	92.07±1.36[a]	61.16±1.99[ef]	80.75±2.07[b]	60.46±1.99[ef]	63.73±2.17d[e]	62.36±2.17[de]	71.40±2.28[c]	63.36±2.17[de]
	F	44.22±0.97[j]	66.72±1.27[cd]	63.51±1.09d[e]	58.98±0.98[gh]	63.67±1.47[de]	45.25±1.61[i]	57.13±1.80[gh]	58.43±1.50[gh]	55.29±2.72[h]	42.37±1.65[j]
2	M	76.32±1.98[i]	153.96±2.97[b]	152.07±2.80[b]	115.26±3.40[de]	177.00±4.28[a]	102.46±4.11[fg]	106.82±4.47[ef]	124.64±4.47[cd]	151.90±4.69[b]	126.18±4.47[cd]
	F	71.95±2.00[i]	117.84±2.62[d]	97.39±2.26[fg]	101.31±2.02[fg]	133.92±3.03[c]	85.45±3.32[h]	95.94±3.71[fg]	119.00±3.09[d]	120.86±5.61[de]	95.11±3.45[g]
3	M	102.32±3.48[k]	239.60±5.21[b]	243.21±4.92[b]	184.47±5.98[cd]	320.92±7.52[a]	153.23±7.23[gh]	183.18±7.85[cd]	192.09±7.85[cd]	195.00±8.24[c]	191.45±7.85[cd]
	F	97.89±3.51[k]	197.31±4.61[c]	146.77±3.97[hi]	184.47±5.98[cd]	187.83±5.32[cd]	122.45±5.83[j]	132.6±6.51[ij]	167.87±5.43[ef]	169.29±9.85[de]	161.37±5.98[fg]
4	M	133.70±4.59[k]	349.96±6.88[b]	349.39±6.51[b]	296.11±7.89[c]	418.33±9.94[a]	214.08±9.55[hi]	272.55±10.38[cd]	254.18±10.38[de]	301.40±10.89[c]	266 .45±10.38[cd]
	F	126.00±4.64[k]	295.69±6.08[c]	223.65±5.25[gh]	245.72±4.68[ef]	239.37±7.03[fg]	161.65±7.69[j]	175.75±8.61[i]	216.09±7.18[hi]	213.57±13.01[hi]	202.26±7.89[i]
5	M	176.11±5.45[l]	488.88±8.16[b]	454.54±7.71[c]	583.16±9.36[a]	489.25±11.78[b]	223.23±9.12[k]	360.09±12.30[de]	306.21±8.33[gh]	515.80±12.90[b]	340.73±12.30[ef]
	F	165.13±5.50[l]	386.50±7.21[d]	313.58±6.22[g]	448.11±5.55[c]	306.21±8.33[gh]	223.23±9.12[k]	227.75±10.20[k]	268.43±8.51[ij]	332.86±15.42ef	246.58±9.36[jk]
6	M	250.55±6.41[m]	654.32±9.59[c]	538.50±9.07[e]	766.47±11.01[a]	580.17±13.86[d]	279.45±10.73[l]	446.27±14.47[g]	375.27±14.47[ij]	716.50±15.18[b]	395.91±14.47[hi]
	F	218.98±6.47[n]	487.34±8.49[f]	391.70±7.32[hi]	642.87±6.53[c]	370.79±9.79[ij]	353.15±13.31[j]	285.75±11.99[l]	317.65±10.01[k]	425.00±18.14[gh]	289.21±11.01[kl]
7	M	345.84±7.50[hi]	822.40±11.23[c]	676.25±10.61[d]	984.16±12.88[a]	653.08±16.21[d]	444.38±15.57[g]	530.45±16.92[f]	430.64±16.93[g]	919.00±17.76[b]	458.36±16.93[g]
	F	289.13±7.57[j]	582.53±9.93[e]	461.60±8.56[g]	831.91±7.64[c]	436.25±11.46[g]	325.35±12.55[i]	340.81±14.04[hi]	363.04±11.71[h]	556.14±21.22[ef]	330.53±12.88[hi]
8	M	416.46±8.07[jk]	944.68±12.08[b]	814.21±11.42[c]	1099.47±13.86[a]	752.08±17.44[d]	509.85±16.75[gh]	627.18±18.21[f]	479.64±18.21[hi]	1115.00±19.10[a]	505.00±18.21[gh]
	F	348.05±8.15[m]	665.22±10.68[ef]	535.70±9.21[g]	951.35±8.22[b]	502.17±12.33[h]	375.95±13.51[lm]	439.50±15.10[ij]	391.30±12.59[kl]	771.43±22.83[de]	370.58±13.86[lm]

M, F represent male and female chicks, respectively.YEC, GO, SS, ML represent:Yoruba Ecotype Chicken, Goliath, Sussex, and Marshall Broiler, respectively. a-m Means with different superscripts for the same week are significantly different,(p< 0.05),

Table 6: Effects of genotype on weekly Feed conversion of local, exotic and crossbred chickens.

GENOTYPE	Wk1	Wk4	Wk8
YEC	1.04±0.02^{de}	1.65±0.12^{de}	1.54±0.07^{cd}
GO	1.21±0.04^{cd}	2.34±0.06^{c}	2.49±0.22^{b}
SS	0.86±0.03^{e}	1.70±0.06^{de}	1.42±0.11^{de}
ML	3.64±0.09^{a}	1.93±0.03^{d}	3.86±0.13^{a}
GOxYEC	1.10±0.033	3.42±0.27^{a}	1.23±0.21^{de}
YECxGO	1.07±0.03^{d}	1.52±0.13^{ef}	1.26±0.13^{de}
SSxYEC	1.41±0.02^{b}	2.84±0.13^{b}	1.82±0.23^{c}
YECxSS	1.06±0.02^{d}	3.27±0.12^{a}	1.12±1.0^{de}
MLxYEC	1.34±0.09^{bc}	1.21±0.21^{f}	1.03±0.19^{f}
YECxML	1.09±0.03^{d}	2.45±0.11^{c}	1.06±0.21^{f}

Table 7: weekly Mortality in local, exotic and crossbred chickens

WEEKS	YECxYEC	GOxGO	SSxSS	MMxML	GOxYEC	YECxGO	SSxYEC	YECxSS	MLxYEC	YECxML
1	4.05	3.28	3.79	6.00	5.00	2.86	3.33	2.86	1.00	8.57
2	2.03	1.64	1.27	2.00	0.00	0.00	3.33	0.00	5.00	5.71
3	1.35	0.00	0.00	0.00	0.00	2.86	0.00	0.00	0.00	0.00
4	0.00	1.64	2.53	4.00	2.50	0.00	0.00	0.00	5.00	0.00
5	0.00	0.00	1.27	2.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	6.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	**14.19**	**6.56**	**8.86**	**14.00**	**10.00**	**5.72**	**6.66**	**2.86**	**11.00**	**14.28**

YEC, GO, SS, ML represent:Yoruba Ecotype Chicken, Goliath, Sussex, and Marshall Broiler, respectively.
^{a,b,c,d,e,f} Means with different superscripts for the same week are significantly different,(p< 0.05)

chickens at 1, 4 and 8 weeks of age. Sussex had the best feed conversion efficiency among the purebred groups. This was followed by Yoruba ecotype chicken. Goliath had the poorest feed efficiency among the purebred chickens. Generally, the crossbred groups were intermediate between Yoruba ecotype and exotic chickens. Crossbred chicks produced from YEC males and GO females were better ($p<0.05$) in feed efficiency than its reciprocal cross at 1, 4 and 8 weeks of age. However, such superiority was not observed beyond week 1 in other crossbred groups.

The results of weekly mortality are presented in Table 7. Most of the mortality occurred in the first two weeks of live. Goliath had the best survivability among the purebred chicks. Crossbred chicks (YECxGO and YECxSS) survived better than purebred local and exotic chicks. Crossbred chicks produced from local YEC cocks were higher in survivability of chicks than the reciprocal crosses.

Discussion

The genotypic difference in the fertility and hatchability of eggs observed in this study agrees with Peters et al.(2004) and Peters et al.(2008) who reported that the strain of the dam have prominent effects on fertility and hatchability of eggs. In a recent study, Ahmedin and Mangistu (2016) observed significant difference between genotypes in the hatchability of fertile egg and hatchability of total egg set. However, the same authors did not observe significant difference between genotypes in the fertility of eggs. The lower

fertility and hatchability obtained for purebred local chicken in this study was at variance with earlier reports on the fertility and hatchability of local and exotic chickens (Peters *et al.,* 2004; Islam and Nishibori, 2009; Ahmedin and Mangistu, 2016). The low fertility and hatchability obtained for Yoruba ecotype chicken in this study may be due to the methods used in sourcing the eggs. It is possible that pre-incubation handling by the local farmers may have influence the hatchability of the purebred YEC eggs. The superior fertility and hatchability of ML x YEC and YEC x ML crossbred chickens over other crossbred groups suggest higher breed complementarity.

The higher hatch weights and weekly body weights of exotic (Goliath and Sussex) genotypes suggests their genetic superiority over the local Yoruba ecotype chicken. Mmereole and Udeh (2009) obtained significant effect of genotype on body weight in their study involving local chicken and its crosses with barred Plymouth Rock. The bigger size of eggs obtained from Goliath and Sussex chickens may account for their higher hatch weights than chicks from Yoruba ecotype chicken. An earlier report by Farooq *et al.,* (2001) shows that a positive correlation exists between egg weight and hatching chick weight in Rhode Island Red and local Desi Fayoumi chicken in Pakistan. The higher body weight of Marshall Chicks at 8 weeks of age suggests that it is a superior meat strain. Heritability of body weight in chicken is fairly high (0.54-0.6). The higher body weight of Marshall Chicks at 8 weeks of age suggests that it is a superior meat strain. Such a higher growth performance (as observed in Marshall Chicks) is expected in chickens that have been previously selected for meat production (Adebambo *et al.,* 2009). The present results showed that the highest genetic gain in body weight through crossbreeding would be achieved by crossing Marshall Cocks with local Yoruba ecotype hens. Sexual dimorphism in body weight of chicks which is in favour of male chicks in this study is consistent with previous reports (e.g. Adebambo *et al.,* 2009). The male bias sexual dimorphism has been attributed to effective male growth hormones compared to female hormones (Singh *et al.,* 1982; Fayeye *et al.,* 2005). Ricklefs (1985) opined that the rapid changes between males and females could partially result from differences in physiological ages between males and females at the same chronological age. According to Benyi, et al. (2015), male broiler utilizes feed more efficiently than female. The comparatively better growth performance of ML x YEC crossbred chicks may therefore be due to its higher feed efficiency. The interaction between sex and genotype suggest a complex interplay between the two fixed effects on bodyweights of chicks at different ages. The variation in feed efficiency among chicks of different genotypes underscores the need for further studies to evaluate the physiologic basis for feed utilisation in chicken. Marks (1993) observed that selection for body weight improves feed utilization and that the increase in total feed intake was due to the nutritional need for maintenance requirements of a large body size. Earlier McCarthy and Siegel (1983) had noted that there is substantial evidence that increased growth rate of selected lines of chicken is mainly associated with increased appetite.

Conclusion

The present results showed that the highest genetic gain in body weight and feed efficiency through crossbreeding would be achieved by crossing Marshall Cocks with local Yoruba ecotype hens. However, higher fertility and hatchability of eggs and survivability of chicks may be achieved by mating Yoruba ecotype cocks with female exotic chickens.

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MODELLING GROWTH CURVES OF NIGERIAN INDIGENOUS NORMAL FEATHER CHICKEN USING BAYESIAN NONLINEAR MODEL

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Abstract

This study was conducted to predict the growth curve parameters using Bayesian Gompertz and logistic models and also to compare the two growth function in describing the body weight changes across age in Nigerian indigenous normal feather chicken. Each chick was wing-tagged at day old and body weights were recorded at the end of each two-week period up to 16 weeks of age. A non-informatics prior was used for the Bayesian inference. The two models were fitted to the weight-age data from day old to sixteen weeks of age for each individual chicken by non-linear Bayesian regression using Winbugs. Models were compared using the mean value, MCE, convergence and the estimate weight when the parameters are fitted to each model. Differences were observed in the growth parameters of chickens. Gompertz having a mean value of 1.70kg and rate of growth (k) of 0.03 for the Asymptotic value (A) while Logistic A mean value (1.88) and k (1.62) which indicate that Logistic has higher growth rate and also attain a mature weight than the Gompertz. MCE for Gompertz ranges from 0.001 to 0.56 and in Logistic from 0.05 to 0.39. Based on all the criteria used for comparing these models, it can be concluded that the mean value for Gompertz model is closer the observed value (1.60kg) than the logistic, also Gompertz model have a lower value of MCE which makes the model more reliable. The Credible interval for Gompertz is more close to the mean value than Logistic function. Gompertz gave the best fit for the age-body relationship for the Nigerian indigenous normal feather chicken, although Logistic function is equally good in predicting the growth curves of the chickens.

Keywords: Normal feather, growth curves, modelling, Nigerian indigenous chicken.

MODÉLISATION DES COURBES DE CROISSANCE DU POULET LOCAL NIGERIAN A PLUMAGE NORMAL À L'AIDE DU MODÈLE BAYESIEN NON LINÉAIRE

Résumé

La présente étude a été réalisée dans l'objectif de prédire les paramètres de la courbe de croissance à l'aide des modèles Gompertz et logistique bayésiens et de comparer les deux fonctions de croissance pour décrire les changements de poids corporels basés sur divers âges chez les poulets nigérians locaux à plumage normal. Chaque poussin a reçu une marque à l'aile à l'âge de un (1) jour, et les poids corporels ont été enregistrés à la fin de chaque période de deux semaines jusqu'à l'âge de 16 semaines. Un prior non informatique a été utilisé pour l'inférence bayésienne. Les deux modèles ont été adaptés aux données sur le rapport âge/poids à partir d'un jour d'âge jusqu'à seize semaines pour chaque poulet individuel par régression bayésienne non linéaire utilisant Winbugs. Les modèles ont été comparés en utilisant la valeur moyenne, la MCE, la convergence et le poids estimé lorsque les paramètres ont été adaptés à chaque modèle. Des différences ont été observées au niveau des paramètres de croissance des poulets. Gompertz a montré une valeur moyenne de 1,70 kg et un taux de croissance (k) de 0,03 pour la valeur asymptotique (A), tandis que la valeur moyenne de Logistic A (1,88) et K (1,62) indiquent que Logistic a un taux de croissance plus élevé et atteint également un poids mature par rapport à Gompertz. La MCE varie de 0,001 à 0,56 pour Gompertz et de 0,05 à 0,39 pour Logistic. Sur la base de tous les critères utilisés pour comparer ces modèles, on peut conclure que la valeur moyenne pour le modèle de Gompertz est plus

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proche de la valeur observée (1,60kg) par rapport à Logistic ; le modèle Gompertz a également une valeur inférieure de MCE, ce qui rend le modèle plus fiable. L'intervalle crédible pour Gompertz est plus proche de la valeur moyenne par rapport à la fonction de Logistic. Gompertz a donné le meilleur ajustement pour la relation âge-corps pour les poulets indigènes nigérians à plumage normal, bien que la fonction Logistic soit également bonne pour prédire les courbes de croissance des poulets.

Mots-clés : plumage normal, courbes de croissance, modélisation, poulet local nigérian

Introduction

Hanotte and Jianlin (2005) reported that over the past 12,000 years, more than 6,300 breeds of livestock belonging to 30 domesticated species were developed following domestication and selection. FAO (2007) reported that the sustainable management, utilization and conservation of a particular population of domestic animals require its characterization. De Vicente et al. (2005) reported that characterization in genetic terms refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors. FAO (2000) reported that poultry contributes the largest animal-source food and is by far the largest group of livestock species contributing about 30% of all animal protein consumed in the world. According to Gueye (2003), chicken species constituted around 98% in Africa. Poultry contributes to improve human nutrition and food security by being a major source of high quality protein from their egg and meat. It serves as a key supplement to revenue from crop and other livestock enterprises, thus avoiding over dependency on traditional commodities with inconsistence prices.

In Frequentist approach, the parameters of interest are fixed and unchanging under all realistic circumstances while Bayesian view the world probabilistically rather than as a set of fixed phenomena that are either known or unknown. In Frequentist approach, no information prior to the model specification while in Bayesian Prior information abounds and it is important and helpful to use it. In Frequentist approach, nothing is more important than repeatability, while in Bayesian every statistical model ever created in the history of the human race is subjective.

Although a lot of studies have been conducted on modelling of growth in Nigerian Indigenous chicken using the classical or frequentist approach, there is no known work that applied Bayesian approach, despite the argument in favour of the Bayesian approach as being more informative and reliable as a result of its ability to incorporate prior in its analysis. It is therefore tangible that this study be carried out so as to provide more useful and information as regards the variation in body weight in Nigerian indigenous normal feather chicken as such knowledge can be useful in improvement and breeding programmes.

Materials and Method

The research was carried out at the Poultry Breeding Unit of the Federal University of Agriculture, Abeokuta, (FUNAAB) located on latitude 7°10 N in Odeda Local Government Area, Ogun State, in South-Western Nigeria. The ambient temperature during the period ranged from 26.9°C in June to 27.1°C in December with average relative humidity of 80%, while the vegetative site was an inter-phase between the tropical rainforest and the derived savannah (AGROMET, FUNAAB, 2015).

Experimental Birds and Management

A total number of 250 Nigerian indigenous normal feather chickens were used for the experiment; chicks were generated through artificial insemination method. Measurements were taken to determine the growth performance of the chicks. The birds were reared from day-old to 16 weeks of age.

The chickens were reared together on a litter floor in an open house. They were medicated similarly and were subjected to the same managerial, hygienic and climatic

conditions. Standard commercial starter feed (metabolisable energy of 2600kj/cal and Crude protein of 23%) was provided for 8 weeks and grower diets (metabolisable energy of 2450 kj/cal and Crude protein of 16%) was also provided from 9 to 16 weeks ad libitum and the birds have free access to water. Chickens were wing-banded at 1 day of age and body weights were recorded at the end of each two-week period.

Widely used non-linear growth models, Gompertz and Logistic were fitted to each individual body weight.

The mathematical relations of these models are as follows:

Gompertz : $W= A*Exp (-Exp(b-kt))$

Logistic : $W= A/(1 + b*Exp(-kt))$

Where W is the corresponding weight at time t. A is the adult value or asymptote, b is the slope and k is the growth rate

Sex effect on the growth curve parameters was analysed using Bayesian analysis of variance. The following fixed effects model was used:

$Yij = \mu + Si + eij$

Yij = a single body weight measurement,

μ = the overall means,

Si = the main effect of sex (1, 2)

eij = the random error.

The Winbug script was used to run the analysis using R-Software as a host.

Results and Discussion

The fitting of Gompertz and Logistic functions offered no computational difficulty for the chicken ecotypes considered in terms of computational time and convergence as these

Table 1: Means, Standard deviation, Credible Interval and Monte Carlo Errors of the parameter estimates of two growth models fitted for Nigerian indigenous normal feather chicken.

Model	Parameters	Mean	Sd	MCE	Median	CI		Convergence
						2.5%	97.5%	
Gompertz	A	1.70	0.38	0.04	1.72	1.32	2.52	
	b	1.41	11.81	0.56	3.67	2.47	10.91	Converged
	k	0.03	0.01	0.001	0.03	0.02	0.0	
Logistic	A	1.88	1.53	0.26	1.55	0.59	6.74	
	b	3.10	3.21	0.39	2.77	0.002	8.20	
	k	1.62	23.28	1.49	0.05	0.01	1.49	

Sd = Standard deviation, MCE = Monte Carlo Errors, CI = Credible Interval

**Growth curve fitted using winbugs. The model equations were y= A * exp(-b * exp(-k*t)) for the Gompertz and y =A / (1+exp(-(-b+k*t))) for the logistics.*

Table 2: Analysis of Variance Showing the Effect of Sex on Gompertz Growth Curve Parameters

Source of Variation	Df	MSS		
		A	b	k
Sex	1	2.61	243.40	5138542.30
Residual	212	1.59	123.76	2982466.90
Pr >F	-	0.20	0.16	0.19

Table 3: Analysis of Variance Showing the Effect of Sex on Logistic Growth Curve Parameters

Source of Variation	Df	MSS		
		A	b	k
Sex	1	2.75	607608	4194.91
Residual	206	0.52	579752.80	144714.69
Pr >F	-	0.02	0.31	0.87

**(p< 0.05)*

Table 4: Least Square Mean and Standard Error for Gompertz and Logistic Growth curve parameters

		Gompertz		Logistic	
Parameter	Subclass	LSM ± SE	N	LSM ± SE	N
A	F	2.04 ± 0.14	122	1.72 ± 0.07[b]	123
	M	2.27 ± 0.06	92	1.95 ± 0.05[a]	85
b	F	4.19 ± 1.33	122	22.77 ± 11.86	123
	M	2.04 ± 0.25	92	132.71 ± 128.18	85
k	F	0.84 ± 0.33	122	46.27 ± 37.04	123
	M	313.80 ± 274.81	92	37.14 ± 35.94	85

[ab] Means within the same row with different superscripts are significantly different (p<0.05), M= Male, F = Female, N= Number, Se = standard error

two curves converged to solutions at a short time interval for the normal feather ecotypes. Gompertz and Logistic functions achieved converged with a low number of iterations.

The means, convergence and their Monte Carlo Errors for the parameters estimated were used as a basis for the comparison of the two models. The means, convergence and their Monte Carlo Errors for the parameters estimated for the growth constant of each function are shown in Table 1.Average mature weight (A) values from Gompertz function was the closest to the observed values followed by Logistic. Highest value of k was observed in Logistic (1.62), which indicate that Logistic have a higher growth rate and also attain a heavier mature weight than the Gompertz which have low A and k value. Fitting the growth curve parameters into each models, Logistic also give a higher weight than Gompertz.

In Gompertz the mean value and credible interval (2.5% and 97.5%) were 1.70 (1.32 and 2.52) for the Asymptotic value, 1.41 (2.47 and 10.91) for b which is the slope and 0.03 (0.02 and 0.07) for k. In logistic mean value and credible interval (2.5% and 97.5%) were 1.88 (0.59 and 6.74) for the Asymptotic value, 3.10 (0.002 and 8.20) for b and 1.62 (0.01 and 1.49). Logistics gives a higher predicted weight of 1.88kg than Gompertz of 1.57kg. Gompertz gave a predicted weight (1.70) closed to the observed weight (1.60kg). MCE for Gompertz ranges from 0.001 to 0.56 and in Logistic from 0.05 to 0.39. Asymptotic value for the Gompertz shows convergence. Gompertz has a low value of MCE which shows the reliability of the model and also the credible interval from the Gompertz is close to the mean value than logistic.

Effect of sex on growth curve parameters presented from Table 4. It is evident that male consistently had higher Asymptotic mean value in the Logistic than the females. The better performance of the male chicken over the female chicken in Asymptotic value (A) for the Logistic in this study was an indication of the effect of superiority of the male over the female when the birds were monitored for better performance. This result is consistence with the report of Gueye *et al.,* 1998. The mean value for the males is 1.95 ± 0.05 while that of female is 1.72 ± 0.07 at 112days.

Conclusion

Based on all the criteria used for comparing these models in the three genetic groups, it can be established that the Gompertz function used in this study gave the best fit for the data analyzed although Logistic functions is equally good in predicting the growth curves of chickens.

Acknowledgement

The authors are thankful to the staff of the Teaching and Research Farm University of Agriculture, Abeokuta for the technical support rendered during the course of this research.

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EFFECT OF CROSSBREEDING AND SELECTION FOR MEAT ON NIGERIAN INDIGENOUS CHICKENS

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Abstract

Indigenous chicken possesses great potentials for genetic improvement through crossbreeding, selection and other breeding programmes. This study was conducted to compare our improved local broiler line with the commercial Anak Titan broiler chicken in terms of growth performance, feed intake and water consumption. A total of 250 birds were used for this study comprising 110 improved local broiler chicken and 140 Anak Titan broiler chicken. The two genotypes were subjected to the same standard management procedure from day old to ten week of age in different pens. Data on seven growth traits (bodyweight, breast girth, keel length, body length, shank length, thigh length, chicken height), feed intake, feed efficiency and water consumption were collected from week one to ten in the two genotypes. Data were analyzed using the Generalized linear model implemented in SAS. As expected, genotype and sex significantly ($p<0.05$) affected bodyweight and all the linear body parameters with the exotic broiler performing better than the improved local broiler. The bodyweight ranged from 47.21g and 37g at week one to 2754.55g and 1573.39g at week ten in exotic and improved local broiler respectively. Breast girth at week one to ten ranged from 11.27cm and 11.76cm to 29.21cm and 27.36cm in exotic and improved local respectively while other linear body parameters followed the same trend. Higher feed intake, efficiency and water intake were recorded in exotic compared to our improved local broiler. The results indicated that our improved local broiler consume lesser feed to achieve improved growth performance when compared to our unimproved local chicken although not as high as that observed in the commercial Anak Titan broiler.

Keywords: crossbreeding, selection, broiler, indigenous chicken, Anak Titan broiler

Introduction

Selection and cross breeding offer means through which genetic variation can be leveraged systematically to improve poultry productivity. The indigenous chickens exhibit high genetic variance in their performance, and therefore have great potential for genetic improvement through cross breeding and/ or selection in breeding and improvement programmes (Omeje and Nwosu, 1983; Nwosu *et al.,* 1984; Ikeobi *et al.,* 1996; Adebambo *et al.,* 1999, 2009; Peters, 2000; Adedeji *et al.,* 2008; Ilori *et al.,* 2016). In Nigeria, the poultry industry produces affordable poultry products such as meat and eggs which is essential for the reduction of protein malnutrition and is one of the fastest growing segments of the agricultural sub-sector (Akinokun, 1990). Similarly, locally-adapted chickens in Nigeria constitute a great number of poultry species produced by rural farmers. These species of poultry have small body and egg sizes, poor growth, poor feed efficiency and poor reproductive performance when compared with the exotic species (Oguike *et al.,* 2000; Ajayi, 2010). These characteristics makes them an undesirable stock in the economic stock market but can easily adapt to rural environment because they are generally hardy, survive on little or no food supplement and adjust to fluctuation in feed availability while the exotic breeds are mostly found in commercial production and are almost exclusively intensively managed examples of which are the normal feather, frizzle feather and the naked neck chickens (Oguike *et al.,* 2000; Ilori *et al.,* 2016). The exotic chicken are early maturing, good egg and meat producer but may not adapt to local environment and not resistant to many diseases. To obtain a balance, the exotic breeds are used to mate the local breed to obtain cross breeds which will combine all the good qualities of exotic and indigenous breeds. Selection for growth traits with the intention of having superior individuals with the best performance has led to rapid transformation in protein supply while the first step before selection is to evaluate their growth performance and their repeatability.

FUNAAB Alpha strains of chicken was developed at the Poultry Breeding unit of the Directorate of the University farm, Federal University of Agriculture, Abeokuta, Ogun state in Nigeria. The selection process for the traits of interest which is the meat and the egg started around 1997 with over 10 generations of selection for improved meat and egg production. There are two types of the FUNAAB Alpha chicken; the egg type and the meat type. The egg type is a dual purpose which was developed through a rigorous, systematic and selective breeding of the Nigerian indigenous chicken without eroding their tropical adaptive features and disease resistance traits. They are phenotypically the same in terms of plumage colours with normal feathered, frizzled feathered and naked neck Nigerian indigenous chickens. The average chick weight at hatch is between 30 – 35g, age at first lay ranged between 16 – 18 weeks, average body weight at first lay is between 1200g – 1728g, and weight of first egg at lay is between 35 – 40g. The average egg lay per year ranges between 200 – 250 eggs. The meat/ broiler type is a two way crosses (50% indigenous and 50% exotic) between selected FUNAAB Alpha and the exotic broilers. The plumage color varies from white to Ash with average body weight of 35- 42g at hatch. The average body weight at 8 week varies between 1.2 to 1.6kg. After the crossbreeding process, selection is then carried out to establish the best performing individuals. The objective of the work is to compare the selected line for meat production with the commercial broiler chicken.

Materials and Methods

Experimental Site

The experiment was conducted at the Poultry Breeding Unit of the Teaching and Research Farms of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm lies within latitude 7°10'N longitude 3° 2'E and altitude 76mm. It is located Odeda Local Government Area of Ogun State with a tropical climate within the derived savannah

zone of South-Western Nigeria. It has a humid climate with mean annual rainfall of 1037mm and temperature of about 34.7°C (Google Earth, 2016).

Experimental Birds and Management

A total of 110 day-old chicks of Anak titan and 110 day-old chicks of FUNAAB Alpha broilers were used for the experiment. The FUNAAB Alpha chicks were purchased from PEARL hatchery at FUNAAB while the Anak Titan were purchased from a reputable hatchery in Abeokuta. The chicks were fed ad libitum on a broiler starter mash containing 3200 kcal/kg Metabolized Energy and 22-23% Crude Protein, lysine (1.30%), crude fat (7.5%) for four weeks. Thereafter, finisher mash containing 2800-3000 kcal/kg Metabolized Energy and 18%-19% Crude Protein, lysine, methionine and cystine plus methionine requirements as 1.25%, 0.86% and 0.86% respectively for the broiler starter and 1.10%, 0.75% and 0.75% for the finisher phase. Clean water was supplied ad-libitum throughout the experimental period.

Data collection

Data were obtained on the following:

Body weight: this was recorded individually on weekly basis from day old to 10 weeks of age using Avery Berkel scale. While the weight gained for each week was calculated by subtracting the weight at the beginning of the week from the body weight at the end of the week.

Breast girth (BG): This is measured as the circumference of the breast around the deepest region.

Keel length (KL): This is the length of the sternum.

Body length (BL): This was taken as the distance between the base of the neck and the cloacae.

Shank length (SL): This was taken as the distance between the hock joint and the tarsal metatarsus.

Thigh length (TL): This was taken as the distance between the hock joint and the pelvic joint.

Chicken height (CH): This was taken as the distance between the footpad and the back.

Feed intake: Feed consumption for each day was obtained from the difference between the feed given per day and the left over.

Feed efficiency: The feed efficiency was calculated using the formula:

Feed efficiency = weight gain divided by feed intake

Water intake: Water intake for each day was obtained from the difference between the water given per day and the left over.

Statistical analysis

Data obtained for growth parameters was analyzed using the General Linear Model of SAS (2013). This model is as specified below:

$$Y\square jk = \mu + Bj + Pk + (BP)jk + e\square jk$$

Where

Y□jk= the parameter of interest

μ = overall mean for the parameter of interest

Bj = fixed effect of j□□ sex (j= 1-2)

Pk= fixed effect of k□□ genotype (1-2)

e□jk = random error associated with each record (normally, independently and identically distributed with zero mean and variance □²e). Duncan Multiple Range Test was used to separate the means that differed significantly (Gomez and Gomez, 1984). Correlation was computed using SAS (2013) to ascertain relationship between measureable traits.

Results

Growth traits as affected by genotype and sex

The results of this study showed significant differences in body weight and linear body measurements. As expected, body weight increased with increase in age of the birds in the two genotypes. Body weight in exotic chickens was significantly ($p<0.05$) higher than that of improved chicken (FUNAAB Alpha) throughout the experimental period (Table 1). The mean body weight ranged from 47.21±0.29g to 2754.55±30.29g for exotic and that of improved chicken from 37±0.42g to 1573.39±28.02g from week 1 to 10 respectively. Body length was also significantly ($p<0.05$) affected by genotype with increase in age of birds with the exotic broiler performing significantly ($p<0.05$) better than the improved chicken except at week 10 (Table 2). Table 3 showed that breast girth was also significantly ($p<0.05$) affected by genotype and followed the same patterns with the previous traits measured. From week one to six, improved chicken had bigger breast girth ($p<0.05$) than the improved but this was later overtaken by the exotic broilers from week seven to the end of the study. Genotype was found to significantly affected ($p<0.05$) thigh length in both genotypes. Exotic chickens were found to be significantly ($p<0.05$) higher in thigh length than that of the improved chicken (Table 4). Shank length and keel length were also significantly ($p<0.05$) affected by genotype with the exotic having better performance (Tables 5 and 6). The chicken height was not significantly different at week 10 ($p>0.05$) but the exotic broiler were significantly taller from the beginning of the study. Sex also significantly ($p<0.05$) affected all the parameters measured with male chicken consistently having higher performance than their female counterparts.

Table 1: Least squares means and standard errors for the effect of genotype and sex of body weight (g) on chickens.

	Genotype		Sex	
Age in weeks	**Exotic (140)**	**Improved (110)**	**Male**	**Female**
0 (day old)	47.21± 0.29^{a}	37± 0.42^{b}	45.89±0.93^{a}	41.70±5.52^{b}
1	127.43± 1.52^{a}	121.67±1.99^{b}	129.73±2.02^{a}	123.28±1.48^{b}
2	314.31±3.94^{a}	219.36±3.61^{b}	314.62±7.0^{a}	258.94±4.38^{b}
3	596.89±7.77^{a}	410.09±7.47^{b}	426.385±36.35^{a}	486.01±8.39^{b}
4	873.76±12.49^{a}	573.27±10.80^{b}	603.12±14.76^{a}	700.61±13.42^{b}
5	875.44±15.21^{b}	886.41±12.49^{a}	726.64±14.62^{b}	1079.158±20.24^{a}
6	1381.03±16.84^{a}	1068.09±12.49^{b}	868.79±25.40^{a}	1107.04±28.18^{b}
7	1637.38±20.21^{a}	1093.09±23.12^{b}	1691.69±43.91^{a}	1289.211±23.21^{b}
8	1973.24±24.15^{a}	1208.09±25.91^{b}	2034.308±53.46^{a}	1509.368±29.67^{b}
9	2362+26.58^{a}	1342.27±26.63^{b}	2406.154±65.26^{a}	1756.526±37.37^{b}
10	2754.55±30.29^{a}	1573.39±28.02^{b}	2804.77±74.79^{a}	2063.87±42.99^{b}

^{ab} *Means in the same row within variable group with different superscripts are significantly different($p<0.05$)*

Table 2: Least squares means and standard errors for the effect of genotype and sex on body length (cm) on chickens.

	Genotype		Sex	
Age in weeks	**Exotic (140)**	**Improved (110)**	**Male**	**Female**
0 (day old)	11.85±0.08	12.17±0.11	11.78±0.14	12.06±0.07
1	13.85±0.09[a]	14.84±0.12[b]	13.89±0.17[a]	14.42±0.09[b]
2	16.05±0.13[a]	18.83±0.14[b]	16.36±0.24[a]	17.56±0.15[b]
3	18.40±0.18[a]	21.39±0.17[b]	18.61±0.31[a]	20.07±0.17[b]
4	20.39±0.19[a]	22.70±0.19[b]	20.52±0.30[a]	21.69±0.18[b]
5	22.38±0.23[a]	24.61±0.23[b]	22.66±0.37[a]	23.58±0.19[b]
6	24.38±0.26[a]	26.22±0.25[b]	24.46±0.42[a]	25.42±0.21[b]
7	26.29±0.29[a]	27.60±0.27[b]	26.36±0.48	27.03±0.22
8	27.91±0.31	28.46±0.29	27.78±0.49	28.28±0.24
9	2362+26.58[a]	1342.27±26.63[b]	2406.154±65.26[a]	1756.526±37.37[b]
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different($p<0.05$)*

Table 3: Least squares means and standard errors for the effect of genotype and sex on breast girth (cm) on chickens.

	Genotype		Sex	
Age in weeks	**Exotic (140)**	**Improved (110)**	**Male**	**Female**
0 (day old)	11.37±0.04[a]	11.76±0.03[a]	11.39±0.08	11.59±0.08
1	13.94±0.11[b]	15.14±0.13[a]	14.13±0.18	14.58±0.11
2	16.06±0.14[b]	17.35±0.12[a]	16.28±0.22[a]	16.74±0.12[b]
3	18.18±0.19[b]	20.12±0.17[a]	18.51±0.28[a]	19.19±0.16[b]
4	20.02±0.21[b]	21.44±0.16[a]	20.27±0.29	20.77±0.16
5	21.92±0.22[b]	22.99±0.19[a]	22.13±0.33	22.47±0.17
6	23.89±0.26	24.44±0.21	24.09±0.38	24.14±0.19
7	25.69±0.26	25.53±0.22	25.98±0.39	25.49±0.19
8	27.48±0.29[a]	26.43±0.23[b]	27.49±0.42	26.87±0.22
9	29.21±0.29[a]	27.36±0.22[b]	29.06±0.41	28.21±0.23
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different($p<0.05$)*

Table 4: Least squares means and standard errors for the effect of genotype and sex on thigh length (cm) of chickens.

	Genotype		Sex	
Age in weeks	**Exotic (140)**	**Improved (110)**	**Male**	**Female**
1	5.11±0.06[a]	4.97±0.04[b]	5.73±0.08[a]	4.69±0.03[b]
2	6.91±0.05[a]	6.25±0.07[b]	6.85±0.08[a]	6.55±0.05[b]
3	8.62±0.71[a]	8.01±0.07[b]	8.66±0.08[a]	8.25±0.06[b]
4	10.23±0.09[a]	8.96±0.07[b]	10.21±0.13[a]	9.50±0.08[b]
5	12.06±0.11[a]	10.26±0.09[b]	11.97±0.17[a]	11.04±0.11[b]

	Genotype		Sex	
Age in weeks	Exotic (140)	Improved (110)	Male	Female
6	13.89±0.13[a]	11.23±0.09[b]	13.76±0.21[a]	12.39±0.14[b]
7	15.65±0.17[a]	11.99±0.10[b]	15.41±0.29[a]	13.62±0.17[b]
8	17.44±0.19[a]	12.76±0.09[b]	16.97±0.35	14.89±0.21
9	19.27±0.17[a]	13.39±0.10[b]	18.58±0.37[a]	16.11±0.24[b]
10	21.15 ±0.17[a]	14.05± 0.10[b]	20.26± 0.41[a]	17.42± 0.28[b]
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different(p<0.05)*

Table 5: Least squares means and standard errors for the effect of genotype and sex on shank length (cm) of chickens.

	Genotype		Sex	
Age in weeks	Exotic (140)	Improved (110)	Male	Female
1	3.96±0.04[a]	3.39±0.04[b]	3.78±0.05	3.69±0.04
2	5.63±0.04[a]	4.62±0.04[b]	5.45±0.07[a]	5.11±0.05[b]
3	7.01±0.07[a]	5.75±0.05[b]	6.82±0.10[a]	6.34±0.07[b]
4	8.49±0.09[a]	6.49±0.04[b]	8.29±0.16[a]	7.40±0.09[b]
5	9.88±0.13[a]	7.53±0.06[b]	9.71±0.21[a]	8.58±0.12[b]
6	11.47±0.15[a]	8.11±0.07[b]	11.16±0.26[a]	9.63±0.15[b]
7	13.02±0.19[a]	8.78±0.07[b]	12.49±0.33[a]	10.74±0.19[b]
8	14.69±0.22[a]	9.36±0.07[b]	14.05±0.39[a]	11.82±0.23[b]
9	16.47±0.19[a]	9.90±0.07[b]	15.45±0.39[a]	13.01±0.26[b]
10	18.24±0.19[a]	10.65±0.07[b]	17.05±0.44[a]	14.35±0.31[b]
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different(p<0.05)*

Table 6: Least squares means and standard errors for the effect of genotype and sex on keel length (cm) on chickens.

	Genotype		Sex	
Age in weeks	Exotic (140)	Improved (110)	Male	Female
1	4.39±0.04[a]	3.59±0.04[b]	4.23±0.06[a]	3.98±0.05[b]
2	6.06±0.04[a]	5.07±0.05[b]	5.92±0.07[a]	5.54±0.06[b]
3	7.75±0.07[a]	6.69±0.07[b]	7.73±0.09[a]	7.14±0.07[b]
4	9.40±0.08[a]	7.31±0.07[b]	9.20±0.15[a]	8.26±0.09[b]
5	10.95±0.11[a]	8.41±0.08[b]	10.73±0.19[a]	9.54±0.12[b]
6	12.59±0.13[a]	9.14±0.09[b]	12.27±0.24[a]	10.69±0.15[b]
7	14.30±0.17[a]	9.51±0.13[b]	13.37±0.32[a]	11.73±0.21[b]
8	15.93±0.19[a]	10.17±0.11[b]	15.28±0.38[a]	12.82±0.24[b]
9	17.66±0.18[a]	10.67±0.11[b]	16.69±0.43[a]	13.95±0.28[b]
10	19.57±0.19[a]	11.43±0.11[b]	18.49±0.48[a]	15.31±0.32[b]
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different(p<0.05)*

Table 7: Least squares means and standard errors for the effect of genotype and sex on chicken height (cm) on chickens.

	Genotype		Sex	
Age in weeks	**Exotic (140)**	**Improved (110)**	**Male**	**Female**
1	9.29±0.04	9.17±0.09	9.22±0.07	9.25±0.67
2	11.47±0.06	11.63±0.11	11.56±0.11	11.54±0.07
3	13.48±0.08[a]	13.90±0.08[b]	13.67±0.13	13.67±0.07
4	15±73±0.12[a]	15.23±0.09[b]	15.58±0.8	15.49±0.09
5	17.47±0.11[a]	16.65 ±0.17	15.58±0.8	15.49±0.09
6	19.07±0.12[a]	17.68±0.15[b]	17.68±0.15	18.36±0.12
7	20.78±0.13[a]	19.36±0.16[b]	20.52±0.22[a]	20.05±0.13[b]
8	22.47±0.14[a]	20.83±0.17[b]	22.20±0.24[a]	21.61±0.13[b]
9	24.09±0.14[a]	21.88±0.19[b]	23.64±0.25[a]	22.97±0.16[b]
10	22.97±0.16	22.83±0.18	25.16±0.27[a]	24.28±0.17[b]
10	2754.55±30.29[a]	1573.39±28.02[b]	2804.77±74.79[a]	2063.87±42.99[b]

[ab] *Means in the same row within variable group with different superscripts are significantly different(p<0.05)*

Feed intake and efficiency as affected by genotype

Table 8 showed that genotype had significant (p<0.05) effect on feed intake. Feed intake was significantly (p<0.05) affected by genotype in all the weeks of the experiment except at week 2, 6 and 7. Feed intake increased with increase in age of the birds in all the genotypes. The exotic broiler had higher feed intake from the beginning of the experiment till week till the end of the experiment. The mean feed intake per day in the week of experiment ranged from 48.73±4.39g, up to 158.64±0.02 for the exotic and 32.38±4.09g, up to 124.95±6.07g for improved, per day for first and tenth week of age respectively. Table 9 showed that genotype also had significant (p<0.05) effect on feed efficiency throughout the weeks of experiment. As expected, efficiency of feed utilization increased with age, the highest feed efficiency recorded at week 10 for both genotypes. The highest significant (p<0.05) feed efficiency of 0.69485±0.03 was recorded for exotic, followed by that of improved with feed efficiency of 0.45437±0.2 at week 10.

Table 8: Least squares means and standard errors of means of the effect of genotype on feed intake (g/day)

	Genotype	
Age	**Exotic(140)**	**Improved(110)**
1	48.73±4.39[a]	32.38±4.09[b]
2	62.14±3.77[a]	62.43±3.69[a]
3	83.92±0.02[a]	86.54±0.02[b]
4	85.41±1.02[a]	87.51±0.34[b]
5	86.92±2.12[a]	88.24±0.02[b]
6	88.92±1.72[a]	88.24±0.03[a]
7	90.92±0.02[a]	89.29±5.59[a]
8	96.90±8.14[a]	93.12±5.59[b]
9	110.09±8.66[a]	111.26±4.12[b]
10	158.64±0.02[a]	124.95±6.07[b]

[a,b]*Means in the same row with different superscripts are significantly different (p<0.05).*
Values in parenthesis = number of observations

Table 9: Least squares means and standard errors of mean of the effect of genotype on feed efficiency

	Genotype	
Age	**Exotic(140)**	**Improved(110)**
1	0.24941±0.03[a]	0.18202±0.01[b]
2	0.26985±0.02[a]	0.22792±0.01[b]
3	0.30003±0.02[a]	0.23234±0.01[b]
4	0.44059±0.03[a]	0.23429±.02[b]
5	0.449789±0.05[a]	0.24444±0.02[b]
6	0.45437±0.03[a]	0.27838±0.02[b]
7	0.49992±0.03[a]	0.30670±0.02[b]
8	0.59211±0.03[a]	0.40444±0.03[b]
9	0.67599±0.03[a]	0.41347±0.04[b]
10	0.69485±0.03[a]	0.45437±0.02[b]

[a,b]Means in the same row with different superscripts are significantly different (p<0.05).
Values in parenthesis = number of observations

Water intake as affected by genotype

Table 10 shows the least squares means in millimeter/day of water intake of the birds as affected by chicken genotype. Water intake was significantly (p<0.05) affected by genotype in all the weeks of the experiment. Water intake increased with increase in age of the birds in all the chicken genotypes. Exotic chicken had the highest water intakes from the beginning to the end of the experiment. However, this was not significant at week 3 (p>0.05)

Table 10: Least squares means and standard errors of the effect of genotype on water intake (ml)/day

	Genotype	
Age	**Exotic(140)**	**Improved(110)**
1	93.3619±7.87[a]	61.5601±1.77[b]
2	93.6288±10.33[a]	61.5601±1.77[b]
3	130.270±12.28	126.323±10.58
4	239.760±19.68[a]	126.323±10.58[b]
5	270.729±5.80[a]	171.118±17.65[b]
6	249.790±0.03[a]	216.346±0.02[b]
7	309.790±0.02[a]	216.346±0.03[b]
8	354.790±0.03[a]	218.770±0.86[b]
9	365.790±0.03[a]	220.588±0.06[b]
10	412.790±0.03[a]	220.588±0.02[b]

[a,b]Means in the same row with different superscripts are significantly different (p<0.05).
Values in parenthesis = number of observations

Discussion

The profitability of poultry enterprise depends on their production at a specific point in time. Having a strain with high growth performance within the shortest possible time will increase profitability and bridge the persistent protein malnutrition especially in the sub-Saharan Africa. The Anak Titan broiler chicken have been developed and improved through a progressive program of breeding and selection for excellent performance and good processing yield to meet multiple market demand around the world.

The differences and superiority exhibited by the exotic broiler chicken used in this study corroborated the fact that they have been selected for better growth performance compared to our improved local broiler counterpart. For the growth performance, the improved was closer to the exotic and had taken advantage of heterosis suggesting that the improved chicken had a high combining ability with the exotic breed. This was expected since the male parent here came from the exotic strain while the local chicken combined significantly well with the exotic strain to achieve an improved body weight. The implication of these acquired attributes for the crossbreds is that they could be further screened as possible candidates for tropical chicken broiler breed development. More vigorous crossbreeding, selection and an improvement of the local chicken would need to be pursued to improve on this. The fact that the improved chicken had low growth rate is expected since our indigenous poultry have gone through more of natural selection for survival to the tropical climate rather than artificial selection for productivity (Ibe, 1998; Ilori *et al.,* 2010, 2016). The body weight of 1.57kg observed in this study at week 10 is only possible at maturity in unimproved Nigerian indigenous chicken. Different researchers have reported that the native chickens are inferior in growth and egg characteristics compared with the exotic strain but have genetic potentials such as adaptability to Nigerian local environment, disease resistance, less feed requirement among other (Nwosu,1987; Sanda *et al.,* 2012). The local chicken body weight has been found by Eshiett and Okere (1989) to be about 1.03 kg at maturity under the intensive management system. The body weight of 1.253kg at week 12 for normal feather chicken, 1.324kg at week 12 and 1.618kg at week 20 for frizzle feather have been reported (Sanda *et al.,* 2012). Mature weight of 1.040kg at 20 weeks has also been reported for naked neck chicken (Gunn, 2008). The results on body measurements followed the same trend. The linear measurements studied (thigh length, body length, shank length, keel length, breast girth and chicken height) showed that the exotic chicken had superiority over the improved chickens except at week one where the superiority was not significant. According to Gous (1997), growth is normally accompanied by an orderly sequence of maturational changes and involves accretion of protein and increase in length and size, not just an increase in body weight. Sexual dimorphism observed in this study for all the traits with male chicken performing better than their female counterparts has earlier been reported (Ibe and Nwosu, 1999; Ilori *et al.,* 2010) to be attributed to differences in hormonal profile, aggressiveness, dominance and competition between males and females especially when both sexes were managed together.

The improved chicken was observed to have recorded the lowest mean for feed intake. This is not surprising as feed intake in animal is directly related to the body mass. The improved local chicken also recorded the least mean value for feed efficiency. This implies that the higher the feed intake needed to achieve a proportional increase in body weight, the lower the feed efficiency obtained and when feed efficiency is low, the quantity of feed to achieve a kilogram body weight is high. However, exotic chicken had highest feed intake with the effective efficiency meaning that they were able to utilize efficiently the feed consumed to achieve a proportionate increase in body weight compared to their counterpart. Ayorinde and Oke (1995) reported that the quantity of feed consumed in kilograms by different strains differed as well as the efficiency

in converting the feed to flesh.

Exotic chicken had the highest water intake throughout the period of the experiment, and this is not surprising as they were originally bred for temperate region where heat radiation is minimal. Heat production is affected by body weight, species and breed, level of production, level of feed intake, feed quality and, to a lesser extent, by the amount of activity and exercise. This implies that the birds increased water consumption to compensate for water loss and to increase the heat dissipation capacity especially with the exotic broiler that is not selected for the tropical climate.

However, water retention is reduced due to the increased electrolyte excretion in urine and faeces (Belay *et al.,* 1992; Belay and Teeter, 1996). At this point, if the amount of water lost is not completely compensated, dehydration and increased body temperature will occur. To overcome this problem, birds consume markedly more water (Zhou *et al.,* 1999; Tanveer *et al.,* 2005), causing plasma expansion, reduced plasma osmolarity and whole blood viscosity. Although birds consume more water to overcome these consequences, water retention is reduced due to increased electrolyte excretion (Belay *et al.,* 1992) and due to continuous loss of water through panting. According to Georgai (2001) and Lott et al. (2003), research has demonstrated that there was a relationship between feed and water consumption. The feed intake varied across weeks between the two genotypes which consequently affected the water intake of the birds. Higher water intake towards the end of the study in improved native chicken might be as a result of their crossbred nature that might have diluted some of their adaptive traits.

Conclusion and Application/Impact

- The improved performance observed in our selected broiler line at 10 week of age is an indication these birds could be further selected for tropical broiler production
- The results of feed intake and efficiency reflected that lesser feed will be required for the improved local broiler production compared to their exotic counterpart
- Lesser water consumption by the improved broiler indicated that the adaptive genes for tropical environment have not been diluted

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PREVALENCE OF BOVINE MASTITIS AND MULTI-ANTIBIOTIC RESISTANT STAPHYLOCOCCUS AND STREPTOCOCCUS SPECIES IN A RESEARCH CENTRE FARM AT NAIVASHA, KENYA

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Abstract

A cross-sectional study was conducted on prevalence of mastitis, its bacterial causes, their antibiotic sensitivities and management practices of sahiwal and dairy cattle kept at a centre of Kenya Agricultural & Livestock Research Organization (KALRO) in Naivasha, Kenya. Clinical mastitis was diagnosed through physical examination of cows' udders and milk. California Mastitis Test (CMT) was used to detect subclinical mastitis (SCM). Bacterial causes were determined by culture and their antibiotic sensitivities investigated by subjecting them to the commonly used antibiotics. Fifty cows were randomly selected from each herd giving a total of 100 cows. Prevalence of clinical mastitis in sahiwals at cow level was 6% (3/50) and subclinical mastitis (SCM) was 54% (27/50). Dairy herd had only SCM with a cow level prevalence of 36%. Prevalence of SCM was significantly different ($p<0.05$) between the herds. Sahiwal herd had 93.8% bacterial recovery rate with Staphylococcus species as the predominant bacteria (86.7%) (n=30). Other isolates included Streptococcus 6.3%, Corynebacterium 3.3%, and Escherichia coli. Mixed infection of Staphylococcus and Streptococcus was found in one case. Milk samples from dairy herd had a bacterial recovery rate of 85.7% with Staphylococcus species as the predominant (55.6%) bacteria. Other isolates included Streptococcus species (38.9%) and Corynebacterium (5.6%). Mixed infection consisting of Staphylococcus and Corynebacterium (5.6%) was also detected. Staphylococcus isolates had highest sensitivity to Gentamycin of 100% while Streptococci had the highest sensitivity for Ampicillin and Gentamycin of 100%, respectively. The isolates showed resistance to some commonly used antibiotics such as sulphamethoxazole, streptomycin and tetracycline. Bovine mastitis is prevalent among cows at KALRO-Naivasha and appropriate control methods needs to be applied to lower this prevalence. Further, this study has shown that Gentamycin and ampicillin are the drugs of choice for treating bovine mastitis in this institute. In fact, knowledge on prevalence of mastitis causing organisms and their antibiotic sensitivities will boost efficacy of therapy and cow productivity.

Key words: antibiotic sensitivity, bacterial causes, dairy herd, mastitis prevalence, Sahiwal herd

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Introduction

The dairy industry is the largest agricultural sub-sector in Kenya, even larger than tea farming (Muriuki *et al.,* 2004). It contributes about 14% of the agricultural Gross domestic product (GDP) and 3.5% of the total national GDP (GoK, 2008). Despite this, the sector is faced with several challenges among them bovine mastitis. Mastitis causes major economic losses in terms of decreased milk production, veterinary costs, premature culling of cows usually the high yielding ones, cost of replacement as well as discarding and down-grading of milk (Shitandi *et al.,* 2004; Mungube *et al.,* 2005; Viguler *et al.,* 2009). Mastitis, an inflammation of the mammary glands, is caused by both infectious and non-infectious agents. The main aetiological agents of mastitis are infectious microorganisms, the majority being bacteria (Malinowski *et al.,* 2006; Cheng *et al.,* 2010; Odongo *et al.,* 2012). The most frequently reported bacterial causes of bovine mastitis in Kenyan dairy herds are Staphylococcus aureus, Streptococcus species and coliforms (Muthee *et al.,* 2005; Gitau *et al.,* 2011; Odongo *et al.,* 2012). A few studies have also reported fungal causes such as Candida albicans and Cryptococcus neoformans (Odongo and Ambani, 1989; Odongo *et al.,* 2012).

Prevalence of bovine mastitis in Kenya has been reported by several authors (Omore *et al.,* 1997; Anakalo *et al.,* 2004; Muthee *et al.,* 2005; Ondiek *et al.,* 2013). High prevalence of mastitis is associated with absence of mastitis control program and surveillance, poor hygiene during milking and lack of preventive measures. However, the types of mastitis, the major risk factors and the extent of resistant strains in Kenya are not well documented (Hart and Kariuki, 1998). Proper data on these issues is needed to inform appropriate mastitis control methods and programs.

Clinical mastitis is easy to detect since it present with clinical signs, while sub-clinical mastitis (also referred to as hidden mastitis) is not and this is more devastating to farmers. While strip cup can detect clinical mastitis through milk changes in colour, smell and consistency, it cannot detect sub-clinical infections since there are no detectable physical changes in sub-clinical mastitis cases. Sub-clinical cases are detected using California Mastitis Test (CMT), an indirect pen-side chemical test used for determining somatic cell counts or numbers in milk (Leslie *et al.,* 2002). The CMT reagent raptures the cells thus releasing DNA responsible for the gel formation. Another method used in diagnosis of sub-clinical mastitis is the direct measurement of somatic cell count (SCC) which is used internationally as a measure of milk quality. Somatic cells are animal body cells present at low levels in normal milk. High SCC values in milk indicate poor quality milk as a result of mastitis (Rice and Bodman, 1993).

Antimicrobials forms the main bulk of therapeutic agents used in treatment and control of mastitis. Antibiotics are largely applied in mastitis therapy and in Kenya some of the most commonly used antibiotics include penicillin, streptomycin, tetracycline, gentamycin, ampicillin and sulphonamides (Ndirangu *et al.,* 2013). Many factors contribute to the success of mastitis therapy. Key factors include, correct diagnosis, stage at which treatment is initiated, clinical presentation, etiology, the drug selected, appropriateness of the route of drug administration and antimicrobial susceptibility of the etiological agent (Miltenburg *et al.,* 1996; du Preez, 2000). Resistance of bacteria to antibiotics is among the main reasons for low efficacy of antibiotic therapy of mastitis. In Kenya only a few studies have reported the antibiotic sensitivities of the bacterial causes of mastitis (Agumbah *et al.,* 1983; Gitau *et al.,* 2011) yet successful mastitis treatment programs require prior in vitro antibiotic sensitivity testing so as to avoid indiscriminate use of antibiotics. In fact sensitivity profiling for commonly used antibiotics is rarely done. The animal health delivery system is such that quacks, para-veterinarians and other less qualified people are the main players (Ndirangu *et al.,* 2013) who lack the capacity to conduct laboratory isolation and antimicrobial sensitivity testing.

Therefore this study was formulated

with two main objectives namely;

1. To determine mastitis management practices and prevalence of mastitis in sahiwal and dairy herds of cattle kept at Kenya Agricultural & Livestock Research Organization-Naivasha Centre.
2. To determine the bacterial causes of mastitis and their antibiotic sensitivities.

Methodology

Study farm

The study was carried out in the livestock farm of KALRO-Naivasha. KALRO Naivasha houses the Dairy Research Institute that is the Dairy Centre of Excellence with state of the art facilities and technologies for addressing dairy production across the East African Region. Naivasha is situated 80 Km North-west of Nairobi. The farm hosts the sahiwal herd and dairy herd. The animals are maintained under extensive and semi-intensive grazing systems on the pasture fields.

Sampling of the study cows and their husbandry practices

Sahiwal herd

At the time of the survey, the sahiwal herd had 192 lactating cows from which 50 cows were selected using systematic random sampling. Under this sampling approach, every 4th cow (192/50=3.84) was selected and included in the study, examined and milk samples collected during milking. The sahiwal cows were kept under free grazing system with the animals grazing in the open field. Milking was also carried out in the grazing fields using portable metallic milking parlour. The cows are hand milked twice a day, in the morning and in the evening. The cows were milked under dusty conditions. Cold un-boiled borehole water was used for cleaning the teats. Non-medicated milking jelly was used with no pen-side mastitis screening test undertaken during milking.

Dairy herd

The dairy herd was made up of Friesians and its crossbreeds with indigenous cattle. The dairy herd had 92 lactating cows out of a sample of 50 cows was selected systematically by picking every 2nd cow (92/50=1.84) during milking. The dairy herd was maintained under semi-zero grazing system, where the animals were grazed in the paddocks during the day and were supplemented with dairy meal (formulated feed) during the morning and evening milking. The cows are machine milked twice a day, in the morning and in the evening. The udders are washed with warm water and dried using a towel before milking. A single towel was used to wipe several cows. There was no use of medicated milking jelly or disinfectant on the udders. Routine pen-side testing of mastitis was not carried out at milking.

Determination of mastitis

This was a cross-sectional study carried out during the month of February, 2013. History of individual cows was first recorded, which included the herd, type of grazing, breed, stage of lactation and parity, among others. The milking hygiene status was also noted and recorded. All the sampled cows were physically examined before their udders were palpated for swellings and abnormal manifestations. Stripping was done on each teat to look for physical changes in milk before milk sample collection. This examination enabled diagnosis of clinical cases of bovine mastitis, detection of teat injuries and loss of udder quarter(s).

Quarter milk samples from cows with no overt signs of clinical mastitis were screened using the California Mastitis Test (CMT) to detect subclinical form of mastitis as described by David et al (2005). A total of 389 milk samples collected from 100 lactating cows in both the Sahiwal and dairy herds were examined using CMT. It was not possible to collect milk from all the 400 udder quarters since some cows had lost and blind teats. The samples were aseptically collected during morning milking from each quarter into individual sterile sample bottles and appropriately labeled. Teats were first washed with warm water and dried with disposable gauze dipped in 70% ethyl alcohol. The first two squirts of milk were discarded.

Then a small sample of milk, about 2 ml was collected from each quarter into a plastic paddle that had 4 shallow cups corresponding to the 4 udder quarters. The CMT reagent of an equal amount was added to the milk and the paddle rotated to form a CMT reagent-milk mixture. After approximately 10 seconds, the score was read while continuing to rotate the paddle.

Mastitis case definition criterion

Clinical mastitis case was defined on the basis of abnormal milk, swollen udders or a combination of the two. In the absence of the above, then clinical mastitis was not detected. Sub-clinical mastitis (SCM) cases were determined from the CMT results based on gel formation. A positive case of SCM was defined at cow level if one or more quarters were CMT positive or at quarter level if the quarter was CMT positive with or without isolation of microorganisms in both cases. CMT results of 1-3 had evidence for gel formation upon mixing milk with the CMT reagent. The CMT results of 1 had mild gel, 2 moderate gel and 3 heavy gel. Negative SCM were those milk samples which did not result in gel formation upon mixing of the milk with the CMT reagent.

Bacterial isolation and identification

Milk samples for bacteriological examination were selected based on their mastitis status with all milk samples from cows with clinical mastitis and those with a CMT score of 2 and 3 used. In total, 53 milk samples (32 from the sahiwal herd and 21 from dairy herd) were selected. The milk samples were refrigerated at 4oC in the laboratory before bacteriological analyses was done. The milk samples were cultured for determination of colony growth and morphological characteristics. Briefly bacterial culture was performed as follows: using a sterile wire loop 1 ml of milk was streaked on to a Petri dish of blood agar enriched with 5% sheep blood and incubated aerobically at 37oC. The plates were examined for growth at 24 hours and those showing no growth were re-incubated and examined the following day. The growth was examined macroscopically (visually) and scored 1-4 depending on abundance of growth. The cultures were further examined to determine their cultural characteristics. Bacteria were identified microscopically through gram-staining reaction, morphology and arrangement of the stained bacterial cells (Gitau *et al.,* 2014). Biochemical tests mainly catalase test, for differentiating gram-positive cocci and rods and indole test, for differentiating gram-negative cocci were undertaken as described by Holt et al (1993).

The small to medium sized colonies that yielded gram-positive cocci, with clustering or in chains and tested positive for catalase were identified as Staphylococcus while those that tested negative were identified as Streptococci. The short gram-negative rods which tested positive for catalase were identified as Corynebacteria (Holt *et al.,* 1993). The gram-negative cocci that tested positive on being subjected to indole test were identified as Escherichia coli (E.coli).

Antibiotic sensitivity testing

Antibiotic sensitivity was determined on 19 bacterial isolates (15 were Staphylococcus species and 4 were Streptococcus species) using 8 different antibiotics. Ten (10) isolates came from the Sahiwal herd and 9 from the dairy herd. The isolates selected for sensitivity testing were those giving a pure culture and with a growth score of 3 or 4. The test antibiotics included ampicillin (25mcg), tetracycline (25mcg), co-trimoxazole (25mcg), streptomycin (10mcg), kanamycin (30mcg), gentamycin (10mcg), sulphamethoxazole (200mcg) and chloramphenicol (30mcg).

The antibiotic sensitivity test procedure used was the Kirb-Bauer disc diffusion method as described by Quinn et al (2000). This involved use of commercially available drugs, impregnated on paper discs that were applied onto the surface of sheep blood agar, uniformly spread with the isolate under test. This was incubated overnight at 37°C and interpretation made depending on the diameter of zone of inhibition of bacterial growth. The effectiveness of a drug was determined by measuring the

diameter of the zone of inhibition around the disc, where the larger the diameter the more effective the drug. Each antibiotic was tested on each of the 19 bacterial isolates. A scale of 0-3 as described by Gitau et al (2011) was used to score the relative efficacy of the different antibiotics against a particular bacterial isolate as shown below.

A zone diameter of < or = 8mm was scored 0 or 'R' for resistant

A zone diameter of 9-15mm was scored 1 or slightly sensitive

A zone diameter of 16-22mm was scored 2 or sensitive

A zone diameter of 23mm and above was scored 3 or very sensitive

Data management and analysis

All the data was entered into MS-excel 2007 where descriptive statistics were generated. Data on prevalence was analyzed according to the two herds used in this study namely sahiwal and dairy herds. The prevalence of mastitis was stratified by stage of lactation and categorized as early lactation (0-3 months), mid-lactation (>3-9 months) and late lactation (> 9 months). The prevalence was also stratified by parity and categorized as few (1-3 calvings), moderate (4-6 calvings) and many (over 6 calvings).

Prevalence was calculated as number of cows with mastitis divided by total number of cows tested and significant differences in mastitis prevalence in the different categories established using Pearson Chi square at 95% confidence levels. Bacteriological analysis samples were classified into two categories of subclinical and clinical mastitic milk. The isolates were classified by Genus. The ability of the test antibiotics to inhibit growth was used to estimate the sensitivity or resistance of Staphylococcus and Sterptococcus to the 8 antibiotics.

Results

Prevalence of clinical mastitis

The prevalence of clinical mastitis in the sahiwal herd at cow level was 6% (3/50) while no case of clinical mastitis was detected in the dairy herd. Majority, (67%;2/3) of the clinical mastitis cases were detected in cows in their early stage of lactation while one was in a cow at mid stage of lactation. Clinical mastitis in two of the cows was evident in one quarter while one cow had clinical mastitis in two quarters, giving a quarter prevalence of clinical mastitis of 2% (4/200).

Prevalence of sub-clinical mastitis

The prevalence of sub-clinical mastitis (SCM) at cow level amongst lactating sahiwal cows was 54% (27/50) and amongst dairy cows was 36% (18/50). The difference in mastitis cases between the two herds was significantly different ($p<0.05$). Among the dairy cows with SCM 44.4% (8/18) were Friesians and 55.6% (10/18) were crossbreeds.

The prevalence of SCM varied with the stage of lactation and parity in both the Sahiwal as well as the dairy herds (Table 1). In the sahiwal herd, SCM was highest amongst cows in their mid lactation stage and in dairy herd it was highest among cows in their late lactation stage. With regard to parity, SCM in both herds was most prevalent in cows with a parity of a few (1-3) times as shown in Table 1.

Teat injuries and loss of quarters

Teat injuries were reported in 18% (9/50) cows examined in the sahiwal herd. The main teat injuries were teat cracks and wounds with a few cows having teat pimples. A total of 12% (6/50) of the cows examined had a blocked quarter. Majority of cows with blocked quarters 67% (4/6) had only one quarter affected. Similarly, 1 cow each had two and three blind quarters, respectively.

On the other hand, 6% (3/50) of the study cows from the dairy herd had teat injuries like cracks, teat pimples and perforations. Lost or blocked quarters were reported in 4% (2/50) of the dairy cows examined. The two

Table 1: Prevalence of sub-clinical mastitis in lactating sahiwal and dairy cattle at KALRO Naivasha by lactation stage and parity

Cow risk factor	Herd type %	
Lactation stage	**Sahiwal (n=50)**	**Dairy (n=50)**
Early (1-3 months)	20 (10/50)[a,c]	0 (0/50)[b,d]
Mid (>3-9 months)	28 (14/50)[a,c]	12 (6/50)[b,f]
Late (>9 months)	6 (3/50)[a,e]	24 (12/50)[b,f]
Total	54 (27/50)[a]	36 (18/50)[b]
Parity		
Few	38 (19/50)[a,b]	30 (15/50)[a,d]
Moderate	10 (5/50)[a]	4 (2/50)[a]
Many	6 (3/50)[a]	2 (1/50)[a]
Total	**54 (27/50)[a]**	**36 (18/50)[b]**

Values with different letter superscripts along the row and column of comparison are significantly different ($p<0.05$)

lost quarters in the two cows were both the right-fore quarters.

Bacterial isolates

A bacterial recovery rate of 93.8% (30/32) for milk samples from the sahiwal study cows was achieved with rest showing no colonial growth on blood agar. Similarly, 85.7% (18/21) of the dairy cattle milk samples had appreciable bacterial growth on blood agar with the rest showing no growth. Among the bacterial isolates recovered, Staphylococcus was the most predominant followed by Streptococcus in both herds (Table 2). Mixed infections were recorded in the two herds of Staphylococcus and Streptococcus in one sahiwal cow, and Staphylococcus and Corynebacterium in a dairy cow.

Antibiotic sensitivities of bacterial isolates from both herds

The in vitro antibiotic susceptibility studies showed that Staphylococcus isolates had the highest sensitivity to Gentamycin of 100% (Table 3). Other drugs to which Staphylococcus isolates exhibited high sensitivities were ampicillin and kanamycin with 93.3% each. With regard to relative drug sensitivity, very high sensitivity was shown to Gentamycin (66.7%), with streptomycin and sulphamethoxazole having no isolate with a score of very high sensitivity as shown in Table 3. Staphylococcus isolates showed highest resistance against sulphamethoxazole (80%) and moderate resistance to streptomycin (40%) and tetracycline (26%).

Streptococcus isolates had highest sensitivity to ampicilin and Gentamycin of 100% each with the other antibiotics having variable sensitivities (Table 3). Further, ampicilin had the highest relative drug sensitivity where 75% of the streptococci were very sensitive to the drug. The streptococci had highest resistance against streptomycin, kanamycin and sulphamethoxazole of 75% each.

Table 2: Common bacterial isolates in the individual udder quarters of lactating sahiwal and dairy cows at KALRO Naivasha

Herd type	Bacterial isolate (%)				
	Staphylococcus	*Streptococcus*	*Corynebacteria*	*E. coli*	Mixed
Sahiwal (n=30)	86.7	6.3	3.3	3.3	3.3
Dairy (n=18)	55.6	38.9	5.6	0	5.6
Mean	71.15	22.6	4.5	1.65	4.45

Table 3: Antibiotic sensitivities of 15 Staphylococcus and 4 Streptococcus isolates from Sahiwal and dairy cows in KALRO Naivasha

Antibiotic	Staphylococcus (n=15)				
	Resistant (%)	Slightly sensitive (%)	Sensitive (%)	Very sensitive (%)	Overall sensitivity (%)
Ampicillin	6.7	20	46.7	26.7	93.3
Tetracycline	26.7	46.7	20	6.7	73.3
Co-trimoxazole	20	13.3	53.3	13.3	80
Streptomycin	40	6.7	53.3	0	60
Kanamycin	6.7	6.7	66.7	20	93.3
Gentamycin	0	6.7	26.7	66.7	100
Sulphamethoxazole	80	13.3	6.7	0	20
Chloramphenicol	20	26.7	46.7	6.7	80
			Streptococcus (n=4)		
Ampicillin	0	25	75	0	100
Tetracycline	50	25	25	0	50
Co-trimoxazole	50	25	25	0	50
Streptomycin	75	0	0	25	25
Kanamycin	75	0	25	0	25
Gentamycin	0	25	50	25	100
Sulphamethoxazole	75	0	25	0	25
Chloramphenicol	50	0	25	25	50

Discussion

The results of this study showed that there were two forms of mastitis occurring in the sahiwal herd namely clinical and subclinical mastitis (SCM). The dairy herd had only subclinical form. The prevalence of SCM was higher than clinical mastitis in sahiwal cows and this is similar to what is reported by other authors (Biffa *et al.,* 2005; Byarugaba *et al.,* 2008). The most probable reason for this is that subclinical form of mastitis usually receives little attention and efforts are concentrated on treatment of clinical cases. In addition, in Kenya there is lack of efficient, diagnostic services and treatment schemes, which facilitates spread of subclinical mastitis to other quarters unchecked. Since subclinical mastitis is not visible to the naked eyes, it implies that farmers cannot easily decide on what order to milk mastitic cows. Therefore, cows with subclinical mastitis are milked first or at the middle and not last as recommended hence assisting in the spread of SCM within the herd unknowingly and faster. In the two herds subclinical mastitis was the most common as reported by previous authors in different parts of Kenya (Ngatia, 1988; Omore *et al.,* 1996; Ondieki *et al.,* 2013). The form of mastitis is an indication of the different degrees of udder infection. The 6% prevalence of clinical mastitis in sahiwal cows reported in this study is similar to 5.6% prevalence of chronic clinical mastitis in cows kept at Egerton University, Njoro, reported by Ondieki et al (2013). However, this was less than the 13.3% reported by Omore et al (1996) for cows from Kiambu County. The probable reason for the lower prevalence may be because the study by Omore was carried out using cows kept under semi and complete zero-grazing whereas the sahiwal cows were under free grazing. Bovine mastitis is more prevalent under zero-grazing

form of cattle rearing. The study by Ondieki et al (2013) further classified the clinical mastitis into acute, sub-acute, chronic and gangrenous types. However, in the current study it was not possible to do this because of the very few cases of clinical mastitis detected in the study.

California Mastitis Test (CMT) and bacterial culture were used in this study to diagnose subclinical mastitis in lactating cows. Subclinical mastitis was defined as the state where udder quarters with no clinical abnormalities produce apparently normal milk that was positive on CMT and bacteriological culture (Mungube *et al.,* 2005; Ayano *et al.,* 2013). Prevalence of subclinical mastitis among the sahiwal cows of 54% reported in this study is similar to 55% reported by Ngatia, (1988). However, the prevalence of subclinical mastitis reported in the two herds of cattle was lower than 87.4% mastitis prevalence reported by Nkoroi and Maitho (2014), for dairy cattle in Nyeri County, Kenya. Since bovine mastitis is a complex disease involving interaction of many factors such as management practices, environment, animal related factors and etiological factors, its prevalence is expected to vary from place to place. The dairy cows investigated in this study had a subclinical mastitis prevalence of 36% which is significantly lower than that of sahiwal cows (54%) used in the same study. The reason for this difference may be the different management systems used in the two herds. The two herds of cattle used in this study were kept under free (for sahiwal herd) and semi-zero (for dairy herd) grazing systems. Non-zero grazing systems of cattle husbandry have been reported in several regions of Kenya such as Kericho, Trans Nzoia, Nyandarua and Nyeri Counties (Wambugu *et al.,* 2011). The production systems are influenced by the agro climatic characteristics of the area, land productivity potential and prevalence of animal diseases. The dairy herd used in this investigation consisted of Friesians and their crossbreeds. This is in agreement with findings by Omore et al (1999) that the breeds in the Kenyan dairy sector are mainly Bos taurus and their crosses with Bos indicus. In addition, the method of milking, either by machine or hand can influence the incidence of sub-clinical mastitis (Rodriguez 1997). The sahiwal cows were milked by hand while machine milking was used on the dairy cows. With regard to grazing system where sahiwal cows were kept under free grazing and dairy cows under semi-zero grazing system the prevalence rates given are in agreement with the results of Shem et al (2001); where prevalence of sub-clinical mastitis was lower in zero grazed cattle than in free grazed cattle with infection rates of 37.5% and 90.6% respectively. However, this differed with the results of Goodger et al (1988), who reported that free range cows were bound to be less affected by mastitis due to ample area per cow compared to zero grazed cows. Another possible reason is breed influence on prevalence of mastitis which can be attributed to differences in mammary gland physiology and anatomy. Indeed occurrence of mastitis may be influenced by hereditary characteristics like milk productivity, teat structures and udder conformation (Radostits *et al.,* 2007; Biffa *et al.,* 2005). The sahiwal cows investigated in this study had pendulous udders and long teats which predisposes them to mastitis hence the high prevalence of mastitis observed in this herd compared to dairy cows. Generally, the high prevalence of SCM reported in this study can also be attributed to the presence of multi-antibiotic resistant Staphylococcus and Streptococcus isolates identified among lactating cows included in the study.

In relation to the stage of lactation, the prevalence of subclinical mastitis among sahiwal cows examined in the current study was found to be highest during mid-lactation stage (4-9 months after parturition). This is consistent with the findings by Mureithi and Njuguna (2016) for cows in peri-urban farms of Thika, Kenya. Usually cows attain peak milk production at this stage. Previous studies have demonstrated that high yielding cows are more prone to subclinical mastitis as a result of increased susceptibility of glandular tissue to infection (Radostits *et al.,* 2007). In the dairy herd subclinical mastitis was most prevalent among cows in late lactation stage. The two findings agree with the observation made by

Ayano et al (2013) that among the cows kept at commercial dairy farms in Ethiopia, subclinical mastitis was more frequently encountered among cows in middle and late lactation than early lactation stage. A probable explanation for this may be the fact that most cows at mid and late lactation are mostly pregnant, although the pregnancy status of the sampled cows was not evaluated in this study. Pregnant cows tend to get stressed and spend most of their time laying down hence predisposing them to mastitis especially in this case where the cows' environment was found to be of poor hygienic status. Further, SCM was prevalent in cows with few parity of 1-3 times. This is consistent with what was reported by Elbably et al (2013) from Egypt and Moges et al (2012) in Ethiopia; but differed with what was reported by others who reported that prevalence of mastitis increases with age or parity (Biffa *et al.,* 2005; Ayano *et al.,* 2013). The variations on effect of lactation stage and parity on mastitis occurrence between the various studies can be attributed to disparities in age, parity and breed of the lactating cows sampled.

Results of the study also found presence of loss of udder quarters of 12% in sahiwal cows and 4% in dairy cows which had blind mammary quarters. This has a direct influence on milk production due to non-factional udder quarters signifying the importance of the problem. Lack of regular screening for SCM found in this study coupled with inadequate follow-up of mastitis cases and persistent challenges of mammary glands by microbial pathogens could be the main predisposing factors to quarter blindness and loss.

Milk samples with a CMT score of 2 and 3 were cultured for bacterial isolation where those from sahiwal herd had an isolation rate of 93.8% and dairy herd had 85.7%. These high bacterial isolation rates from CMT positive samples means that CMT is a good diagnostic tool for detection of subclinical mastitis and culture methods may be used to confirm and aid in proper mastitis therapy. On the other hand failure to isolate bacteria from some CMT positive milk samples could partly be attributed to low concentration of bacteria in milk, intermittent shedding and intracellular location of pathogens, and presence of inhibitory substances in milk (Radostits, 2007). The bacterial isolates in this case are important pathogens associated with mastitis in dairy cattle. Staphylococcus spp., Streptococcus spp. and E. coli reported in the present study are similar to those reported elsewhere in Kenya (Gitau *et al.,* 2012; Ondiek *et al.,* 2013; Nkoroi and Maitho, 2014). The major mastitis causing bacterium isolated was Staphylococcus species which is in agreement with the results of similar studies conducted in different parts of Kenya (Gitau *et al.,* 2012; Odongo *et al.,* 2012; Ondieki *et al.,* 2013; Nkoroi and Maitho, 2014; Mureithi and Njuguna, 2016). A similar finding was also reported by Ayano et al (2013) for dairy cattle in Ethiopia and Byarugaba et al (2008) for cows in Uganda. Among the two herds investigated in the present study the prevalence of Staphylococcus species was higher among milk samples from the sahiwal cows of 86.7% than those from the dairy herd which was 55.6%. This variation may be attributed to the differences in the levels of hygiene and milking practices among the two herds. In the sahiwal herd the cows were milked by hand and milking hygiene standard was generally poor characterized by being done in the open under dusty conditions, use of cold un-boiled borehole water and wiping several cow udders with one cloth. In the dairy herd there was machine milking and good milking hygiene. Staphylococus is spread among cows or between quarters directly during the milking process from infected udders or quarters to clean ones through the milkers' hands or wiping towels (Nkoroi and Maitho, 2014). A high proportion of these bacteria in milk is related to poor hygiene practices (Ateba *et al.,* 2010).

The present study also identified other bacterial causes of mastitis which included Streptococci, Corynebacterium and E.Coli. Other studies carried out in dairy farms in Kenya, have also reported Streptococci and E. coli as other bacterial causes of bovine mastitis in addition to Staphylococci (Shitandi and Gathoni, 2004; Ondieki *et al.,* 2013; Mureithi

and Njuguna, 2016).

The antibiotic sensitivity results showed that the most effective antibiotics in treatment of mastitis among cows in the study site are Gentamycin, ampicillin and kanamycin. Other in vivo studies have reported Gentamycin to be the most effective drug (Ondiek *et al.,* 2013; Gitau *et al.,* 2011). The present study further showed that the bacterial isolates had low sensitivity to tetracycline, streptomycin and sulphamethazoxale. Similar findings were reported by Gitau et al (2011) where sensitivity was highest for Gentamycin and kanamycin while it was moderate to low for penicillin, tetracycline, streptomycin and penicillin-dihydrostreptomycin. This can be explained by the fact that there has been extensive use and misuse as well as availability of tetracycline, streptomycin and penicillin for many years in Kenya while Gentamycin and kanamycin were introduced recently thus resistance to the latter two drugs has not developed extensively. Further the high cost of mastitis treatment can partly be related to development of resistance to the common and cheap antibiotics like penicillin and tetracycline that was demonstrated in the bacterial isolates. High incidence of the use of non-efficacious and sub-therapeutic regimes of antimicrobials in developing countries by all cadres of veterinarians and para-veterinarians is an important concern. Indeed the antibiotic resistance observed in this study is a cause for worry and measures to mitigate development of antibiotic resistance are required including prudent use of drugs and prior antibiotic sensitivity testing before commencing mastitis therapy.

Conclusions

- The study revealed a relatively high prevalence of bovine mastitis in the study area, particularly the subclinical form.
- Many previous studies have focused on intensive dairy farms and there is a tendency to link high prevalence of the disease with exotic cattle but this study showed that mastitis is prevalent in sahiwal cows under free grazing thus it has shown that mastitis is assuming equal importance in extensive cattle production systems.
- The specific mastitis etiological agents found were Staphylococcus spp., Streptococcus spp., Corynebacterium and E. coli.
- The Isolated bacteria were most sensitive to Gentamycin and ampicilin and therefore these are the drugs of choice for mastitis therapy in the study area.

Recommendations

- Effective mastitis preventive and control measures needs to be instituted in the study area. Such measures should include; improvement of milking hygiene, teat disinfection, routine pen-side testing for mastitis specifically during milking, effective treatment of mastitis cases both clinical and subclinical, culling of older cows with repeated mastitis episodes, prompt treatment of teat/udder injuries among others.
- There is no adequate information regarding mastitis in local and dual purpose dairy cows, hence there is need for more research.
- Maintenance of environmental hygiene should be instituted to reduce intrammamary infections with Staphylococci and E. coli.

Importance of the findings 'Impact'

The findings of the study have revealed that the disease affecting the mammary glands of cows, known as mastitis, occurs mainly in the form that cannot be observed (sub-clinical mastitis) which necessitates use of tests to detect. This study showed that mastitis is prevalent in sahiwal cows under free grazing thus demonstrating that mastitis is assuming equal importance in extensive cattle production systems. Factors putting cows at the risk of contracting mastitis were identified and information is useful in formulating appropriate and improved control strategies. Bacteria causing the disease in the study area

were identified and antibiotics that are effective in treatment identified hence these drugs can be used by veterinarians in Naivasha without undertaking antibiotic sensitivity tests.

Acknowledgments

This study was funded by World Bank through Eastern Africa Agricultural Productivity Project (EAAPP). The authors are grateful to technical staff of Kenya Agricultural & Livestock Research Organization at Veterinary Research Institute and Masinde Muliro University of Science and Technology for their part in collecting and culturing of milk samples. Further they are grateful to the management and staff of KARLO-Naivasha for allowing them to use the cows at the centre and for their assistance during sample collection.

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PATTERN OF WANING OF MATERNAL ANTIBODIES AGAINST NEWCASTLE DISEASE OF CHICKS FROM SELECTED HATCHERIES IN KUMASI, GHANA.

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Abstract

This study was conducted to measure and compare the rate at which maternal antibodies against Newcastle Disease (ND) wane in chicks from some selected hatcheries in Kumasi, Ghana. Sera from 150 unvaccinated cockerels collected from three hatcheries (A, B, C) were screened using standard methods of heamagglutination inhibition technique. Two separate vaccination schedules against ND were employed in advising poultry farmers at the Regional Veterinary Laboratory were compared for their suitability. Descriptive statistics was employed. All the chicks from the three hatcheries had maternal antibodies against ND at day-1 and that the maternal antibodies lasted till day-21. Hatchery A had the highest maternal antibody titre of 9.97 GMT at day-1, followed by B with 7.84 GMT and C having the lowest GMT of 6.70. Based on the vaccination schedule, schedule (I) which puts the first Newcastle vaccination at 8 days old (week 1) would not be favourable for any of the chicks that were obtained from the three hatcheries due to the presence of protective maternal antibody levels (4.0 GMT) in sera obtained from chicks during the first week of age. Schedule (II) which puts the first ND vaccination at day-15 favored hatcheries A and C. This is because the MAb titres of chicks from hatcheries A and C decayed below the protective titres after day-14. This study represents the first report on waning pattern of Newcastle disease specific MAb in chicks in Ghana and it also showed that vaccination schedule should take cognizance of MAb evaluation for the control incessant ND outbreaks.

Keywords: Decay, Maternal antibodies (MAb), Newcastle Disease (ND), Vaccination schedule, Ghana

SCHEMA D'AFFAIBLISSEMENT DES ANTICORPS MATERNELS CONTRE LA MALADIE DE NEWCASTLE CHEZ DES POULETS DES ECLOSERIES SÉLECTIONNÉES À KUMASI AU GHANA

Resume

La présente étude a été réalisée dans l'objectif de mesurer et de comparer les taux auxquels les anticorps maternels contre la maladie de Newcastle (MNC) diminuent chez les poussins dans certaines écloseries sélectionnées à Kumasi au Ghana. Les sérums de 150 jeunes coqs non vaccinés prélevés dans trois écloseries (A, B, C) ont été examinés à l'aide de méthodes standard de la technique d'inhibition de l'hémagglutination. Deux modèles de vaccination distincts contre la MNC, utilisés pour donner des conseils aux aviculteurs au Laboratoire vétérinaire régional, ont été comparés pour déterminer leur pertinence. Des statistiques descriptives ont été utilisées. Tous les poussins des trois écloseries avaient des anticorps maternels contre la MNC au jour 1 et les anticorps maternels se sont maintenus jusqu'au jour 21. L'écloserie A a montré le titre d'anticorps maternel le plus élevé d'une GMT de 9,97 au jour 1, suivi de B avec une GMT de 7,84, C ayant la plus basse GMT de 6,70. Selon le calendrier de vaccination, le calendrier (I) qui fixe le premier vaccin contre la maladie de Newcastle à 8 jours (semaine 1) ne serait pas favorable aux poussins obtenus dans les trois écloseries en raison de la présence de taux d'anticorps maternels protecteurs (GMT de 4,0) dans les sérums prélevés sur les poussins pendant la première semaine d'âge. Le calendrier (II) qui fixe la première vaccination contre la MNC au jour 15 était favorable pour les écloseries A et C. C'est parce que les titres des anticorps maternels (Mab : maternal antibodies) des poussins des écloseries A et C se sont dégradés en dessous des titres protecteurs après le jour 14. Cette étude représente le premier rapport sur le schéma d'affaiblissement des MAb spécifique à la maladie de Newcastle chez les poussins au

Ghana, et elle a également montré que le calendrier de vaccination devrait prendre en compte l'évaluation des MAb pour le contrôle des poussées incessantes de MNC.

Mots-clés : détérioration, anticorps maternels (MAb), maladie de Newcastle (MNC), calendrier de vaccination, Ghana

Introduction

Evaluation of rate of waning of maternal antibody to most pathogens in chicks has been very useful in developing effective and efficient vaccination protocols for most poultry disease. This is particularly important in disease endemic areas with promising poultry industry such as found in most developing countries including Ghana where frequent disease outbreaks have hindered the harnessing of the potential of this sector towards the growth of the economy (Adei and Asante 2012 ; Killebrew et al 2010).

Newcastle disease has been identified as the major diseases that stall the progress of the poultry industry in Ghana. (Atuahene et al, 2010). In 2014, there were 93 outbreaks of Newcastle disease which affected 92,696 birds out of which 2,382 died in Ashanti region alone (MOFA, 2015). This shows the severity of the impact of Newcastle disease outbreaks in this region, the hub of poultry production in Ghana despite various vaccinations protocols in place.

Currently, there are two main vaccination programs often employed in the control of |Newcastle diseases in Ghana; one which puts the first vaccination at day 8 and the second at day 14. These vaccination schedules show lack of uniformity due to limited information on the rate of waning of maternal antibodies against Newcastle disease in chicks obtained from major hatcheries. Hence, persistence outbreaks observed could stem from improper timing of vaccination leading to maternal antibody interference and neutralization of vaccine antigens. Other possible causes include vaccine failure and vaccine breaks stemming from improper handling of vaccines..

Previous reports on maternal immunity and rate of decay in chicks are extensive (Gharaibeh et al, 2008; Jalil et al, 2009; Addison *et al.,* 2010; Maas *et al.,* 2011). These studies clearly showed that the half-life of some viral poultry disease mAbs ranged from 3 to 8days (Alan *et al.,* 1978; Fahey *et al.,* 1987; Darbyshire and Peters, 1985; Al-Natour *et al.,* 2004). However those of Newcastle specific antibodies in chickens have been reported by some researchers to be detectable up to day 28 post hatch (Oni and Adedipe, 2012). These reports also showed that mAb titers vary slightly from region to region based on the endemicity and vaccination programmes adopted to prevent outbreaks of the disease. In Ghana however, there's no published information regarding the effectiveness of vaccination programmes and waning of maternal antibodies to most economic important poultry pathogen. This study therefore addresses this gap by providing information on the effectiveness of the vaccination programmes and the waning pattern of maternal antibodies in chicks obtained from major hatcheries towards the understanding of the role of maternal antibody interference on the reported ND outbreaks often observed in Ghana.

Significance of the study

This study discovered that the existing vaccination protocol commonly used to advise farmers in the hub of poultry production in Ghana is not in synchrony with the waning of maternal antibodies of chicks obtained within that zone. Hence, detection and rate of waning of maternal antibodies in chicks is important to the assessment of poultry vaccination schedules. This study therefore will also help researchers to uncover the role of maternal antibody interference in the control Newcastle disease outbreaks of which many researchers and poultry farmers often do not explore. Thus approach to control of ND should always stress the importance of maternal antibody interference towards the development of newer vaccination schedule to be adopted.

Materials and Methods

Study areas

This study was conducted on the premises of the Regional Veterinary Services Directorate in Kumasi, Ashanti region. Kumasi lies between longitude 1.30'W and 1.35'E and latitudes 6.35'N and 6.40'S. It recorded a human population of 2,035,064 in 2010 and an estimated population of 2,396,458 in 2013 the highest in the region (KMA, 2014) The average temperaturet ranged between 21.5^{0}C and 30.7^{0}C while the average humidity varied between 84.16% at sunrise and 60% at sunset (GSS, 2014).

Study design

A purposeful multistage sampling technique was used in this study. Ashanti region was purposefully selected for the numerous hatcheries it has. In the Ashanti region, six major hatcheries were identified and three of these major hatcheries (A, B and C) were selected. In each of these three major hatcheries, fifty (50) one-day old unvaccinated cockerels each were screened for ND antibodies using haemagglutination inhibition technique for a period of three weeks. The chicks were fed on commercially prepared feed, water was provided ad libitum. The structure had appropriate ventilation and lighting. Also adequate biosecurity was employed to prevent the introduction of disease or infectious agents to the unvaccinated birds.

Vaccination programmes

Vaccination programmes used for the parent stocks and that used to advise the farmers were obtained from records of the hatcheries selected and from the Regional Veterinary laboratory respectively.

Screening of sera and data analysis:

The sera were collected and screened using Heamagglutination inhibition technique as described in OIE standard protocol. Microsoft Excel 2013 spreadsheet was used to categorise the data and calculate the geometric mean titres. A graph of the results of the maternal antibody geometric mean titres of the chicks from each hatchery was drawn using Microsoft Excel 2013 spreadsheet.

Ethical approval:

All applicable international, national, and/or institutional guidelines for the care and use of animals were observed.

Results

Vaccination programmes used for the parent stock of the three hatcheries:

In comparing MAbs of chicks from the three hatcheries, vaccination schedule of the parent stock were put into consideration. It was observed that Hatchery A administetred first Newcastle vaccine (HB1) at week 1 which was followed by 4 booster vaccinations with Lasota and Newcavac vaccines while Hatchery B gave the first Newcastle vaccine (HB1) at week 2 then followed by 3 booster vaccinations with Lasota and Newcavac vaccines. Birds from Hatchery C were given first Newcastle vaccine (HB1) at week 1 then boosted with 2 vaccination doses of Lasota and Newcavac vaccines respectively. (Tables 1& 2)

Table 1: The parent stock vaccination programmes of the three hatcheries

ND Vaccination Schedule For Hatchery A Parent Stock	ND Vaccination Schedule For Hatchery B Parent stock	ND Vaccination Schedule for Hatchery C Parent Stock
HB1(week 1)	HB (week 2)	HB1 (week 1)
Lasota (week 4)	Lasota (week 6)	Lasota(week 3)
Newcavac(week 10)	Newcavac(week 10)	Newcavac (week 12)
Newcavac (week 16)	Newcavac(week 18)	-
Newcavac (week 22)	-	-

Table 2: Vaccination programmes advised by the regional veterinary laboratory personnel in the Ashanti Region

AGE IN DAYS	PROGRAMME I	PROGRAMME II
8 (week 1)	HB1	-
15 (week 2)	-	HB1
29 (week 4)	Lasota	-
42 (week 6)	-	Lasota
70 (week 10)	Lasota	Lasota
102 (week 16)	Newcavac	Newcavac

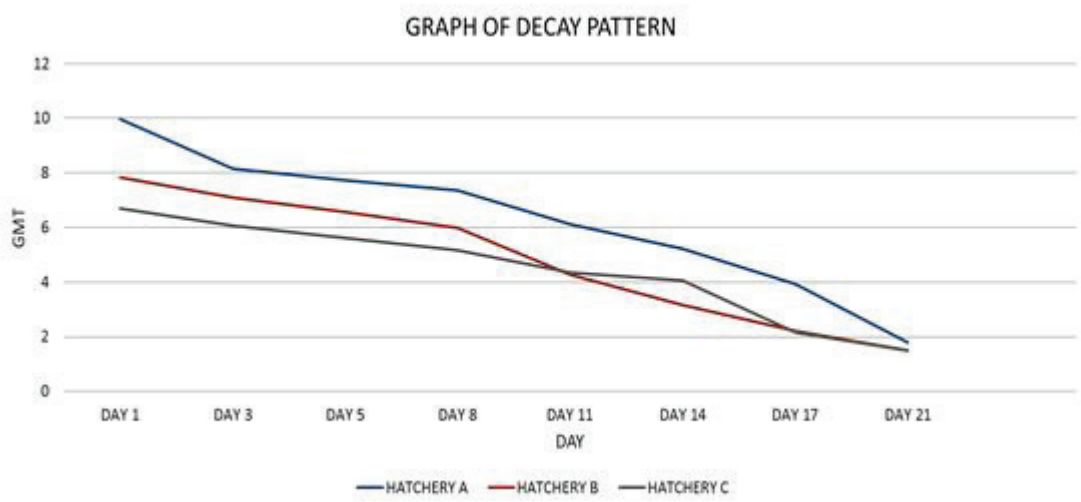

Figure 1: The decay pattern of maternal antibodies of chicks from hatchery A, B and C

From figure 1, it was observed that all the chicks from the three major Hatcheries (H-A, H-B and H-C) had maternal antibodies present at day-1 which lasted till day-21. It was also observed that Hatchery A had the highest maternal antibody titre of 9.97 GMT at day-1, followed by Hatchery B with 7.84 GMT and Hatchery C having the lowest GMT of 6.70. Pattern of waning of MAbs follows the reverse with H-C chicks weaning below 4.0GMT (Protective level) at day11, H-B chicks at day 14 while H-A chicks at day 17.

Discussion

This study revealed the presence of circulating maternal antibodies in all the chicks from the three hatcheries sampled at Day-1 through to Day-21. Evaluations of maternal antibodies in newly hatched chickens have been extensively studied with various reports from different countries with a fairly uniform waning time of about four or five days to about 10 days with an exception of infectious Bursa disease viral antibody lasting till about 30 days (Alan *et al.,* 1978; Fahey *et al.,* 1987; Al-Natour *et al.,* 2004; Addison *et al.,* 2010; Maas *et al.,* 2011; Ahmed, 2011; Gharaibeh and Mahmood, 2013). Although the level of maternal antibodies in newly hatched chicks varies largely upon the vaccination protocol of the breeder stock and the concentration of the maternal titre which often almost halved in newly hatched chicks.

Genetic line as well as the role of the male parent had been suggested to be factors that determine maternal immunity in offspring chicks (Saino *et al.,* 2002; Leandro *et al.,* 2011) Genetic influence over maternal immunity

dynamics was linked with physiological activity and metabolic rate of the chick (Bumstead *et al.,* 1991; Hamal et al ., 2006) which in turn affects the rate of absorption and decay of antibodies from the yolk (Boa-Amponsem *et al.,* 1997; Grindstaff *et al.,* 2003; Davison *et al.,* 2008) Hence, input of genetic material from the male could play a major role in the maternal antibody dynamics of the offsprings (Ahmed, 2015). However, the role of genetic line was not investigated in this study but should be encourage especially in poor resource countries like Ghana.

From this study, it was observed that maternal antibodies in all chicks lasted till day 21, however, titer of MAbs in chicks at day 1 ranked highest in H-A chicks, lower in H-B chicks and lowest in H-C chicks. This was probably due to the variation in vaccination protocol where live and inactivated ND vaccinations, also booster doses were administered to parent stocks at various timing in each of the three hatcheries. These variations reflected in chick's titre at day old and duration of protective level. (Table1).

Antibody tires above 4.00 GMT are generally considered protective (OIE, 2012) and as such MAb titres in chicks from hatchery B and C were protective until day Day-11 and 14 respectively. This corresponds well with a report which advised that ND vaccination done after Day 14 would have minimal MAb interference (Gharaibeh and Mahmood, 2013). Hence program II will best suit chicks from Hatchery B and C while none of the programme will be useful for chicks from Hatchery A since their MAb tires decayed below protective tires after Day-17. The implication of this shortfall in synchronization of vaccination schedule with maternal antibody titer in chick may be the reason for incessant ND outbreak due to MAbs interference (Gharaibeh and Mahmood, 2013; MOFA 2015).

This study showed that maternal antibody interference could be a possible contributing factor to the outbreaks of Newcastle Disease observed in Ghana. However, larger study with wide coverage considering factors such as evaluation of vaccine efficacy, types of strain and involvement of other regions known for poultry production as well as other hatcheries in Ghana is suggested.

Conclusion

Therefore, this study underscores the importance of evaluation of maternal antibodies dynamics to different disease pathogens such as in this case- Newcastle disease virus with a view of developing protective vaccination schedules. Hence regular evaluation of MAbs is recommended in other to adjust vaccination schedules in line with hatchery protocols and prevalence of outbreaks within the region. Also further studies on decay of MAbs to other economically important diseases such as Infectious Bursa Disease are recommended.

Acknowledgements:

We acknowledge all staff of the Veterinary laboratory, Kumasi, Ghana.

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BIOMETRICAL STUDY OF FEMALE REPRODUCTIVE TRACT (BOS INDICUS) IN CAMEROON

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Abstract

The study aims to determine the biometry of 501 genital tracts of local female zebus (Bos indicus) slaughtered at the Ngaoundere municipal slaughterhouse (Cameroon). Average length (cm), width (cm), thickness (cm) and weight (g) were 2.83 ± 0.03, 1.96 ± 0.02, 1.28 ± 0.01 and 5.17 ± 0.11 in right ovary; and 2.64 ± 0.03, 1.78 ± 0.02, 1.15 ± 0.01 and 4.02 ± 0.09 in left ovary, respectively. Using electronic Vernier calipers, 16957 follicles were counted with an average of 16.92 ± 0.32 follicles per ovary. Small (<3 mm), medium (3 to 8 mm) and large (> 8 mm) follicles were 8.53 ± 0.20, 8.06 ± 0.21 and 0.29 ± 0.02, respectively. 20.4% were pregnant. Regarding non pregnant animals, average oviduct, uterine horn, uterine body, cervix, vagina and vulva commissure lengths (cm) were 19.27 ± 0.14, 20.32 ± 0.20, 1.24 ± 0.02, 6.92 ± 0.08, 19.45 ± 0.08 and 4.91 ± 0.03, respectively. The diameters (cm) of cervix and vagina were 3.23 ± 0.04 and 3.49 ± 0.03, respectively. Younger (aged ≤ 4 years old) and thin (body condition score-BCS 1 and 2) animals presented smaller dimensions of genital tract compared to older with a BCS ≥ 3. The right ovaries were heavier in weight as compared to left ones. Pregnant animals presented a higher follicular population and ovaries with a corpus luteum were larger in dimensions. The results indicated that local female zebus in Cameroon had a particular genital biometry.

Keys words: biometry, genital tract, local zebus, Ngaoundere, Cameroon.

BIOMÉTRIE DES ORGANES REPRODUCTEURS FEMELLES (BOS INDICUS) AU CAMEROUN

Résumé

L'étude visait à déterminer la biométrie de 501 tractus génitaux de femelles zébus locaux (Bos indicus) abattues à l'abattoir municipal de Ngaoundéré (Cameroun). Les longueurs moyennes (cm), la largeur (cm), l'épaisseur (cm) et le poids (g) étaient de 2,83 ± 0,03 ; 1,96 ± 0,02 ; 1,28 ± 0,01 et 5,17 ± 0,11 pour l'ovaire droit ; et 2,64 ± 0,03 ; 1,78 ± 0,02 ; 1,15 ± 0,01 et 4,02 ± 0,09 pour l'ovaire gauche, respectivement. Utilisant un caliper électronique type Vernier, 16957 follicules ont été comptés à la surface ovarienne avec une moyenne de 16,92±0,32 follicules par ovaire. Les petits (< 3 millimètres), moyens (3 à 8 millimètres) et grands (>8 millimètres) follicules étaient de 8,53±0,20 ; 8,06 ± 0,21 et 0,29± 0,02 ; respectivement. 20,4% de femelles examinées étaient gestantes. Concernant les animaux non gestants, les longueurs moyennes (en cm) de l'oviducte, des cornes utérines, du corps utérin, du cervix, du vagin et de la commissure vulvaire étaient de 19,27± 0,14 ; 20,32± 0,20 ; 1,24 ± 0,02 ; 6,92± 0,08; 19,45 ± 0,08 et 4,91± 0,03, respectivement. Les diamètres (en centimètre) du cervix et du vagin étaient de 3,23 ± 0,04 et 3,49± 0,03, respectivement. Les animaux plus jeunes (âgé de moins de 4 ans) et maigres (notes d'état corporel 1 et 2) ont présenté de plus petites dimensions du tractus génital comparés aux plus âgés présentant une note d'état corporel supérieure ou égale à 3. Les ovaires droits étaient plus lourds que ceux de gauche. Les animaux gestants ont présenté une plus grande population folliculaire et les ovaires portant un corps jaune avaient des dimensions plus grandes. Ces résultats ont indiqué que les femelles zébus locaux au Cameroun ont une biométrie génitale particulière.

Mots-clés: biométrie, tractus génital, zébus locaux, Ngaoundéré, Cameroun.

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Introduction

The world cattle population was estimated to be about 1.3 billion heads, with about 15 percent in Africa (Jaji *et al.,* 2012). With 7 million cattle, they represent 10% of the total livestock in Cameroon (MINEPIA, 2009). However their productivity remains very low. Increasing demands for food, restriction of natural resources and global warming are contemporaneous challenges for animal production (Garcia, 2013). The characterization of genotypes of livestock is the first approach to a sustainable use of its animal genetic resource (Tolenkhomba *et al.,* 2012). An important step in the knowledge and valorization of animal genetic resources is the phenotypic characterization (description of morphobiometric and zootechnical trait). According to Food and Agriculture Organization (2012), this characterization is essential for identifying these animal genetic resources and the management planning at the local, national, regional and international level. It is also important to characterize cattle phenotypically because information on the phenotypic traits is easily accessible by the communities (Zulu 2008; Soro *et al.,* 2015). The reproductive performance depends upon the normal structure and functions of genital organs of an animal (Siddiqui *et al.,* 2005). The knowledge of biometrical status of female genital tract is essential to perform artificial insemination, pregnancy diagnosis and dealing with the infertility problems (Memon, 1996). There are many reports about the biometrical values of the reproductive tracts of cows in different countries of the world but not a solitary report is available in Cameroon. The present study was undertaken to determine the biometry of the reproductive tracts of different female local zebus in Cameroon.

Materials and methods

Study area

The study was conducted using samples collected at the Ngaoundere Municipal Slaughterhouse (NMSH) and analyzed at the veterinary laboratory of IRAD-Wakwa Regional Center (physiology and reproduction biotechnology department) in Adamawa region of Cameroon. The cattle slaughtered at the NMSH were from the Vina Division (76.8%) and Mayo Rey Division (23.2%). Ngaoundere is situated between Latitude 7°19'39N and Longitude 13°35'4E and have an average annual rainfall of 1496.7 mm. The temperatures varied from 15.2°C to 29°C with an average humidity of 58.2%.

Characteristics of animals

A total of 501 local zebus of different breeds based on phenotypic criteria as described by Lhoste (1969)] were randomly selected for this study. The body condition score (BCS) and age (years) have been determined as described by Natumanya *et al.,* (2008) and Moussa Garba *et al.,* (2013), respectively. The pregnancy status has been determined by the presence of a fetus.

Biometry of genital tract

Harvest of reproductive organs

After slaughter, the entire reproductive organs included ovaries, oviducts, uterus, vagina and vulva of each cow was separated from the pelvic viscera and ligamentous attachments, and then collected and deposited on the inspection table in the slaughterhouse. Concerning pregnant cows only ovaries were removed.

Vulvo-vagina, uterine and oviduct dimensions

All measurements of the vulvar commissure, vagina, cervix, uterine body, uterine horns and oviducts length and diameters of each non-pregnant cows (n= 399) were carried out following the techniques described by Mc Entee (1983) using a graduated tape in centimeter (Moussa Garba *et al.,* 2013).

The length of any oviduct was taken from the top of the fimbria to the tubal-uterine horn junction. The length of the uterine horn was the distance from the middle of the point of bifurcation of the uterus utero-oviducal junction. The length of the uterine body was taken from its bifurcation to the internal os of the cervix. The length of the cervix was the

distance from the tip of internal os to the tip of external os of the cervix and the diameter was recorded. The length of the vagina was taken as the distance from the external os of the cervix to the ventral commissure of the vulva and the diameter was recorded. The length of vulvar commissure was measured from ventral to dorsal commissure of the vulva.

Ovary dimensions

After slaughter, the ovaries right and left (n=1002) were removed at their junction with the ovarian ligament as close to the ovarian tissue as possible after fimbria was removed. Ovary was individually placed in conical tubes containing an isotonic medium (NaCl 0.9%) supplemented with penicillin-streptomycin (0.5 mg / ml). These tubes were placed in a flask at a temperature of 30-32 °C and transported to the laboratory within 2 hours following slaughter (Kouamo *et al.,* 2014).

In the laboratory, excessive tissues attached to the ovaries were carefully trimmed off and ovaries were weighed using an electronic scale, Mettler PC 2000. The length, width and thickness in centimeter of the ovaries were measured using electronic Vernier calipers as per Bhattacharya and Luktuke (1960). The length of the ovary was taken as the distance from the anterior pole to the posterior pole along an axis parallel to the ovarian mesenterial attachment (base). Width of the ovary was taken as the greater distance from the medial to the lateral surfaces or borders. Thickness of the ovary was recorded as the greatest distance along an axis vertical to the longitudinal axis (base) at its center or distance from attached to the free borders expressed. The ovaries were thereafter allocated into two size groups (<2.25x1.75x1.25 and >2.25x1.75x1.25) as described by Samad and Raza (1999). The diameter and color of each corpus luteum (CL) permits to classify them into three types as described by Nguyen and Hanzen (2013): hemorrhagic CL or CL1 (diameter less than 2 cm, reddish color), diestrus CL or CL2 (diameter between 2 and 3 cm, yellowish or orange) and decreased CL (corpus albicans) or CL3 (diameter less than 1 cm and whitish color). Follicular population was determined using electronic Vernier calipers and follicles were classified into 3 categories: small (diameter < 3mm), medium (diameter between 3 to 8 mm) and large (diameter > 8 mm) as described by Duygu *et al.,* (2013).

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) Version 20. The analysis of variance and Duncan's test statistics were used to analyze appropriate data sets. The level of significance was 5%.

Results

Characterization of slaughtered animals

Different breed of zebus have been determined during the study (table 1). BCS and average age (minimum-maximum) of the animals were 3.14 ± 0.04 (1-5) and 6.62 ± 0.11 years old (2-15), respectively. Of the 501 female zebus examined, 102 (20.4%) were pregnant. Out of 1002 ovaries examined, 535 (53.4%) carried a CL (table 1).

The average weight of animal was 363.86 ± 3.05 kg. Table 2 presented the variations of the weight.

Biometry of genital tract

Table 3 presents the reproductive organs biometrics of female local zebu. The size (length, width and thickness) of the right ovary was significantly higher than those of the left ovary. No significant difference was founded between the length of the right and left uterine horns and oviducts.

Effects of breed, BCS, age, physiological status, ovarian symmetry, weight and size of the ovary on the biometrics of the genital tract

Weight, width and thickness of the ovary were significantly lower in thin animals (table 4). In addition, thin animals had fewer small follicles ($P < 0.05$) than cows with BCS ≥3 (tables 4 and 5). Ovarian dimensions and the length or diameter of the oviducts, uterine horns, body, cervix, vagina, vulva commissure

were significantly lower in animals less four years compared to other age groups (tables 4 and 5). In addition, cows aged over 8 years had fewer follicles means compared to those aged between 4-8 years old and the average number of large follicles increased with age (tables 4 and 5).

Ovarian dimensions were significantly higher ($p < 0.05$) in the ovaries with a CL and in pregnant cows. In addition, the average number of total follicles, small, medium and large follicles was higher in pregnant cows. The average number of large follicles was significantly ($P < 0.05$) higher on the right ovary. Ovaries less than 3 g had few follicular population (small, medium).

Table 1: Characterization of slaughtered animals

Factors		Number examined	%
Breed	Gudali	359	71.7
	Akou	91	18.2
	Djafoun	34	3.4
	Bokolo	17	6.8
BCS	Thin	100	20.0
	Normal	237	47.3
	Fat	164	32.7
Age (years)	< 4	56	11.2
	4 to 8	357	71.3
	> 8	88	17.6
Physiological status	Pregnant	102	20.4
	Non pregnant		
		399	79.6
Corpus luteum (CL)	CL1	26	2.6
	CL2	229	22.9
	CL3	280	27.9
	Absent	467	46.6

CL1: hemorrhagic CL; CL2: diestrus CL; CL3: corpus albicans.

Table 2: Variation of weight following breed, BCS, age and physiological status

Factors		Number examined	Means weight
Breed	Gudali	359	
	Akou	91	349.04 ± 6.25^{b}
	Djafoun	34	346.36 ± 11.66^{ab}
	Bokolo	17	380.85 ± 15.49^{ab}
	P value		0.030
BCS	Thin	100	325.89 ± 6.16^{a}
	Normal	237	356.61 ± 4.14^{b}
	Fat	164	397.49 ± 4.96^{c}
	P value		0.000

Factors		Number examined	Means weight
Age (years)	< 4	56	297.72 ± 10.66[a]
	4 to 8	357	367.43 ± 3.29[b]
	> 8	88	391.48 ± 6.19[c]
	P value		0.000
Physiological status	Pregnant	102	400.95 ± 5.74[a]
	Non pregnant	399	354.26 ± 3.39[b]
	P value		0.000

In each column different letters (a, b, c) indicated significant difference between group (p<0.05).

Table 3: Genital biometry of local zebu

Factors		Number examined	Means ± SEM	Minimum – Maximum
Vulva commissure length (cm)		399	4.91 ± 0.03	3.00 – 7.00
Vagina length (cm)		399	19.45 ± 0.08	16.00 – 25.50
Vagina diameter (cm)		399	3.49 ± 0.03	2.00 – 6.00
Cervix length (cm)		399	6.92 ± 0.08	3.50 – 12.00
Cervix diameter (cm)		399	3.23 ± 0.04	1.50 – 6.00
Uterine body length (cm)		399	1.24 ± 0.02	0.50 – 3.00
Uterine horn length (cm)	Average	399	20.32 ± 0.20	12.00 – 38.00
	Right	399	20.40 ± 0.20[a]	12.00 – 38.00
	Left	399	20.24 ± 0.19[a]	12.00 – 38.00
	P value		0.712	
Oviduct length (cm)	Average	399	19.27 ± 0.14	12.00 – 29.20
	Right	399	19.36 ± 0.15[a]	12.00 – 29.20
	Left	399	19.21 ± 0.14[a]	12.00 – 29.20
	P value		0.414	
Ovary width (cm)	Average	501	4.58 ± 0.08	1.00 – 12.81
	Right	499	5.17 ± 0.11[a]	0.99 – 2.93
	Left	501	4.02 ± 0.09[b]	0.94 – 17.44
	P value		0.000	
Ovary length (cm)	Average	501	2.72 ± 0.02	1.20 – 4.85
	Right	499	2.83 ± 0.03[a]	1.36 – 5.08
	Left	501	2.64 ± 0.03[b]	1.00 – 5.50
	P value		0.000	
Ovary width (cm)	Average	501	1.86 ± 0.02	0.85 – 2.95
	Right	499	1.96 ± 0.02[a]	1.00 – 3.51
	Left	501	1.78 ± 0.02[b]	0.85 – 3.70
	P value		0.000	

Factors		Number examined	Means ± SEM	Minimum – Maximum
Ovary thickness (cm)	Average	500	1.21 ± 0.01	0.50 – 2.10
	Right	499	1.28± 0.01[a]	0.50 – 2.90
	Left	501	1.15 ± 0.01[b]	0.50 – 2.60
	P value		0.000	
Number of follicles	Average follicular population	1002	16.92 ± 0.34	0.00 – 70.00
	Small (< 3 mm)	1000	8.53 ± 0.19	0.00 – 40.00
	Medium (4 to 8 mm)	1000	8.06 ± 0.49	0.00 – 50.00
	Large (> 8 mm)	1000	0.29 ± 0.14	0.00 – 3.00

In each column different letters (a, b) indicated significant difference between group (p<0.05). SEM=standard error of means.

Table 4: Effects of breed, BCS, age and physiological status on oviduct, uterus and vulvo-vagina dimensions

		N	Ovary weight (g)	Ovary length (cm)	Ovary width (cm)	Ovary thickness (cm)	Oviduct length (cm)
Breed	Gudali	281	4.60 ± 0.10[a]	2.73 ± 0.03[a]	1.86 ± 0.02[a]	1.22 ± 0.01[a]	19.27± 0.17[a]
	Akou	78	4.71 ± 0.20[a]	2.74 ± 0.06[a]	1.90 ± 0.03[a]	1.20 ± 0.02[a]	19.31±0.31[a]
	Djafoun	30	4.27 ± 0.21[a]	2.65 ± 0.08[a]	1.81 ± 0.06[a]	1.19 ± 0.04[a]	18.82±0.53[a]
	Bokolo	10	4.35 ± 0.36[a]	2.80 ± 0.09[a]	1.86 ± 0.08[a]	1.18 ± 0.04[a]	20.20±0.77[a]
	P value		0.665	0.808	0.616	0.740	0.606
BCS	1-2	87	4.14 ± 0.20[a]	2.65 ± 0.05[a]	1.79 ± 0.03[a]	1.14 ± 0.02[a]	19.21±0.33[a]
	3	205	4.35 ± 0.12[a]	2.69 ± 0.03[a]	1.83 ± 0.02[a]	1.19 ± 0.01[b]	19.07±0.18[a]
	4-5	107	5.19 ± 0.16[b]	2.84 ± 0.05[b]	1.96 ± 0.03[b]	1.28 ± 0.02[c]	19.70±0.27[a]
	P value		0,000	0.005	0.000	0.000	0.170
Age (years)	< 4	54	3.26 ± 0.21[a]	2.30 ± 0.05[a]	1.61 ± 0.03[a]	1.09 ± 0.03[a]	16.78±0.34[a]
	4-8	291	4.67 ± 0.10[b]	2.74 ± 0.03[b]	1.89 ± 0.02[b]	1.23 ± 0.01[b]	19.28±0.14[b]
	> 8	54	5.08 ± 0.23[b]	2.97 ± 0.07[c]	1.93 ± 0.04[b]	1.22 ± 0.01[b]	21.67±0.37[c]
	P value		0.000	0.000	0.000	0.000	0.000
Physiological status	Pregnant	102	6.13 ± 0.16[a]	3.17 ± 0.05[a]	2.14 ± 0.03[a]	1.37 ± 0.02[a]	----------
	Non pregnant	399	4.19 ± 0.09[b]	2.61 ± 0.02[b]	1.79 ± 0.02[b]	1.18 ± 0.02[b]	----------
	P value		0.000	0.000	0.000	0.000	

In each column different letters (a, b, c) indicated significant difference between group (p<0.05). N = number.

Discussion

The average length (cm) of oviducts, uterine horns, uterine body, cervix, vagina and vulva commissure were similar to those reported in tropical zebu. Thus, Moussa Garba *et al.,* (2013) obtained an average length (cm) of 19.1, 21.6, 8.2, 18.9 and 5.9 for oviducts, uterine horns, cervix, vagina and vulva commissure, respectively. Riasat *et al.,* (2006) reported an average length (cm) of 20.23 and 6.0 ± 0.22 for the uterine horns and the cervix, respectively. However, Khaton *et al.,* (2015 a,b) in dairy cows reported an average length (cm) of 21.03, 25.56, 3.12 and 22.59 of the oviducts, uterine horns, uterine body and vagina, respectively. Carvalho *et al.,* (2005) also reported in the European breeds an average length (cm) of 20.5-23.1, 35.3-39 and 4.1 of the oviducts, uterine horns and body, respectively. The average diameter of the cervix was similar to that obtained by Moussa Garba *et al.,* (2013). However Khaton *et al.,* (2015 a,b) reported a higher vagina diameter compared to this study. The difference could be due to genotype and age. Genotype and environmental factors play a crucial role in productive and reproductive performance of cows. The adaptation of the reproductive function in the animal's environment may justify the difference between local and European breeds. Indeed, the physical development depend of the age of animal. Young animal had lower dimensions of reproductive organs than adult.

Uterine horn length (cm)	Uterine body length (cm)	Cervix length (cm)	Cervix diameter (cm)	Vagina length (cm)	Vagina diameter (cm)	Vulva commissure length (cm)
20.07± 0.09^{a}	1.22 ±0.22^{a}	6.81 ± 0.09^{a}	3.20 ± 0.05^{a}	19.34± 0.09^{a}	3.45 ±0.04^{a}	4.87 ± 0.04^{a}
20.81 ±0.47^{a}	1.28 ±0.06^{a}	7.27 ± 0.16^{a}	3.33 ± 0.08^{a}	19.71 ± 0.18^{a}	3.54 ±0.07^{a}	5.04 ± 0.07^{a}
20.98 ±0.97^{a}	1.25 ±0.10^{a}	6.81 ± 0.33^{a}	3.12 ± 0.13^{a}	19.59± 0.30^{a}	3.63 ±0.12^{a}	4.91 ± 0.13^{a}
21.49 ±1.08^{a}	1.30 ±0.17a	7.42 ± 0.72^{a}	3.47 ± 0.20^{a}	20.18 ± 0.72^{a}	3.72 ±0.21^{a}	5.23 ± 0.25^{a}
0.259	0.797	0.077	0.311	0.102	0.247	0.115
19.71 ±0.36^{a}	1.28 ±0.05^{a}	6.85 ± 0.16^{a}	3.16 ± 0.08^{a}	19.55 ± 0.18^{a}	3.48 ±0.08^{a}	4.88 ± 0.09^{a}
20.15 ±0.26^{a}	1.23 ±0.03^{a}	6.91 ± 0.11^{a}	3.20 ± 0.05^{a}	19.41 ± 0.10^{a}	3.45 ±0.04^{a}	4.91 ± 0.05^{a}
21.13 ±0.43^{a}	1.22 ±0.04^{a}	6.97 ± 0.15^{a}	3.34 ± 0.08^{a}	19.45 ± 0.15^{a}	3.57 ±0.07^{a}	4.94 ± 0.06^{a}
0.784	0.676	0.706	0.200	0.784	0.284	0.814
16.33 ±0.26^{a}	0.89 ±0.04^{a}	5.10 ± 0.09^{a}	2.79 ± 0.10^{a}	17.96 ± 0.07^{a}	3.07 ±0.07^{a}	3.81 ± 0.06^{a}
20.58 ±0.22^{b}	1.25 ±0.03^{b}	6.88 ± 0.07^{b}	3.26 ± 0.04^{b}	19.21 ± 0.06^{b}	3.49 ±0.04^{b}	4.95 ± 0.03^{b}
22.90 ±0.44^{c}	1.53 ±0.07^{c}	8.90 ± 0.19^{c}	3.48 ± 0.09^{c}	22.22 ± 0.17^{c}	3.90 ±0.08^{c}	5.84 ± 0.06^{c}
0.000	0.000	0.000	0.000	0.000	0.000	0.000
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Table 5: Effects of ovarian symmetry, weight and size of the ovary on the follicular population

Factors		Number examined	Average number of follicles/Ovary			Total follicles/ Ovary
			Small (< 3mm)	Medium (3-8 mm)	Large (> 8 mm)	
Breed	Gudali	359	9.87 ± 0.38[a]	7.43 ± 0.24[a]	0.36 ± 0.02[a]	16.55 ± 0.52[a]
	Akou	91	9.08 ± 0.82[a]	7.66 ± 0.56[a]	0.34 ± 0.04[a]	16.29 ± 1.18[a]
	Djafoun	34	9.29 ± 0.93[a]	7.10 ± 0.75[a]	0.28 ± 0.06[a]	16.11 ± 1.45[a]
	Bokolo	17	9.91 ± 1.54[a]	5.53 ± 0.94a	0.41 ± 0.11[a]	13,82 ± 1,82[a]
	P value		0.804	0.385	0.622	0.741
BCS	1-2	100	8.04 ± 0.63[a]	7.04 ± 0.52[a]	0.30 ± 0.03[a]	14.41 ± 0.99[a]
	3	164	10.07 ± 0.47[b]	7.26 ± 0.30[a]	0.35 ± 0.03[a]	16.85 ± 0.65[a]
	4-5	237	10.13 ± 0.56[b]	7.79 ± 0.37[a]	0.38 ± 0.03[a]	16.92 ± 0.76[a]
	P value		0.036	0.387	0.366	0.086
Age (years)	< 4	56	9.54 ± 0.96[a]	7.64 ± 0.65[ac]	0.46 ± 0.06[a]	17.04 ± 1.36[a]
	4 à 8	357	9.77 ± 0.38[a]	7.66 ± 0.26[a]	0.35 ± 0.02[b]	16.67 ± 0.53[a]
	> 8	88	9.46 ± 0.32[a]	6.12 ± 0.46[bc]	0.28 ± 0.40[b]	14.82 ± 0.76[a]
	P value		0.926	0.022	0.025	0.265
Physiological status	Pregnant	102	11.17±0.65[a]	7.89±0.44[a]	0.21±0.03[a]	17.38±0.90[a]
	Non pregnant	399	9.31±0.37[b]	7.26±0.24[b]	0.38±0.02[b]	16.13±0.51[b]
	P value		0.019	0.233	0.000	0.260
CL	Absent	465	8.37±0.28[a]	8.33±0.32[a]	0.32±0.03[a]	16.99±0.48[a]
	Present	535	8.68±0.27[a]	7.83±0.26[a]	0.27±0.03[a]	16.86±0.48[a]
	P value		0.425	0.224	0.070	0.838
Ovary symmetry	Right	499	8.78±0.29[a]	8.30±0.28[a]	0.35±0.02[a]	17.46±0.44[a]
	Left	501	8.29±0.25[a]	7.82±0.30[a]	0.23±0.02[b]	16.39±0.45[a]
	P value		0.206	0.245	0.000	0.095
Ovary weight (g)	<3	280	5.89±0.27[a]	4.91±0.25[a]	0.25±0.03[a]	11.05±0.39[a]
	3-5	376	8.59±0.29[b]	7.81±0.27[b]	0.33±0.03[a]	16.73±0.44[b]
	>5	344	10.62±0.38[c]	10.90±0.42[c]	0.28±0.03[a]	21.89±0.61[c]
	P value		0.000	0.000	0.120	0.000
Ovary size (cm)	<2,25x1,75x1,25	170	5.69±0.35[a]	5.10±0.26[a]	0.28±0.03[a]	11.06±0.52[a]
	>2,25x1,75x1,25	322	10.66±0.38[b]	11.28±0.43[b]	0.34±0.03[a]	22.20±0.63[b]
	P-value		0.000	0.000	0.194	0.000

In each column different letters (a, b, c) indicated significant difference between group (p<0.05).

The weight and the size (length, width and thickness) of ovaries obtained were similar to those obtained by Natumanya *et al.,* (2008) in the Ankole zebu; lower than those reported in European breeds with an average ovarian weight ranging from 10-19 and 5-15 g (Pierson and Ginther, 1987) and measuring 3-43 mm, 19-23 mm and 13-19 mm for length, width and thickness, respectively (Dominguez, 1995) and greater than those reported by Moussa Garba *et al.,* (2013): 2.9 ± 1.8 g in Niger zebu. These variations could be due to the effect of nutritional status and breed. Indeed, BCS significantly influenced dimensions of ovary in this study. This is explained by the fact that under nutrition not only negatively impacts the general health of the animals but also their ovaries. In addition, the European breeds are heavier than local breeds, therefore the weight and size of their ovaries would be higher. The

right ovary was heavier than the left. In cattle, the majority of ovulations are observed on the right ovary (Drion *et al.,* 2000). This confirms the fact that the right ovary is more active than the left ovary. The age, presence of the corpus luteum and physiological status significantly influenced the dimensions of the ovaries. These results are comparable to those reported by Pierson and Ginther (1987), Kouamo *et al.,* (2014) and Khaton *et al.,* (2015 a, b). This proves that the development of the animal and its reproductive organs are correlated to age. In addition, the corpus luteum increased in volume due to the intense production of progesterone in pregnant animals.

The average number of follicles counted on the surface of the ovaries and the small, medium and large follicles were comparable to those obtained by Carvalho *et al.,* (2008) in Nellore zebu and Natumanya *et al.,* (2008) in the Ankole zebu. In contrast, the number of large follicles appeared to be different from that obtained in the European breed (Dominguez, 1995). The difference may be due to adaptation of ovarian function in cow's environment, to plane of nutrition, to species differences and possibly to differences in methodology of counting. Thin animal (BCS 1 and 2) presented few number of follicles as report by Moussa Garba *et al.,* (2013). This confirms the major impact of energy intake on the dynamics of follicular growth (Maina, 2008). Indeed, underfeeding reduced pituitary gonadotropin secretion responsible for follicular development. This study showed that cows aged over 8 years old had fewer medium and large follicles than those under 8 years old. Indeed, fertility in all species decreases with age following the failure of the hypothalamic-pituitary axis, the deficiencies of ovarian hormones and apoptosis, therefore the number of follicles also decreases (Armstrong, 2001). Other factors such as weight and ovarian size also significantly affect the average number of total follicles (Kouamo *et al.,* 2014).

Conclusion

This study indicated that local zebus have a particular biometry of reproductive organs and lower than the European breed. Thus, in order to improve success rate of assisted reproductive technologies, it is necessary to prepare and adapt materials for artificial insemination or embryo transfer such as vaginal speculum, insemination gun to local breeds.

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RESPONSES OF BROILER CHICKENS UNDER HOT HUMID TROPICAL CLIMATE AS AFFECTED BY AZADIRACHTA INDICA LEAF

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Abstract

Environmental temperature is the most critical variable influencing the performance of broiler chickens in the hot humid tropical climate. Neem (Azadirachta indica) has been reported to possess several benefits including antioxidants which may be able to alleviate the effects of heat stress on broiler chickens during hot dry season. A study was carried out to evaluate the effect of Azadirachta indica leaf powder (NLP) on the growth, haematology and serum biochemistry parameters of broiler chicks. Two hundred and forty day-old broiler chicks were randomly assigned to four treatments which contained 0, 5, 10 and 15g/Kg diets in a Completely Randomized Design experiment. Each treatment was replicated four times with fifteen birds in each replicate. Feed and water were provided ad libitum. The results showed that the levels of creatine kinase of the birds fed 0 and 5g NLP were similar but higher than those of the birds 10 and 15g NLP. The final body weights of the broiler chickens fed 10 and 15% NLP were similar but significantly higher than those of the birds fed 0 and 10g NLP. The weights gains and feed conversion ratios of the birds followed a similar trend to that of the final body weights. The feed conversion ratios of the birds fed 10 and 15g NLP were similar but significantly lower ($P < 0.05$) than those fed 0 and 5g NLP. It was concluded that broiler chickens exposed to heat stress in the hot–humid climate responded favorably to inclusion of neem leaf in their diets up to 10g/Kg diet.
Keywords: Chicken, physiology, performance, antioxidant, hot-dry season.

RÉACTIONS DES POULETS DE CHAIR EN CLIMAT TROPICAL CHAUD ET HUMIDE AFFECTÉS PAR LA FEUILLE D'AZADIRACHTA INDICA

Résumé

La température environnante est la variable la plus critique qui influence la performance des poulets de chair en climat tropical chaud et humide. Il a été signalé que le margousier (Azadirachta indica) possède plusieurs qualités, y compris des antioxydants qui peuvent atténuer les effets du stress thermique sur les poulets de chair pendant la saison sèche chaude. Une étude a été réalisée pour évaluer l'effet de la poudre foliaire d'Azadirachta indica (PNL) sur la croissance, l'hématologie et les paramètres de biochimie sérique des poussins de chair. Deux cents quarante poussins d'un jour ont été assignés de manière aléatoire à quatre traitements contenant des régimes de 0, 5, 10 et 15 g / Kg dans une expérience consistant en un dispositif complètement aléatoire. Chaque traitement a été répété quatre fois, avec quinze oiseaux dans chaque répétition. L'alimentation et l'eau ont été fournies ad libitum. Les résultats ont montré que les niveaux de créatine kinase des oiseaux recevant 0 et 5g de PNL étaient similaires mais plus élevés que ceux des oiseaux recevant 10 et 15 g de PNL. Les poids corporels finaux des poulets de chair recevant 10 et 15% de PNL étaient similaires, mais nettement plus élevés que ceux des oiseaux recevant 0 et 10 g de PNL.. Les gains de poids et les ratios de conversion alimentaire des oiseaux ont suivi une tendance similaire à celle des poids corporels finaux. Les ratios de conversion des oiseaux recevant 10 et 15 g de PNL étaient semblables mais nettement inférieurs ($P < 0,05$) à ceux nourris avec 0 et 5 g de PNL. Il a été conclu que les poulets de chair exposés au stress thermique en climat chaud-humide ont répondu favorablement à l'inclusion de la feuille de margousier dans leur alimentation jusqu'à 10g / Kg.
Mots-clés : poulet, physiologie, performance, antioxydant, saison chaude-sèche

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Introduction

Fast growing commercial broilers are highly sensitive to high temperature during growing-finishing phase in the tropical environment. Climatic conditions (Thaxton and Siegel, 1970; McFarlane and Curtis, 1989; Mashaly *et al.,* 2004) and nutrition (Maxwell *et al.,* 1992; Hangalapura *et al.,* 2005) impose a state of stress response associated with a number of modifications to metabolic (Davison *et al.,* 1983; Lin *et al.,* 2006; Shini *et al.,* 2008), physiological (Puvadolpirod and Thaxton, 2000; Post *et al.,* 2003; Mumma *et al.,* 2006; Virden *et al.,* 2007; Shini *et al.,* 2008) and immunological functions (Siegel, 1961; Glick, 1967; Thaxton and Siegel, 1970; Mashaly *et al.,* 2004; Hangalapura *et al.,* 2005). Environmental temperature is the most important variable affecting feed intake and thus weight gain of broilers. High ambient temperature reduces feed intake, live weight gain, and feed efficiency (Donkoh, 1989), thereby adversely affecting the performance of broilers. It has been suggested that decrease in growth rate could be attributed partly to decrease in feed intake (Hurwitz *et al.,*1980). Thyroid activity has been reported to decrease due to high ambient temperature (Evans and Ingram, 1977; Bowen and Washburn, 1985). McNabb and King (1993) indicated that plasma triiodothyronine and thyroxine are associated with ambient tem¬perature. Studies have shown that the circulating concentrations of thyroid hormones are reduced at high tem¬peratures (Heninger *et al.,* 1960; Bowen *et al.,* 1984). Additionally, it has also been reported that corticosterone concentra¬tion increases during heat stress (Edens and Siegel, 1975). Heat stress also results in reduced plasma protein and increased blood glucose concentrations (Donkoh, 1989).

Various methods are available to alleviate the effect of high environmental temperature on broiler chickens. However, since it is expensive to cool animal build¬ings especially in the developing countries, such methods are focused mostly on the dietary manipulation. Additives from plants have been utililized as a result of their bioactive compounds content such as flavonoids, tanins, saponin, etc (Sharma et al. (2012). Neem (Azadirachta indica) has been reported to possess several benefits including antioxidants which may be able to alleviate the effects of heat stress on broiler chickens during hot dry season (Donkoh, 1989; Sahin *et al.,* 2001; Flachowsky, 2000, 2002). Neem leaves and its constituents have been reported to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties (Elangovan *et al.,* 2000; Subapriya and Nagini, 2005). The leaves extract contain nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Mitra *et al.,* 2000). As a result of the major roles played by poultry in bridging the protein gap in developing countries (Onyimonyi *et al.,* 2009), the present study was designed to investigate the ameliorative effect of neem leaf on broiler chickens during hot dry season in Nigeria.

Materials and Method

Experimental Site

This study was conducted at the Teaching and Research Farms Directorate of the Federal University of Agriculture, Abeokuta, Nigeria. The study area falls within South-Western Nigeria at Latitude 7°13'49.46"N, Longitude 3°26'11.98"E and on altitude of 76m above sea level. The climate is humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity is 34°C and 82% respectively. The average daily ambient temperature and relative humidity during experimental periods were 33.25oC and 82.75% respectively.

Preparation of neem leaf meal powder

Neem leaves (Azadirachta indica) were harvested from young neem trees around the Federal University of Agriculture, Abeokuta, Nigeria. The leaves were removed from the stalk, sorted and cleaned from any extraneous materials and then air-dried under a shed until they were crispy to touch, while retaining their greenish coloration. The leaves were milled

using a laboratory mill. The powdered leaf was sieved to remove unwanted matter by using 710 µm sieves size in a repeated manner to get fine powder, a product herein referred to as neem leaf powder (NLP). NLP was thoroughly mixed with other ingredients.

Experimental Birds, Materials and Management

Two hundred and forty Marshal broilers day-old-chicks (mixed sex) were purchased from a reputable hatchery in Nigeria. Four experimental diets were formulated with the inclusion of neem leaf powder (NLP) at 0, 0.5, 1, and 1.5% for treatments 1, 2, 3 and 4 respectively. The birds were randomly assigned to the dietary treatment at day 21. Each treatment had 60 birds comprising four replicates of 15 birds each. Feed and water were provided for the birds ad libitum throughout the period of the experiment. The chicks were fed broiler starter for 28 days and broiler finisher feed between 29 and 56 days. The birds were raised on a deep litter system. The experiment lasted for 56 days

Data collection

Feed Intake

The amount of feed consumed each day was weighed before it was given to the birds. The amount left in the feeders was subtracted from the feed offered in the previous day as feed intake for the day. Feed intake was recorded on replicate basis.

Body Weights

The initial and final weights of the birds were taken by weighing the birds before the birds were assigned to different dietary treatment supply of feed and water at day 21 and 56 respectively.

Collection of blood samples

8ml blood samples were collected from 2 chickens per replicate each via brachial veins over a period of 30 sec to 1 min using hypodermic syringes. Blood collection was done at the 6th week of the experiment. 4 ml of blood was drawn into a heparinized tubes to prevent coagulation while the remaining 4 ml was left in the syringe to coagulate. Blood samples were then analysed for packed cell volume (PCV), Hb, white blood cells (WBC), red blood cells (RBC), serum protein, serum glucose, serum albumin and globulin, serum creatinine, serum urea, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), serum creatine kinase, Aspartate Transaminoferase (AST), Alanine Transaminoferase (ALT).

Relative Weight of Organs

Weight of spleen, liver, kidney, lungs, bursa of Fabricius, thymus and heart were taken and relative weight was determined as a percentage of live weight of the birds.

Data Analysis

All the data collected were subjected to analysis of variance using SAS (1999) statistical package while significant means were compared using Duncan's Multiple Range Test. Each treatment was replicated four times with fifteen birds in each replicate.

Results and discussion

Serum Chemistry

The effect of different levels of NLP on serum chemistry of broiler chickens at finisher phase is shown in Table 2. The serum protein level was higher ($P < 0.05$) in the broiler fed 15g NLP than those in control group. Serum albumin was higher ($P < 0.05$) in the birds offered 15g NLP than those fed 5 and 10g NLP.. The globulin level was higher ($P < 0.05$) than that of the control group. Serum triglyceride was similar ($P > 0.05$) in the birds fed 15, 10 and 0g NLP but that of the birds fed 10g NLP was higher ($P < 0.05$) than that of 5g NLP. The level of AST was higher ($P < 0.05$) than those of the birds in 0 and 5g NLP treatment groups. Serum ALT level was similar in the birds fed 15 and 10g NLP but higher ($P < 0.05$) than those of the birds fed 5g NLP. The levels of creatine kinase of the birds fed 0 and 5g NLP higher than those of the birds 10 and 15g NLP whose values were similar.

Table 1: Diet composition for the starter and finisher phase of broiler chicken

Ingredients	Starter phase (%)	Finisher phase (%)
Maize	47.00	50.00
Soya bean meal	18.50	12.00
Groundnut meal cake	15.00	11.00
Fish meal(72% CP)	2.10	2.10
Wheat offal	10.35	17.90
Bone meal	3.00	3.00
Oyster shell	3.00	3.00
Salt	0.25	0.25
*Premix	0.25	0.25
Methionine	0.30	0.30
Lysine	0.25	0.20
	100	100
Calculated		
Crude protein (%)	23.05	19.91
ME (MJ/Kg)	11.73	11.71
Ether extract (%)	3.93	3.89
Crude fibre (%)	3.67	3.79
Calcium (%)	1.75	1.74
Phosphorus (%)	0.43	0.41

** kg of premix contains: vitamin A: 10,000,000 IU; vitamin D3: 2,000,000 IU; vitamin E: 20,000 IU; vitamin K: 2,250 mg; thiamine B1: 1,750 mg; riboflavin B2: 5,000 mg; pyridoxine B6: 2,750 mg; niacin: 27,500 mg; vitamin B12: 15 mg; pantothenic acid: 7,500 mg; folic acid: 7,500 mg; biotin: 50 mg; choline chloride: 400 g; antioxidant: 125 g; magnesium: 80 g; zinc: 50 g; iron: 20 g; copper: 5 g; iodine: 1.2 g; selenium: 200 mg; cobalt: 200 mg.*

Table 2: Serum parameters of broiler chickens fed varying levels of NLP

Parameters	**Levels of inclusion**				
	0	**5**	**10**	**15**	**SEM**
Protein(g/l)	52.45^{b}	56.44^{ab}	57.70^{ab}	66.75^{a}	2.34
Albumin(g/l)	36.05^{ab}	32.60^{b}	30.90^{b}	43.15^{a}	2.02
Globulin(g/l)	16.40^{b}	23.85^{a}	26.80^{a}	23.60^{a}	1.59
Triglyceride(mg/dl)	145.80^{ab}	127.25^{b}	196.60^{a}	139.60^{ab}	11.52
AST (iμ/l)	112.45^{b}	126.34^{b}	169.75^{a}	189.26^{a}	11.96
ALT (iμ/l)	36.70^{bc}	26.15^{c}	54.35^{ab}	66.75^{a}	6.52
CK (iμ/l)	216.35^{a}	188.50^{a}	129.15^{b}	106.30^{b}	16.98

^{a,b,c} means within rows with different superscripts differ significantly ($P < 0.05$)
CK- creatine kinase, AST-Aspartate Transaminoferase, ALT-Alanine Transaminoferase

Haematology

Table 3 shows the effect of different levels of NLP on haematology of broiler chickens during hot dry season. Packed cell volume, white blood cells, haemoglobin, heterophil, Lymphocyte, eosinophil, basophil, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were not affected by different levels of NLP.

Table 3: Haematological parameters of broiler chicken fed varying levels of NLP

Parameters	levels of inclusion of neem				
	0	5	10	15	SEM
Pack cell volume(%)	30.50	33.50	35.00	37.00	1.35
White blood cell (Cumm3)	10.85	11.55	12.05	11.30	0.41
Red blood cell(x1012/L)	2.60	2.95	2.85	3.45	0.16
Haemoglobin (g/dl)	9.85	10.95	11.20	11.20	0.32
Heterophil	30.00	26.50	32.50	39.00	2.36
Lymphocytes (%)	70.00	72.00	65.00	60.50	2.13
Eosinophil (%)	0.00	0.00	0.50	0.00	0.13
Basophil (%)	0.00	0.00	1.00	0.50	0.26
MCV (µ3)	117.85	114.41	123.38	107.23	3.74
MCH(µµG)	38.04	37.66	39.55	32.46	1.48
MCHC(%)	32.34	32.84	32.04	30.27	0.48

[a, b, c]: means within rows with different superscripts differ significantly (P < 0.05)
MCV-Mean corpuscular volume, MCH- Mean corpuscular haemoglobin, MCHC -Mean Corpuscular haemoglobin concentration.

Growth Performance

The effect of varying levels of NLP on growth performance of broiler chicken at the starter phase is shown in Table 4. The final body weights of the broiler chickens fed 10 and 15% NLP were significantly higher than those of the birds fed 0 and 10g NLP. The weights gains and feed conversion ratios of the birds followed a similar trend to that of the final body weights. The feed conversion ratios of the birds fed 10 and 15g NLP were significantly lower (P < 0.05) than those fed 0 and 5g NLP.

Table 5 shows the effect of varying levels of NLP on lymphoid and vital organs of broiler chickens. The liver, kidney and spleen were not affected by different varying levels of NLP. The weight of the birds fed 15g NLP was significantly higher (P<0.05) than the birds fed 10g. Moreover, the birds fed 5, 10 and 15g were significantly lower (P > 0.05) than the birds in the control group. The relative weight of thymus in the birds in different treatment groups had the same trend with that of the lungs.

Table 4: Effect of varying levels of NLP on growth performance of broiler chickens

Parameters	Levels of Inclusion					
	0	5	10	15	SEM	P value
Initial body weight (g)	470.30	465.82	466.17	466.06	1.385	0.6596
Final body weight (g)	1703.13[b]	1784.99[b]	2080.87[a]	2076.04[a]	49.199	0.0002
Weight gain (g)	1232.83[b]	1319.17[b]	1614.70[a]	1609.98[a]	49.705	0.0002
Feed intake (g)	2910.13	2974.55	2962.68	2802.53	30.125	0.1601
Feed conversion ratio	2.36[a]	2.26[a]	1.84[b]	1.74[b]	0.075	0.0001

[a, b:] means within rows with different superscripts differ significantly (P < 0.05)

Table 5: Effect of varying levels of NLP on lymphoid and vital organs of broiler chickens

Parameters	0	5	10	15	SEM
Liver(%)	2.6	2.30	2.33	2.13	0.09
Kidney(%)	0.12	0.09	0.12	0.11	0.01
Spleen(%)	0.02	0.01	0.01	0.01	0.00
Lungs(%)	0.54^{a}	0.51^{ab}	0.49^{ab}	0.45^{b}	0.01
Heart(%)	0.71^{a}	0.38^{b}	0.35^{b}	0.33^{b}	0.06
Thymus(%)	4.08^{a}	3.86^{ab}	3.66^{ab}	3.30^{b}	0.12

$^{a, b, c}$ *means within rows with different superscripts differ significantly ($P < 0.05$)*

Discussion

The better growth performance of the birds fed 10 and 15g NLP in the present study could probably be due to the high protein content of neem leaves as Banjo (2012) reported that the use of Moringa improved performance of broiler chickens due to its protein content. Schaaf et al. (2000) has reported that the composition of fresh neem leaves is protein-7.1%. The similarity in the feed intake across the treatment groups is in agreement with the findings of Bonsu et al. (2012) who reported that neem leaf meal had no significant effect on the feed intake of the birds. The lack of variations in feed consumption among the treatment groups suggests that the concentration of toxicants (Ogbuewu *et al.,* 2011) in the NLP was within the tolerable limits of the birds. Contrary to the improved body weights gain and feed conversion ratios recorded in in the birds in this trial, Bonsu et al. (2012) revealed that dietary inclusion of neem leaf powder at 2 and 2.5% levels adversely affected body weight gain and feed conversion ratios. The difference in this trial and other trials reported in literature could be attributed to the season, type of bird, concentration of the text ingredient and environment in which the experiment was carried out. The improvement in the growth performance of the birds observed in this study may also be explained by the fact NLP may have enhanced the immunity and the antioxidant enzyme activities (Durrani *et al.,* 2008; Onyimonyi *et al.,* 2009) of the birds and that the birds could tolerate the bitter and astringent taste of neem leaves at 10 and 15g NLP. The improved performance in this study agrees with the findings of Onyimonyi et al. (2009) who reported that varying dietary neem leaf meal improved performance of broiler chickens. Durrani et al. (2008) also reported that neem leaf extract improved performance of broiler chickens.

Globulin concentration is taken to be direct correlate of humoral immune competence (Swechha et al. 2014). The higher levels observed in the birds fed 10 and 15g NLP suggests a higher immune competence. This observation corresponds to the superior performance recorded in the birds in these treatment groups. The improved performance can therefore be attributed to the antioxidant activities of some components of neem leaves like vitamins C, E, phenols, nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Subapriya and Nagini, 2005; Rocha *et al.,* 2010)

It has been shown that hyperthermia-associated myopathy is characterized by an increase in the plasma activity of the skeletal muscle-derived isoenzyme creatine kinase, reflecting Ca2+-mediated alterations in muscle membrane integrity (Sandercock et al. 2001). In the present study, variations in the activity of creatine kinase was observed. The lower levels recorded in the birds fed 10 and 15g NLP compared with control chickens indicate less skeletal muscle damage during hot dry season. This observation suggest that the bioactive compounds in neem leaf powder ameliorated the effect of heat stress on the birds during hot dry season in the tropical environment. Similar increases in plasma CK activity have been

reported for hyperthermia-associated stress conditions in pigs (Shibata 1996).

Conclusion

Broiler chickens exposed to heat stress in the hot–humid climate responded favorably to inclusion of neem leaf in their diets. Inclusion of neem leaf powder in the diets of broiler chickens up to 10g/Kg diet enhanced the performance of broiler chickens during hot dry season.

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PATTERN OF PULMONARY LESIONS IN SOME WILDLIFE RODENTS AND DUIKERS IN GHANA

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Abstract

Respiratory disease has been presumed as a limiting factor in the domestication of some wild rodents; however, there is dearth of information on the pattern and burden of pulmonary lesions in these animals especially in West Africa where they are delicacies. A total of 96 rodents including 48 giant rats, 28 cane rats, 5 squirels and 15 duikers acquired from game meat market in Kumasi, Ghana were investigated for lung lesions. The lungs were observed macroscopically for distribution of consolidation and sections were further processed for microscopic evaluation using standard techniques. Results showed that 56 (58%) of lung samples had lesions with higher prevalence in giant rats than duiker. The young and male animals were observed to be more affected. Microscopic changes in the lung include pulmonary congestion, interstitial pneumonia, bronchopneumonia and atelectasis. The knowledge of the pattern of pneumonia in wildlife rodents and duikers calls for the need for a national surveillance on the nature of respiratory diseases of wildlife and it also may inform choices of conservational policies on these animals. .

Keyword: Lung, Pneumonia, Rodents, Duiker, Ghana, Nigeria

SCHEMA DE LÉSIONS PULMONAIRES CHEZ CERTAINS RONGEURS ET CEPHALOPHES SAUVAGES AU GHANA

Resume

Il est présumé que la maladie respiratoire est un facteur contraignant dans la domestication de certains rongeurs sauvages ; cependant, il existe une pénurie d'informations sur le modèle et le fardeau des lésions pulmonaires chez ces animaux, en particulier en Afrique de l'Ouest où ils sont considérés comme des délicatesses. Au total, 96 rongeurs, dont 48 rats géants, 28 aulacodes, 5 écureuils et 15 céphalophes, obtenus sur le marché de la viande de gibier à Kumasi au Ghana, ont été étudiés en vue de détecter la présence de lésions pulmonaires. Les poumons ont été observés macroscopiquement pour déterminer la distribution de la consolidation et les sections ont été soumises à une évaluation microscopique utilisant des techniques standard. Les résultats ont montré que 56 (58%) des échantillons de poumons avaient des lésions avec une prévalence plus élevée chez les rats géants que les céphalophes. On a noté que les animaux jeunes et mâles étaient plus affectés. Les changements observés par microscopie au niveau des poumons comprennent la congestion pulmonaire, la pneumonie interstitielle, la bronchopneumonie et l'atélectasie. La connaissance du type de pneumonie chez les rongeurs et les céphalophes sauvages révèle la nécessité d'une surveillance nationale de la nature des maladies respiratoires de la faune, et elle peut également éclairer les choix de politiques de conservation de ces animaux.

Mots-clés : poumon, pneumonie, rongeurs, céphalophe, Ghana, Nigeria

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Introduction

In many parts of the world, dramatic changes in farming practices have encroached into the habitat of wild animals with ensuing disruption of the ecosystem. This aberration in the ecosystem has further impinged on the wellbeing and health status of wildlife.

Wild animals have been used as a resource throughout the course of human existence (Ajayi 1971) with conspicuous role in the rural as well as national economics of tropical Africa (Akinpelu 2007). The populations of numerous wildlife species have undergone a precipitous decline in the past century, resulting in significant reduction of some and extinction or near - extinction for others (Kellman and Tackaberry 1997). Many of these problems have been attributed directly to habitat loss and over exploitation due to increasing human activities (Omonona and Jarikre 2015).

Generally, there is relationship and interaction between health status, local environment and husbandry techniques especially as it concerns the productive life of animals kept in captivity (Onyeanusi et al, 2001). This portends a clearer understanding of wild animal diseases especially those in captivity; furthermore, domestication often deprives wild animals of their normal unlimited and unrestricted free access to natural food items, minerals and preferred habitats unlike the wild where the animals use this access to avoid disease and pests infestations (Uloko and Audu, 2010).

Our observations over the years presume respiratory disease as a limiting factor in the domestication of some these wild rodents (Jayeoba *et al.,* 2015) with isolation of some bacteria including Pasteurella spp. from their airways (Jayeoba *et al.,* 2014). Nonetheless, there is dearth of information on the pattern and burden of pulmonary lesions in these animals (Emikpe *et al.,* 2016). Thus this present study aimed at describing the respiratory pathology observed in some wild rodents and the duiker from Kumasi, Ghana.

Materials And Methods

Study animals

The animals were gotten from markets in the Kumasi Metropolis and Sekyeredumase, Ghana. This areas fall within the forest zone characterized by high rainfall patterns. The presence of forests and grasses in the area favour game animals such as grasscutter, giant rat, squirrels, and duikers among others. The sex and age of the animals was determined using standard techniques as described by (Adebayo et al 2009). This study lasted for a year: October 2015 to September 2016.

Sampling

The pluck of the carcasses from the rodents and duikers were removed for thorough observation. The lungs were examined grossly for changes in consistency, texture, color and degree of consolidation. The extent of pneumonia was determined by visual observation and palpation adapting the method used by Jarikre et al (2016). Samples from the lungs were processed routinely for histopathology. The slides were evaluated using the Olympus light microscope (CX21) attached to a digital computerized camera (AmScope, MU900).

Statistics

Descriptive statistics was used to describe the age, sex, season, year influences on the occurrence of pneumonia. Chi square test was used for the test of significance and Pearson's correlation for degree of association.

Results

Of the total of 96 animal carcasses examined; 28 were cane rat, 15 duiker, 48 giant rats, and 5 squirrels. Some of the pulmonary lesions observed include pneumonia, atelectasis, congestion and emphysema

Pulmonary lesions

56 (58%) showed pulmonary lesions and 40 (42%) normal (table 1). The young were more affected and while the male also showed

pulmonary lesions more than the female animals ($p<0.05$). There was negative correlation of age and pulmonary lesions (-0.1, $p=0.05$).

Microscopically, the lung lesions included pulmonary congestion, interstitial pneumonia, bronchopneumonia and atelectasis (table 2). The normal lungs showed typical empty, distended to collapsed alveoli and airways characteristic of mammalian lung (fig 1).

The pulmonary congestion was characterized by diffuse venular and capillary congestion, with moderate accentuation of the alveolar septae (figure 2). A few also showed haemorrhage into the airspaces. Interstitial pneumonia observed showed thickening or accentuation of the alveolar septa due to congestion of alveolar capillaries, haemorrhages and/or infiltration of mononuclear inflammatory cells including lymphocytes and macrophages (fig 3). Disruption of alveolar lining and formation of hyaline membrane was also common depending on the severity and extent of alveolar epithelial necrosis and repair from type II pneumocytes.

Bronchopneumonia was characterized by patchy to diffuse hyperaemia, necrosis of bronchi, bronchiolar and alveolar epithelial cells with presence of exudate and cellular debri in airway and alveolar spaces. The exudate included oedema, neutrophils, and abundant alveolar macrophages (fig 4). Atelectatic lung appeared slightly congested with alveolar walls lying in close apposition.

The prevalence of pulmonary lesion was higher in giant rat (39%) and low in duiker (1%). The incidence of interstitial pneumonia was also high (30%) as compared to a few bronchopneumoina (3%). The occurrence of bronchopneumonia was more in the giant rats.

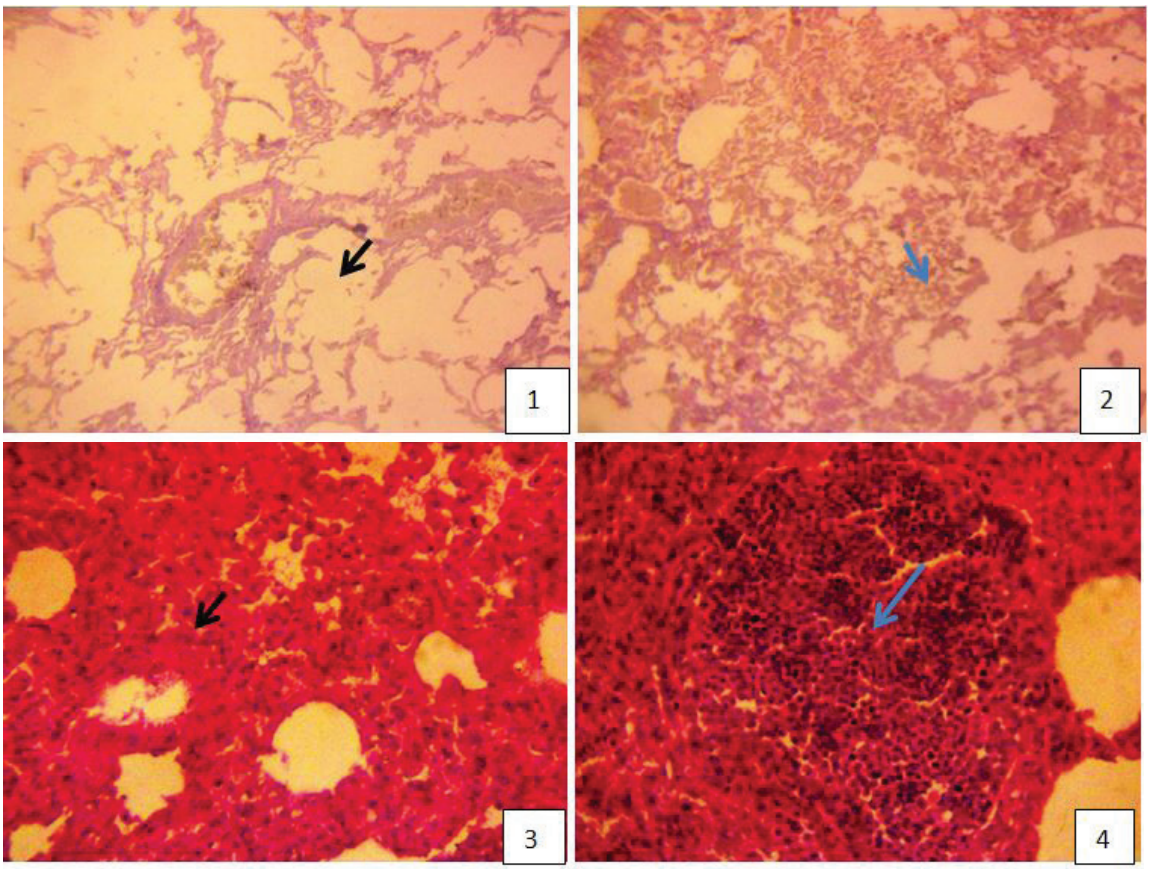
Table 1: The pattern of pneumonia in relation to age and sex from the examined wildlife animals

	Normal	Pulmonary lesion	Total	p value	Correlation
Young	20	32	52	0.489	-0.071 (0.494)
Adult	20	24	44		
Male	21	39	60	0.037	-0.175 (0.089)
Female	19	17	36		

Table 2: The type and distribution of lung lesions in the examined wildlife animals

	CR	D	GR	SQ	Total	p value	Correlation
Normal	13	14	11	2	40	0.00	+0.263 (0.010)
Pulmonary lesion	15	1	37	3	56		
Congestion	5	1	9	0	15	0.006	
Interstitial	7	0	20	2	29		
Bronchopn	0	0	3	0	3		
Atelectasis	3	0	5	1	9		

CR- cane rat; D- duiker; GR- giant rat; SQ- squirrel;



Figures: 1- normal lungs with air-distended alveoli (arrow). **2-** pulmonary vascular and capillary congestion with expanded septae (arow). **3-** interstitial pneumonia due to vascular, inflammatory changes (arrow) and accentuation of alveolar septae. **4-** bronchopneumonia characterized by massive infilling of neutrophils and macrophages in alveolar spaces. HE x100, 400

Discussion

This study appears to be the first report describing the pattern of pulmonary lesions in some of these wildlife species. This study has been able to show that pulmonary lesions are of considerable importance in the management and conservation of wildlife. More so, it is a pointer to what account for mortality of these wildlife in the wild and natural habitat.

The African giant rats (AGR) are hardy physically however; their ecology and survival instincts may predispose them to irritants capable of inducing pneumonia. 39% pulmonary lesions were observed. More so, the occurrence of interstitial pneumonia and bronchopneumonia in this rodent underscores the involvement of pulmonary pathogens like viruses and bacteria which should be further characterized towards the development of appropriate vaccination in near future especially as domestication of these species intensifies. On the other hand, the partly low occurrence of pneumonia in the cane rat (grasscutter) as compared to AGR may not be unconnected to the domestication success of the specie. Upper respiratory tract infection is an established condition in cane rats. 16% pulmonary lesions were observed in the cane rat. Our observation was quite similar to Okorie, (2004) who reported that the occurrence of snuffles (Pneumonic pasteurellosis) and Uloko and Audu (2010) with prevalence of pneumonia of 23.80% in grasscutter in some parts of Nigeria.

Hence pneumonia was suggested to be the fore most cause of mortality in grasscutter however in this study; pneumonia was more in giant rats.

Out of the five squirrels examined three had pulmonary lesions, thus highlighting the importance of pneumonia in this species also. The low occurrence of pulmonary lesions in the duiker (1%) suggests that the animals examines may be immune-competent and or properly adapted in the wild.

The difference in the pattern of pneumonia underscores the role and contribution of different causative agents especially viruses which may account for interstitial pneumonia observed in rodents examined. Some studies had incriminated Murine Parainflunza virus 1 (Sendai virus), which had been previously described in rodents (Perccy and Barthold 2007), however efforts is ongoing to elucidate the aetiology of pneumonia in these species.

The occurrence of bronchopneumonia in grasscuter is a significant observation indicating the role of possibly bacterial complicated viral pneumonia in this specie which also needed to be properly elucidated. The influence of sex and age were also important for appropriate intervention and preventive measures, in this investigation, high prevalence of pneumonia was observed in young animals, this may probably be as a result of little or no immunological memory prior to exposure of the causative agent. Nonetheless, toxicity and other environmental stressors may be involved in the pathogenesis of pneumonia in wildlife. Furthermore, the roles of the causal agents including viruses and bacteria would inform appropriate control measures suitable for domestication and conservation of the wildlife species.

Conclusion

This study elucidates the pattern of pneumonia in wildlife rodents and duikers which calls for the need for a national surveillance on diseases of wildlife and need for control policies which are very instrumental to choices of conservational policies on these animals.

Acknowledgment:

We appreciate contribution of Mr A. Nwagbara in processing of the tissues.

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SEMEN QUALITIES OF KALAHARI RED BUCKS FED Moringa oleifera LEAF MEAL-BASED CONCENTRATES DURING DRY SEASON

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Abstract

To determine the semen qualities of Kalahari Red goats fed Moringa oleifera leaf-based concentrates during dry season, sixteen (16) Kalahari Red bucks were fed MOLM-based concentrates at 0, 5, 10 and 15 % for 100 days in a Completely Randomised Design. Ten (10) induced does were used as teasers on 60th, 77th, 84th, 91st, and 98th day for semen quality. The highest semen volume of 1.9 ml per ejaculate was observed in Kalahari Red bucks fed 15 % MOLM inclusion level. 10 % of MOLM elicited sperm concentration of 3.42 x 109/mls ($P < 0.05$), progressive motility ($P > 0.05$) of 98.00 % and the lowest abnormality of 5.00 %. The lowest value of 0.71 µmol/ml malondialdehyde was recorded for the group fed 15 % inclusion level of MOLM. It could be concluded that MOLM improved semen qualities of Kalahari Red bucks.

QUALITÉS DU SPERME DES CHEVRES KALAHARI ROUGE NOURRIES AUX CONCENTRES A BASE DE FEUILLES DE Moringa oleifera PENDANT LA SAISON SECHE

Résumé

Dans la perspective de déterminer les qualités du sperme des chèvres Kalahari rouges nourries aux concentrés à base de feuilles de Moringa oleifera pendant la saison sèche, seize (16) chèvres Kalahari rouges ont reçu des concentrés à base de feuilles de Moringa oleifera (MOLM) à 0, 5, 10 et 15% pendant 100 jours selon un dispositif entièrement aléatoire. Dix (10) chèvres induites ont été utilisées comme teasers aux 60ème, 77ème, 84ème, 91ème et 98ème jours en vue de déterminer la qualité du sperme. Le volume de sperme le plus élevé de 1,9 ml par éjaculat a été observé chez les mâles rouges Kalahari recevant un niveau d'inclusion de MOLM de 15%. Le niveau d'inclusion de MOLM de 10% a provoqué une concentration de sperme de 3,42 x 109/mls ($P < 0,05$), une motilité progressive ($P > 0,05$) de 98,00% et la plus faible anomalie de 5,00%. La valeur la plus basse de 0,71 µmol / ml de malondialdéhyde a été enregistrée pour le groupe recevant un taux d'inclusion de MOLM de 15%. On peut conclure que MOLM a amélioré les qualités du sperme des chèvres rouges Kalahari.

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Introduction

The Kalahari Red goat was imported into Nigeria in 2011 by the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB) and the goat project is anchored in the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) with the ultimate aim of multiplication and improvement of the indigenous breeds viz; Red Sokoto and West African dwarf goats (Oduguwa, 2014). The Kalahari Red goat (KR) is a breed mostly found in South Africa which serves many purposes including milk, meat, and skin production. They have characteristics such as good foraging abilities and are regarded as a "minimum care maximum profit" breed. Interest in the Kalahari Red breed is widespread. It breeds all year round and can kid 3 times in 2 years (Tollie, 2012). Kalahari Red goat is an exotic breed of goat which can be used to produce more meat and milk than the indigenous goats. In order to meet the ultimate aim of multiplication and improvement of the indigenous breed of West African Dwarf goats by Kalahari Red goat its semen quality must be ascertain to be of good quality. Although information is available on semen characteristics of goats, little is known about fertility parameters of Kalahari Red bucks raised in the tropical countries. Moringa is known to contain phenolic compounds and flavonoid that can affect the reproductive performance of herbivores by directly interacting with steroid hormone systems (Oberdorster *et al.,* 2001). However, the concentra¬tions of testosterone were similar across the groups (5.65 – 5.95 ng/L) when WAD bucks were supplemented with Moringa leaf at 0 g/kg concentrate, 100, 200, and 300 g/kg respectively for 8 weeks (Abimbola *et al.,* 2013). Moreover, there is paucity of information regarding feeding values of air-dried Moringa oleifera leaf meal for Kalahari Red goats in dry season when there is severe feed scarcity. Hence, the objective of this study was to determine the semen qualities of Kalahari Red goats fed Moringa oleifera leaf-based concentrates during dry season.

Materials and method

Experimental Site

The experiment was carried out at the Kalahari Unit of the Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Ogun State, which is located in the tropical rainforest zone in Nigeria within 7o13'47.41"N, 3o23'43.48"E. The climate is humid and located in the forest zone of South-Western Nigeria. The mean precipitation and the temperature are 1,112.70 mm and 23.5 0C respectively. Relative humidity averaged 81.50 % throughout the year. Seasonal distribution of rain is approximately 110.90 mm (9.97 %) in the dry season (October – March) (OORBDA, 2012).

Harvesting and Processing of Moringa oleifera Leaf meal

Fresh leaves of Moringa oleifera (Nigerian ecotype) were collected from previously established plot at Kalahari Unit. The harvested Moringa leaves were air-dried by spreading on a tarpaulin and cemented floor in a roofed and well ventilated room. The leaves were frequently turned until they were crispy to touch while retaining their greenish colouration. The leaves were then hand-milled to obtain a product herein referred to as Moringa oleifera leaf meal (MOLM) which was stored in air-tight sacs until ready to use.

Experimental Diets preparation

The concentrates contained Palm Kernel Cake (24.00 kg),Corn Bran(16.00kg),Wheat offals(50.00 kg),Soybean Meal (6.00 kg),Bone meal (1.00 kg),Salt (2.75) Livestock premix (0.25 kg). Four experimental diets were formulated which contained Moringa oleifera leaf meal at the rate of 0 , 5 , 10 and 15 % respectively.

Management and Feeding of Experimental Animals

Sixteen (16) Kalahari Red bucks weighing 27.27±0.68 kg were used in the experiment for a duration of 100 days. The

animals were managed under intensive system. Weighed quantities of experimental diets and groundnut haulms (basal diets) were served to the animals at 08:00 hours and 14:00 hours respectively and water was given ad libitum. The bucks were randomly allotted into four different diets of 0, 5, 10 and 15 % with four replicates per treatment.

Induced Oestrous and Semen Collection

Semen samples were collected from bucks randomly from each treatment with the aid of an artificial vagina (AV) between 7.00 am and 11.00 am to ensure optimum quatity and quality on 60th, 77th, 84th, 91st and 98th days of the experiment. Warm water (38 0C) was poured into the AV with the aid of a plastic funnel and locked. It was inflated by mouth through the air-valve until the inner linning of the AV was closed enough and the stopper tightened. The inner latex rubber of the AV was then lubricated with petroleum jelly which is a heavy organic solvent that does not contaminate semen when it is in contact with it, and at the same time retains heat. A doe on heat was used as a teaser which was tied on the neck and right fore leg to a pole in order to restrain the animal. On introduction to the teaser, each buck made vigorous upward and forward thrust and a lounging movement which signified the occurrence of ejaculation. Ejaculates were pooled at the first or second mount in a graduated test tube attarched to the base of the rubber-like funnel inserted to the AV. The pooled semen samples were diluted at 32 0C in a one-step process with a Tris-based extender: Tris-hydroxymethyl-aminomethane (2.42 g), citric acid (1.36 g), glucose (1.00 g), procaine penicillin (0.028 g), egg yolk (20 mls) and distilled water (80 mls) to make up 100 ml. The pooled ejaculates were transferred into test tubes and placed in warm (37 0C) water bath.

Determination of Semen volume and colour

The volume of semen harvested was measured using the graduated collection tube that was attached to the base of the rubber-like funnel inserted to the AV. The semen colour was noted immediately after collection as chalky, cream or milky and transparent.

Determination of Sperm Motility

Sperm motility was determined as described by Bearden and Fuquay (1997). Briefly, semen was placed in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37 0C on arrival to the Animal Physiology Laboratory and accessed for sperm motility using Celestron PentaView microscope (LCD-44348 by RoHS, China) at × 400 magnifications. A semen mount was made using 5µl semen and the semen was placed directly on a microscope slide and covered with cover slip. For each sample, five microscopic fields were examined to observe progressive sperm motility and the mean of the ten successive evaluations was recorded as the final motility score.

Determination of Sperm Concentration

1 ml aliquot of semen in a Tris-based extender (0.1 % V/V) was used to determine sperm concentration using a spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 650 nm wavelength against egg yolk as blank according to Chemineau et al. (1991).

Determination of Sperm Abnormality

Sperm abnormality was evaluated as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin smears. A thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. Abnormality of sperm cells located in the head, midpiece and tail were observed under Celestron PentaView LCD microscope (×400 magnifications).

Determination of Sperm live/dead ratio

A micropipette was used to place 5 µl of semen and a sterile glass stirring rod was used to place a small droplet of 5 µl of eosin-negrosin stain (1 % eosin and 5 % nigrosin in 3 % sodium citrate dehydrates solution) side by side on a microscope slide(25.4 × 76.2 mm). a slide was used to mix them gently and was

used to form a smear which was air-dried for 30 minutes. The liveability (live/dead ratio) was observed using the light microscope starting with low power (×10) to higher magnification (×400). The side scrolls were adjusted until a clear vision of the spermatozoa were seen as described by Oyeyemi et al.(2011).

Determination of Malondialdehyde Concentration

MDA concentration as index of lipid peroxidation in the stored semen was measured in a thiobarbituric acid reactive substances (TBARS) according to Papastergiadis et al.(2012). For this assay, 0.10 ml of sperm suspension was incubated with 0.10 ml of 150 mM Tris-HCl (pH 7.1) for 20 minutes at 37 0C. Subsequently, 1.00 ml of 10 % trichloroacetic acid (TCA) and 2.00 ml of 0.375 % thiobarbituric acid was added followed by incubation in boiling water for 30 minutes. Thereafter, it was centrifuged for 15 minutes at 3000 revolution per minutes inside the blank tube and the absorbance was read with UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532 nm.

Statistical Analysis

Initial weights were used as covariates and variant means were separated us¬ing duncan multiple range test method. In addition, Microsoft office excel 2007 was used for plotting graphs while Systat version 5.02 was used for regression analyses.

Statistical Model

$$Y_{ij} = \mu + M_i + \square_{ij}$$

Where: Y_{ij}= Dependent variable; μ = Sample mean; Mi= Effect of the ith inclusion of air-dried Moringa oleifera leaf meal in the concentrate at 0, 5, 10 and 15 %); □ij= Random Error

Results

Figure 1 shows semen volume per ejaculate of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates. On the 60th day 0 % had 0.30 ml, 5 % had 0.50 ml, 10 % had 1.00 ml and 15 % had 1.50 ml. On the 77th day, 0 %, 5 %, 10 % and 15 % had 1.00 ml, 1.70 ml, 0.70 ml and 1.80 ml ($P < 0.05$) respectively.

Table 1 shows sperm concentration (× 109) of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates. On the 60th day of the experiment, semen concentration of bucks fed 5 % MOLM had the highest value of 5.37 × 109 which was followed by 10 % (4.53 × 109) while the least value was from 15 % (1.41 × 109). On the 91st and 98th days, the sperm concentration of 0 and 15 % inclusion levels recorded 1.41 ×109 each per ejaculate. 0n the 91st day 10 % MOLM recorded the least mean value of 0.67 × 109 .

Figure 2 shows sperm concentration (× 109) of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates. The results showed that the sperm concentration were significantly influenced by substitution of Moringa oleifera in the concentrates. 10 % Moringa oleifera leaf meal-based diet recorded the highest concentration (3.42 × 109) which was followed by 5 % (2.60 × 109). 0 and 15 % had the mean values of 2.05 × 109 and 2.06 × 109 respectively.

Table 2 shows semen characteristics of Kalahari Red bucks fed Moringa oleifera leaf meal-based concentrates. The results showed that substitution level of test ingredients significantly ($P < 0.05$) affected the liveability of the spermatozoa. Abnormal head ($P < 0.05$) was highest in bucks fed 5 % MOLM (6.08 %) while the lowest value was observed in 10 % (1.15 %). The mid-piece values for 0, 5, 10 and 15 % Moringa oleifera leaf meal meal-based diets were 2.50 %, 3.47 %, 2.00 % and 4.84 % respectively. The highest mean values of 5.65 % for cut-tail was recorded in 5 %. The semen colour ranged from milk to cream.

Figure 3 shows malondialdyhide (MDA) concentration of spermatozoa of Kalahari Red bucks fed Moringa oleifera leaf meal-based concentrates. MOLM significantly ($P < 0.05$) influenced malondialdyhide concentrations. The highest mean value of malondiadyhide 2.96 μmol/ml was recorded for bucks fed control diet while the lowest value was recorded for the group fed 15 % MOLM (0.71 μmol/ml).

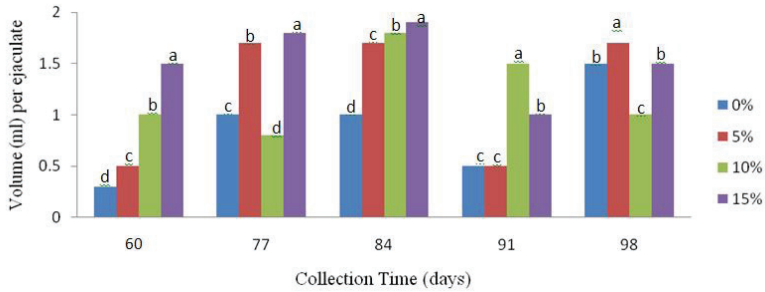

Figure 1: Semen volume per ejaculate of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates

Table 1: Sperm concentration (×109) of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates

***Moringa oleifera* leaf meal inclusion level**	**Collection period (days)**				
	60	**77**	**84**	**91**	**98**
0 %	1.72±1.92^{c}	0.82±0.00^{c}	4.86±3.85^{b}	1.41±0.00^{a}	1.41±0.00^{b}
5 %	5.37±0.60^{a}	1.41±0.00^{a}	4.84±0.00^{b}	1.16±0.00^{b}	0.24±0.00^{c}
10 %	4.53±0.06^{b}	1.41±0.00^{a}	5.25±0.22^{a}	0.67±0.00^{c}	5.25±0.42^{a}
15 %	1.41±0.00^{d}	0.88±0.00^{b}	5.19±2.72^{a}	1.41±0.00^{a}	1.41±0.00^{b}
P-Value	<.00	<.00	<.00	<.00	<.00

^{a,b,c,d} means in the same row with different superscripts differ significantly (P < 0.05)

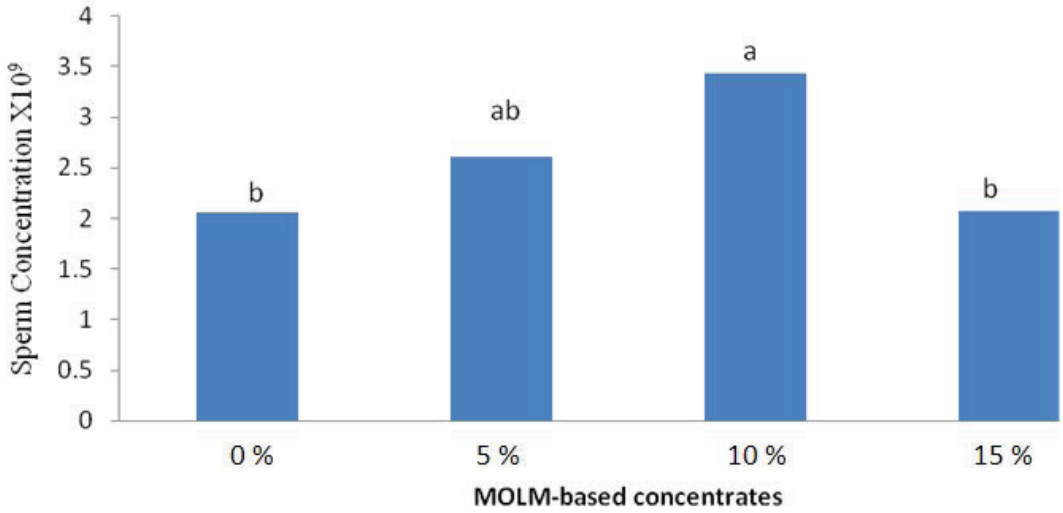

Figure 2: Semen concentration of Kalahari Red bucks fed air-dried Moringa oleifera leaf meal-based concentrates

Table 2: Semen characteristics of Kalahari Red bucks fed Moringa oleifera leaf meal-based concentrates

	Moringa oleifera leaf meal inclusion level				
Parameters (%)	**0 %**	**5 %**	**10 %**	**15 %**	**SEM**
Progressive motility	88.00	94.00	93.33	98.00	1.59
Liveability	91.38^{bc}	88.05^{c}	93.00^{ab}	96.04^{a}	0.79
Abnormal Head	3.00^{b}	6.08^{a}	1.15^{b}	3.80^{ab}	0.51
Mid-Piece	2.50	3.47	2.00	4.84	0.59
Cut Tail	5.62	5.65	5.00	4.75	0.58
Semen Colour	Milky	Chalky	Creamy	Creamy	

^{a,b} *means in the same row with different superscripts differ significantly (P < 0.05)*

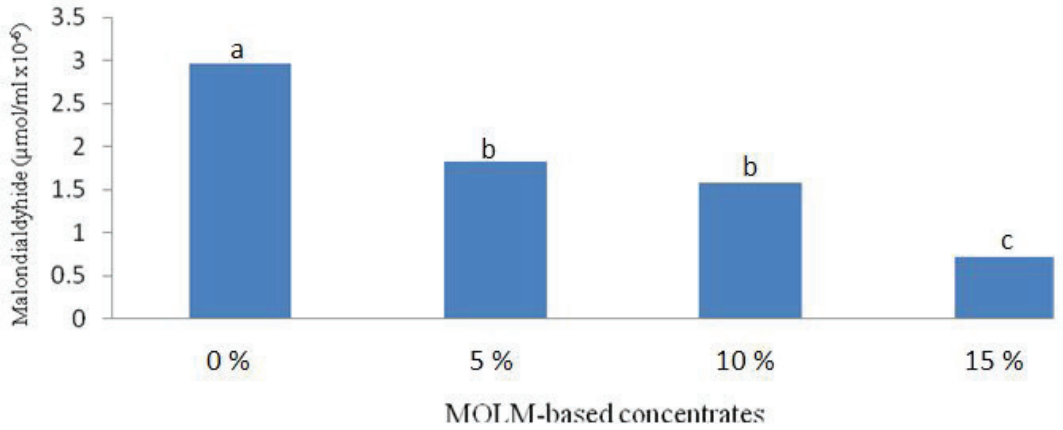

Figure 3: Malondialdyhide (MDA) concentration of spermatozoa of Kalahari Red bucks fed Moringa oleifera leaf meal-based concentrates

Discussion

Induced does were introduced to the bucks as teasers after sex deprivation. In order to ensure meaningful effect of Moringa oleifera-based concentrates on the bucks, semen collection did not commence until the 60th day of the experiment owing to the fact that the time required for successive diffentiation of a germ cell called the spermatogenic cycle, varies among mammalian specie. It is approximately 35 days in mouse, 54 days in bull, 74 days in man and 50 to 65 days in goats (Franca *et al.,* 1999). This difference in sperm concentration was due to the variation in nutrient combination or mixture. It has been clearly established that reproductive performance is influenced by nutrition in goats (Zygoyiannis and Katsaounis, 1986). In bucks, severe protein deficiency will adversely affect sperm production because spermatozoa are high in protein content (Smith, 1988). It has been shown that feeding of high protein concentrates can increase the production of spermatozoa .The mean volume per ejaculate of buck was in the range of 0.3 ml to 1.9 ml. This is higher than 0.43 ± 0.03 ml to 0.45 ± 0.22 ml reported by Khandoker et al. (2006) for Black Bengal buck. This difference could be due to the inclusion level of Moringa oleifera leaf meal in the diets which was buttressed by Peters (2002) who observed that semen production largely depends on several factors such as nutritional status, general health condition, endocrine balance and soundness of the sex organs. Heat stress could be responsible for deleterious effects on semen quality (Moretti *et al.,* 2007). However, the dilution of goat semen in extenders containing egg yolk can be deleterious to sperm cells. This occurs owing to the presence of phospholipase A secreted by

the bulbourethral glands which is an egg yolk coagulating enzyme (LeBoeuf *et al.,* 2000) and is responsible for the reduced viability of sperm cells (Corteel, 1981). The colour of the semen observed throughout the period of collection was not different from the control. This agrees with the reports of Oyeyemi et al. (2011) on the colour of semen in bucks. Therefore, it can be suggested that the continued use of MOLM did not have any effect on the colour of semen of Kalahari Red bucks. The increase in the percentage of abnormal spermatozoa during drought could be attributed to increase in testicular temperatrure that provokes specific degeneration with the appearance of abnormalities (Chemineau and Cagnie, 1991). Thus, males may show transient sterility due to high temperature. Abnormalities observed in this study have important consequences on quality of the spermatozoa and fertility. The abnormalities of the sperm cells observed in this study were far below (6.08 %) the normal range of 20 % reported by Oyeyemi et al. (2006) for effective fertility in West African Dwarf bucks. Substitution levels of Moringa oleifera leaf meal in the diets influenced malondialdyhide concentrations in the semen. The highest mean value of malondiadyhide (2.96 µmol/ml) was recorded for bucks fed 5 % MOLM while the lowest value was recorded for the group fed 15 % MOLM (0.71 µmol/ml). Malondialdehyde is used as a potential marker of oxidative stress. Reactive oxygen specie play a dual role in male infertility (Sanoeka and Kurpisz, 2004). On one hand, ROS plays a keyrole in processes such as capacitation and fertilization. On the other hand, excessive production of ROS can inflict severe damage to spermatozoa (Pasqualotto *et al.,* 2000; Agarwal *et al.,* 2003). Spermatozoa contain large quantities of polyunsaturated fatty acids (PUFA); therefore, they are susceptible to ROS-induced damage (Sanoeka and Kurpisz, 2004). It has been suggested that ROS induce membrane lipid peroxidation in sperm. The toxicity of generated fatty acid peroxides are important causes of sperm malfunction (Agarwal and Saleh, 2002). Increased plasma MDA levels along with decreased antioxidant defense point towards continuous oxidative stress. Oxidative stress (OS) as a result of an inappropriate bal¬ance between oxidants and antioxidants in the semen can lead to sperm damage, impairs the structure and function of spermatozoa and eventually male infertility (Agarwal *et al.,* 2003). Antioxidants are the phytochemicals which protect the cells against oxidative damages. They are the first line of defence against free radical damages. Perry et al.(1999) reported that the Moringa oleifera leaf meal contains numerous nutritional antioxidant compounds in substantial amount, such as ascorbic acid, flavonoids and phenols (Veliogu *et al.,* 1998). The scavenging action of plant constituents has been found to relate to polyphenolic compounds (Jonathan *et al.,* 2012). In this study, Moringa olifera leaf meal showed antioxidant activity, however, the magnitude of antioxidative potency varied with the inclusion levels. This could be due to the difference in concentrations and type of antioxidative compounds present in the air-dried leaves.

Conclusion

MOLM-based concentrates did not pose any health challenge but improved the semen qualities of Kalahari Red bucks.

Acknowledgement

The authors are grateful to Tertiary Education Trust Fund (TETFund) for funding the research, the fund was facilitated by Directorate of Grants Managements (DGM), and IFSERAR, FUNAAB. Also to IFSERAR, for providing the Kalahari Red goats with all necessary materials on the farm.

Conflict of Interest Statement:

The authors declare that they have no conflict of interest.

Statement of Animal Rights:

All applicable International, National, and Institutional guidelines for the care and use of animals were followed in the conduct of this research.

Informed Consent:

Informed consent was obtained from all individual participants included in this study.

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SEROLOGICAL AND MOLECULAR INVESTIGATION OF PESTE DES PETITS RUMINANTS IN ADAMA DISTRICT, EASTERN SHOA ZONE OF OROMIA, ETHIOPIA.

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Abstracts

Peste des petits ruminant (PPR) is one of highly contagious viral disease of small ruminants associated with severe economic losses world including Ethiopia. The present cross-sectional study was conducted in four localities of Adama district, Oromia Region, Ethiopia between November 2015 and April 2016 to determine the sero- prevalence and antigen detection of PPRV in small ruminants using c-ELISA and RT-PCR. A total of 384 sera and 100 nasal swab samples collected from small ruminants were examined. Out of which, 30.2% and 42% were found to be positive by c-ELISA and RT-PCR respectively. The prevalence of PPR was not significantly different between species (χ^2 = 8.23; p = 0.364) and sexes (χ^2 = 6.98; p = 0.404). However, the prevalence was significantly different between study localities (χ^2 = 8.147; p = 0.043), age groups (χ^2 =27.198; p = 0.000) and body conditions (χ^2 = 17.274; p = 0.000). Prevalence was higher in Soloke (40%),in young small ruminants (48.33%) and poor body condition animals(47.77%) than other study localities (Amede, Sefera and Bokushenen),adult small ruminants and medium/ good body conditions, respectively. This study revealed a high sero-prevalence of PPR in small ruminants in the selected study areas. This disease is detrimental to small ruminant welfare and causes substantial economic losses, thereby affecting the livelihood of poor farmers and pastoralists. The need for implementing feasible control measures is, therefore, eminent to minimize the losses associated with the disease.

Key words: c-ELISA, RT-PCR, Sero-prevalence, small ruminants, PPR.

ETUDE SÉROLOGIQUE ET MOLÉCULAIRE DE LA PESTE DES PETITS RUMINANTS DANS LE DISTRICT D'ADAMA DANS LA ZONE SHOA D'OROMIA EN ÉTHIOPIE

Résumé

La peste des petits ruminants (PPR) est l'une des maladies virales hautement contagieuses des petits ruminants associée à de graves pertes économiques dans le monde, y compris en Éthiopie. La présente étude transversale a été menée dans quatre localités du district d'Adama de la région d'Oromia en Éthiopie, entre novembre 2015 et avril 2016, dans le but de déterminer la séroprévalence et la détection de l'antigène du virus de la peste des petits ruminants (PPRV) chez les petits ruminants en utilisant la technique c-ELISA et RT-PCR. Au total, 384 sérums et 100 échantillons d'écouvillons nasaux prélevés sur de petits ruminants ont été examinés. Parmi ceux-ci, 30,2% et 42% ont été trouvés positifs respectivement par c-ELISA et RT-PCR. La prévalence de la PPR n'était pas significativement différente entre les espèces (χ^2 = 8,23; p = 0,364) et les sexes (χ^2 = 6,98; p = 0,404). Cependant, la prévalence était significativement différente entre les localités de l'étude (χ^2 = 8.147; p = 0.043), les groupes d'âge (χ^2 = 27.198; p = 0.000) et les conditions corporelles (χ^2 = 17,274; p = 0,000). La prévalence était plus élevée à Soloke (40%), chez les jeunes petits ruminants (48,33%) et chez les animaux pauvres (47,77%), respectivement par rapport aux autres localités de l'étude (Amede, Sefera et Bokushenen), aux petits ruminants adultes et aux conditions corporelles moyennes / bonnes. Cette étude a révélé une forte séroprévalence de la PPR chez les petits ruminants dans les zones d'étude sélectionnées. Cette maladie porte préjudice au bien-être des petits ruminants et entraîne des pertes économiques considérables, ce qui affecte les moyens de subsistance des agriculteurs et des pasteurs démunis. Il est donc nécessaire de mettre en œuvre des mesures de contrôle

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réalisables pour minimiser les pertes associées à la maladie.

Mots-clés : c-ELISA, RT-PCR, séroprévalence, petits ruminants, PPR.

Introduction

Ethiopia is believed to have the largest livestock population in Arica. This livestock sector has been contributing considerable portion to national export earnings and the livelihoods of rural households (CSA, 2015). According to CSA (2015) report, sheep constitute about 16.1% of the total livestock population of the country. Unlike the large population and importance of sheep in the country their productivity is low. This low productivity is a reflection of diseases, poor nutrition, poor animal production system and lack of veterinary care (Sisay, 2007). Studies conducted in some parts of Ethiopia have shown that respiratory disease caused by peste des petits ruminants (PPR) is the major economic importance and impose a significant constraint towards sheep and goat production in the country in the near future (Biruk, 2014;Megersa et al., 2011).

Peste des petits ruminants (PPR) is an acute, highly contagious and, infectious, frequently fatal and transboundary viral disease of major economic importance. The disease has high morbidity and mortality rates in small ruminants, especially in endemic countries (Abdalla *et al.,* 2012; Sibel and Harun, 2010). The virus is belongs to the genus Morbilli virus, family Paramyxoviridae , and is closely related to the Rinder pest virus, , human measles virus, Morbilli viruses of marine mammals and distemper virus of canidae and other wild carnivores. The disease is characterized by pyrexia, ocular and nasal discharges, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosa, enteritis, diarrhoea and bronchopneumonia followed by either death or recovery from the disease (Abdalla *et al.,* 2012).The PPR virus spreads through close contact between susceptible and infected animals. The outbreaks are associated with social, cultural and economic activities that promote host contacts such as livestock trade, cultural festivals, husbandry practices as well as environmental and climatic factors (Garrett *et al.,* 2013; Ohta, 1982).

In Ethiopia, Megersa *et al.,* (2011) and Biruk (2014) reported outbreak of the disease on a small ruminant in pastoral and agropastoral and estern Amhara Region of Ethiopia, respectively, but the status of the disease is not yet reported in Adama district, eastern Shoa zone of Oromia Region. Therefore, this study was conducted to determine the prevalence of PPR and major risk factors associated with the disease in and around Adama district.

Materials and methods

Study area

The study was conducted from November 2015 to April 2016 in four villages of Adama district, Oromia region, 99 km south east of the capital, Addis Ababa. Geographically, the area is located at a latitude of 8°32'29" N and a longitude of 39°16'08"E and has an elevation ranging from1600 to1712 m.a.s.l. (Figure 3). The area experiences a bimodal rainfall pattern with a short rainy season from February to April and long rainy season from the middle of June to the end of September .The remaining months are dry periods. The area gets an annual average rainfall of 851mm and the mean annual maximum and minimum temperature are about 26°C and 14 °C, respectively. According to CSA (2015), the population of small ruminants in Adama district is 93857. Crop-livestock farming is the main farming system of the area and small ruminants are the first dominant species kept by farmers.

Study animals

The study was carried out on local indigenous sheep and goats having different sex, age, body condition scores, origin and kept under extensive management system that were selected randomly from the total population of sheep and goats.

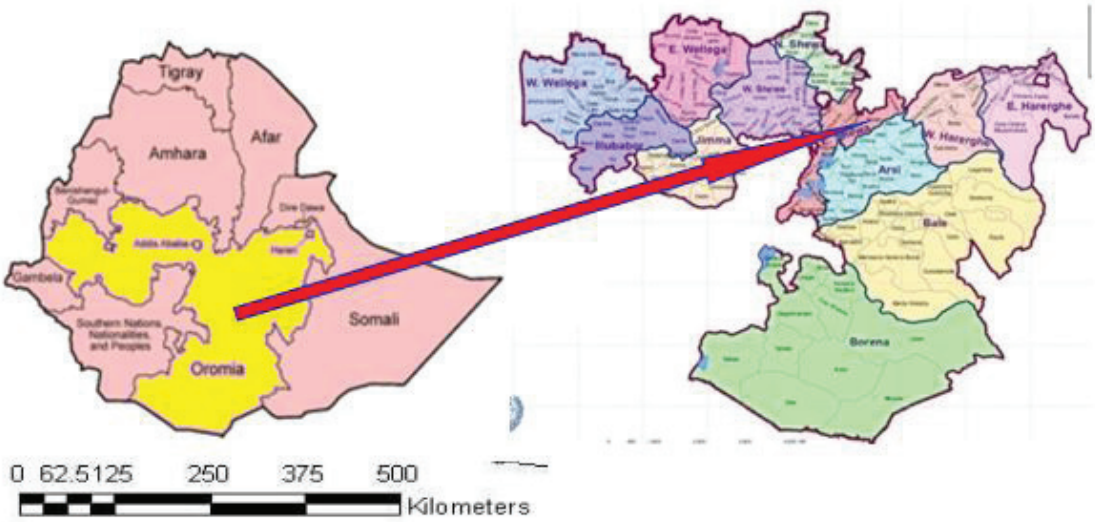

Figure 1: Map of study area.

Study design and sample size determination

A cross sectional study design with simple random sampling technique was conducted between November 2015 and April 2016 on 384 small ruminants (179 sheep and 205 goats) to determine the prevalence and associated risk factors of PPR in the study area. The desire sample size required for his study was determined based on the expected prevalence 50%, the 5% desired absolute procession and 95% confidence interval(CI)) according to Thrusfield (2005).

$$n=\frac{(1.96^2 * P_{ex}(1-p_{ex})}{d^2}$$

Where
1.962=the value of z at 95% Confidence level
n = required sample size
P_{ex} = expected prevalence
d = desired absolute precision

Accordingly, 384 small ruminants were included in the current study. The age of each animal was estimated based on the dentition formula) as given by Abegaz and Awgchew (2009); sheep and goats with only milk teeth were classed as lambs (less than 1 year old), and those with one to four pairs of permanent incisors were classed as adult, whereas animals with a full set of teeth were classed as old. The body condition scoring was classified as lean (poor), medium and fatty (Good) according to Thompson and Meyer (1994). All sampled animals included to this study were not vaccinated against PPR prior to sample collection.

Sample collection

Blood samples were collected aseptically from jugular vein of each sampled animal using disposable needles and non-heparinized vaccutaner tube and then brought to nearby veterinary laboratory in an icebox. At laboratory, blood samples were kept overnight to clot at slant position at room temperature. Then the separated serum was carefully collected in cryovial without mixing with the clotted blood. Finally, the serum samples were transported in an ice box to the National Animal Health Diagnostic and Investigation Center (NAHDIC) laboratory, Sebeta. Upon arrival; the serum was centrifuged to remove the remaining red blood cells before being transferred to new cryovials and stored at □20°C until further processing took place.

In addition to serum samples, 100

nasal swab samples were collected from the suspected animals for isolation (presence) of PPR viral antigen. The swab samples were collected using sterile swabs which were placed in a viral transport media (VTM) containing PBS, antibiotic and antifungal and transported to NAHDIC laboratory where viral antigen extraction was carried out. While collecting serum and nasal swabs, data like sex, age, species, body condition score, health status and origin of the animals were properly recorded.

Sample processing

Amonoclonal antibody (MAb) based competitive Enzyme Linked Immunosorbent Assay (cELISA) (OIE, 2013) was used for the detection of antibodies directed against the nucleoprotein of the PPR virus using approved competitive ELISA kit as described by Libeau *et al.,* (1995). The calculation of the competition percentage (S/N %) values were read with ELx800 Absorbance Microplate Reader (Biotek® Instruments, Inc. USA) with an inference filter of 450 nm. The OD (optical density) values of each sample were converted to S/N % by using the following formula:

$$S/N\% = [OD_{Sample} / OD_{NC}] \times 100$$

Serological examination

The samples with S/N less than or equal to 50 % were considered as positive.

The same procedure was used to convert the OD values to percentage inhibition (PI) for PPR detection by using the following formula:

$$PI = [100 - OD_{Sample} / OD_{NC}] \times 100$$

The samples with PI >50% were considered as positive.

Viral antigen detection (nucleic acid)

Nasal swab samples(100) were examined for the presence of PPRV RNA by one step reverse transcription- polymerase chain reaction (RT-PCR) assays (OIE, 2013). Primarily, the RNA extraction from samples was done using commercial RNA extraction kit (Qiagen® RNeasy Mini Kit, courtaboeuf, France) as per the manufacturer's instructions. The RNA obtained was converted to cDNA using a reverse transcriptase enzyme. The reverse transcription and PCR were carried out sequentially in the same tube. The cDNA was amplified using PPRv specific NP3 and NP4 primers as previously described by Couacy-Hymann *et al.,* (2002). The forward and reverse primers used were: PPRV-NP3 5'- GTC TCG GAA ATC GCC TCA CAG ACT – 3' and PPRV NP4 5' CCT CCT CCT GGT CCT CCA GAA TCT-3' , respectively.

Data management and analysis

All collected raw data were organized and enter in to MS-Excel spread sheet computer program and analyzed using SPSS 20 soft-ware version. Descriptive statistics was used to summarize the data. Relative frequency of a specific category of a given factor was calculated as the proportion of cases out of the total cases. Factors related to occurrence of PPR were investigated using Chi-square test (χ^2) and level of significant was considered at $P<0.05$.

Result

Sero-prevalence of PPR

In the present study a total of 384 sheep and goats sera (179 sheep and 205goats) were collected from four sites of Adama district, Oromia Region, Ethiopia to screen specific antibodies for PPRV using cELISA serological tests. Of total serum samples tested, 116, (30.2%) were positive for the presence of specific antibodies against PPRV. The sero-prevalence of PPR was higher in Soloke (40%) as compare to the other sites. There was a

Statistical significant difference between sample sites (χ^2 = 8.147; p< 0.043) (Table 1).

The statistical analysis of putative risk factors showed no significant difference in the prevalence of PPR between species (χ^2 = 0.823;

Table 1: Prevalence of PPR in study sites

Study sites	No animal examined	No positive	Prevalence%	χ^2	P – value
Amede	80	24	30%		
Bokushenen	128	43	35.6%		
Sefera	97	19	19.6%	8.147	0.043
Soloke	79	30	40%		
Total	**384**	**116**	**30.2%**		

Table 2: Prevalence of PPR based on various risk factors

Risk factors	No animal examined	No positive	Prevalence%	χ^2	P – value
Species					
Goat	205	66	32.1%		
Sheep	179	50	27.9%	0.823	0.364
Sex					
Female	211	60	28.44%		
Male	173	56	32.37%	0.698	0.404
Age					
Young	120	58	48.33%		
Adult	264	58	21.97%	27.198	0.000
B. condition score					
Poor	90	43	47.77%		
Medium	157	38	24.2%	17.274	0.000
Good	137	35	25.55%		

p = 0. 364) and sexes (χ^2 =0.698; p = 0.404). On the other hand, statistical analysis revealed a significant difference in sero-prevalence of PPR between different age groups (χ^2 = 27.198; p = 0. 000) and body condition scores (χ^2 =17.274; p = 0.000) (Table 2).

Viral antigen detection Using RT-PCR

From a total of 100 nasal swabs examined with RT-PCR for detection of PPRV nucleic acid, 42 (42%) samples were positive

Table 3: Results of RT-PCR for detection of PPR viral nucleic acid in suspected field samples

	Nasal swabs(n =100)				
Risk factors	**No animal examined**	**No positive**	**Prevalence%**	χ^2	**P – value**
Goat	57	26	45.6%	0.711	0.399
sheep	43	16	37.2%		
Total	**100**	**42**	**42%**		

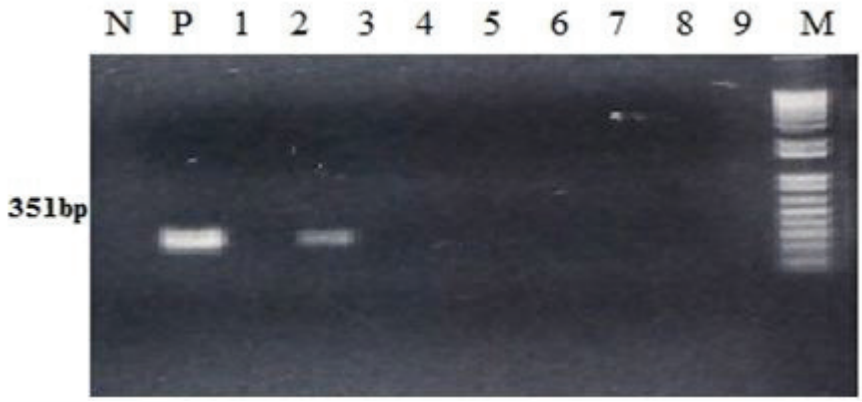

Figure 2: Agarose gel electrophoresis of PCR.

(Table 3). Of the 42 positive cases, 26 and 12 nasal swabs were belongs to sheep and goats, respectively (P>0.05). Figure 2 shows the photograph of the gel electrophoresis of the PCR products that was analyzed. The fragment size of the amplified products was 351bp.

Products (351bp) amplified with NP3 and NP4, PPR specific primers.

Lane M=100bp DNA molecular weight marker; Lane N= Negative control; Lane P= Positive control; Lane 1-9= Field samples.

Discussion

Peste des petits ruminants cause significant economic losses through morbidity, mortality and weight loss in goat and sheep worldwide including Ethiopia. The present study conducted in four sites of Adama district of Oromia region, Ethiopia disclosed an overall sero-prevalence of 30.2% PPR in small ruminants. Similar findings have been reported by other workers in and out of Ethiopia viz;30.5% in Assela , Ethiopia (Megersa *et al.,* 2011) , 28.1% in Eastern Amhara (Biruk, 2014), 30.4 % in Algeria (Karadjadj *et al.,* 2015), 33% in India(Singh *et al.,* 2004), 35.23% in Sindh, Pakistan (Nizamani *et al.,* 2015), 26% in Bangladesh(Banik *et al.,* 2008), 32.8% in India (Balamurugan *et al.,* 2012) and 34.2% in Pakistan (Munir *et al.,* 2012a). However, the result is higher than the findings of Kivaria *et al.,* (2013) 22.1% in Tazania, Özkul *et al.,* (2002) 22.4% in Turkey and Abraham *et al.,* (2005) and Waret-Szkuta *et al.,* (2008) 6.8% and 6.4% in Ethiopia, respectively. On other hand, our result is lower than Berihun *et al.,* (2014), Waret-Szkuta *et al.,* (2008), Bahadar *et al.,* (2009) and Khan *et al.,* (2007) who reported 47.5% in southern Tigray , 52.5% in Dolo Odo, Somalia, Ethiopia, 50% and 43.33% in , Pakistan ,respectively. Such inconsistency in the prevalence rates of PPP could be attributed to the diagnostic test and sampling methods used, the prevalence variability within the population studied, susceptibility of different breeds to the disease and the management practices within deferent geographical regions.

In this study, 32.1% and 27.9% serum samples from goats and sheep were positive for PPR, respectively although the difference was not significant. A higher sero-prevalence in goat as compared to sheep has been reported by several other researchers as well (Awa *et al.,* 2002; Balamurugan et al 2011; Biruk 2014; Farougou et al 2013; Rashid *et al.,* 2008; Shamaki *et al.,* 2004 ;Swai *et al.,* 2009). The higher sero-prevalence in goats than sheep might be due to the fact that new born kids account a large proportion of the goat flock each year which increase the size of susceptible population. On Other hand, our findings are not in accordance with Singh *et al.,* (2004), Abubaker *et al.,* (2011b) and Nizamani *et al.,* (2015) who reported a lower sero-prevalence in goats than sheep. Higher recovery rate in sheep might be due to longevity as a result of which a higher

proportion of sheep shows positive for anti-PPRV antibodies than goats.

This study revealed that PPR sero-prevalence in small ruminants was higher in Soloke (40%) as compared to other study sites (P< 0.05). The difference in sero-prevalence in study sites could be due to variation in small ruminant population, animal health service and the movement of small ruminants from endemic areas (Afar region) for grazing, watering and marketing purpose. On the other hand, this variation could be due to larger numbers of PPR-affected animals' blood samples were collected from highly affected area (Soloke).

The current study revealed a prevalence rate of 32.37% and 28.44% in male and female animals respectively although the difference was not significant(p>0.05).This finding agrees with findings of Osman (2005), Swai *et al.,* (2009) and Sarker and Islam (2011) who reported high sereo-prevalence rates in males than in female animals. This difference could be due to the fact that the high demand of male animals for meat propose driven them to the market and contribute to the higher infection rate than female which maintained at home for breeding purpose and also due to genetic variation of the animals (Sarker and Islam, 2011).In contrast to our finding, Munir *et al.,* (2008) and Mahajan *et al.,* (2012) reported higher sero-prevalence of PPR in females as compared to males. This variation could be related to the sample size and the physiological demand on female in the form of lactation, pregnancy and estrus can increase susceptibility to PPR infection (Susan, 1998).

With regard to age category, the PPR sero-prevalence amongst young animals (48.33%) was much higher than adults (21.97%) with statistical significance (p<0.05). This could be accounted by the fact that the susceptibility of animals to infection by the PPR virus is linked to age (Hilan *et al.,* 2006; Kihu *et al.,* 2015). Young animals (<12 months old) seem more prone to PPR than adults. The high sero-positivity detected in the young stock was likely to be due to maternal antibodies against PPRV and also the increased susceptibility of young animals to PPRV might be due to malnutrition, poor immunity and poor management systems (Sarker and Islam, 2011).This result is not consistent with the findings of Abubakar *et al.,* (2011b) and Tounkara *et al.,* (1996) who noted higher prevalence in adult small ruminants. This age sero-prevalennce discrepancy could be explained as sheep and goats exposed to natural infection to PPRV at a very young age may carry antibodies for 1-2 year following exposure and remains positive for a long time (Singh *et al.,* 2004).

In the present study an attempted made to know whether body condition influence on prevalence of PPR in small ruminants; and it was found that 47.77%, 24.2% and 25% in poor ,medium and good body condition animals, respectively (P<0.05).The high sero-positivity in poor body condition animals likely to be due to immune suppression and malnutrition. Poorly nourished animals are prone to different infectious diseases including PPR.

To confirm the presumptive diagnosis, RT-PCR diagnosing technique was used for the detection of PPR viral antigen in100 suspected animals nasal swab samples. The results showed that 42% of samples were positive for PPRV antigen. This indicates that PPR virus is circulating within and between the small ruminant flocks. This result is comparable to the findings of Luka *et al.,* (2011),Anees *et al.,*(2013) and Biruk (2014) who reported 51.2%, 25% and 46.4% prevalence, respectively by the amplification of the nucleoprotein (N) gene and confirmed the presence of PPRV in clinical samples tested, using a set of primers specific for the F gene of the PPRV.

Conclusion and recommendation

PPR is an economically important viral disease of small ruminants worldwide including Ethiopia; and it is one of the animal diseases whose control is considered important for poverty alleviation in countries like Ethiopia. In the current study, both serological and molecular studies revealed that PPR is circulating between small ruminants in the

study area. In view of this, epidemiological future of PPR should be studied for the better understanding of the problem in the country; and proper and regular vaccination with PPR homologous vaccine that is recommended by the OIE should be implement in high risk areas of the country..

Acknowledgment

Authors wish to thank National Animal Health Diagnostic and Investigation Center (NAHDIC) Sebeta, Ethiopia for providing the kits and necessary facilities to carry out this work.

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SEROPREVALENCE OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN PLATEAU STATE, NORTH-CENTRAL NIGERIA

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Abstract

This survey was undertaken to establish the prevalence of contagious bovine pleuropneumonia (CBPP) in Plateau State, Nigeria by screening 528 cattle for Mycoplasma mycoides subspecies mycoides (Mmm) antibodies using competitive enzyme-linked immunosorbent assay (c-ELISA). Sera samples were collected from 6 randomly selected local government areas (LGAs) of the State over a 14-month period (May, 2013 – June, 2014). Results showed an overall CBPP seroprevalence of 14.39% for the State. Antibodies to Mmm were detected in all the LGAs sampled with prevalence ranging from 7.50% to 31.58%, indicating a significant association ($P<0.05$). Seasons ($P<0.05$, $\chi 2=34.00$) and sample collection points ($P<0.05$, $\chi 2=8.848$) were also significantly associated with the seroprevalence of CBPP. There was however, no significant association ($P>0.05$, $\chi 2=0.47$) between the sex of cattle and seroprevalence of CBPP. This study confirms that CBPP is widespread, and the findings could serve as a baseline for initiation of effective control programmes against the disease to improve cattle health and production in the State.

Key words: Contagious bovine pleuropneumonia, seroprevalence, Plateau State.

SÉROPRÉVALENCE DE LA PLEUROPNEUMONIE CONTAGIEUSE BOVINE DANS L'ETAT DU PLATEAU DANS LE NORD-CENTRE DU NIGERIA

Résumé

La présente étude a été réalisée dans le but de déterminer la prévalence de la pleuropneumonie contagieuse bovine (PPCB) dans l'État du Plateau au Nigeria en examinant 528 bovins pour rechercher la présence des anticorps de Mycoplasma mycoides sous-espèce mycoides (Mmm) par dosage immuno-enzymatique de compétition (c-ELISA). Des échantillons de sérums ont été prélevés dans 6 zones de gouvernement local (LGA : Local Government area) sélectionnées de manière aléatoire dans cet État sur une période de 14 mois (mai 2013-juin 2014). Les résultats ont montré une séroprévalence globale de PPCB de 14,39% pour cet État. Les anticorps contre Mmm ont été détectés dans toutes les LGA échantillonnées, avec une prévalence allant de 7,50% à 31,58%, ce qui fait penser à l'existence d'une association significative ($P <0,05$). Les saisons ($P <0,05$, $\chi 2 = 34,00$) et les points de collecte des échantillons ($P <0,05$, $\chi 2 = 8,848$) ont également été significativement associés à la séroprévalence de la PPCB. Cependant, on n'a noté aucune association significative ($P> 0,05$, $\chi 2 = 0,47$) entre le sexe des bovins et la séroprévalence de la PPCB. Cette étude confirme que la PPCB est répandue et que les résultats pourraient servir de référence pour le lancement de programmes efficaces de lutte contre la maladie afin d'améliorer la santé et la production des bovins dans cet État du Nigéria.

Mots-clés : pleuropneumonie bovine contagieuse, séroprévalence, État du Plateau.

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Introduction

Contagious bovine pleuropneumonia (CBPP), caused by Mycoplasma mycoides subspecies mycoides (Mmm), is a debilitating and highly contagious disease of cattle manifested by anorexia, fever, and severe respiratory signs such as dyspnoea, polypnoea, cough and nasal discharge (Lefevre, 2000). The disease is a serious threat and impediment to livestock production and development in Sub-Saharan Africa, and some Asian countries (Windsor, 2000; March *et al.,* 2003). This is due to cattle mortality, weight loss, reduced fertility, organ condemnation, and cost of treatment and vaccination programmes (Amanfu, 2009; Dedieu *et al.,* 2010), with affected countries being excluded from international trade (OIE, 2014). In Nigeria, estimated CBPP morbidity and mortality rates of 50% and 25% respectively have been documented, with economic analyses estimating its current annual financial burden to be N2.2 billion (Fadiga *et al.,* 2011).

Despite vaccination campaigns in Nigeria, CBPP continues to occur with increased frequency leading to heavy losses (Aliyu *et al.,* 2000). The occurrence of CBPP in all agro-ecological zones is also a powerful reminder that the disease is still a long way from being eradicated in Nigeria (Tambuwal, 2009; Egwu *et al.,* 2012). This is thought to be as a result of several factors including absent or improperly implemented control programs, poor vaccination coverage (9.7-38.6%) of several localities due to inadequate vaccine supplies and constraints in field mobility and support funds, lack of vaccine efficacy, ignorance of farmers, poor management systems (Aliyu *et al.,* 2000; Fadiga *et al.,* 2011; Tambuwal *et al.,* 2011), and reduced disease surveillance in the field, abattoir and the laboratory (Amanfu, 2009; FDL, 2010). Other factors include ethnic and intertribal wars in recent times, as well as insurgency in some States in the Northern part of the country which resulted in the displacement of people and their livestock creating difficulty for mass vaccination, and increasing the distribution of CBPP across many parts of the country in addition to introduction of the disease to naïve areas (Ankeli, 2015).

The control of CBPP is therefore urgent (Kairu-Wanyoike *et al.,* 2014), which requires not only strict cattle movement control, local quarantine of affected herds and vaccination using potent live attenuated strains (Vilei and Frey, 2010), but also its diagnosis in live cattle and identification of causal agent in tissues using reliable, specific and sensitive diagnostic strategies (Aliyu *et al.,* 2003; Mbulu *et al.,* 2004; Bischof *et al.,* 2009). Serological diagnosis plays a key role in survey and control programmes to combat CBPP (Bruderer *et al.,* 2002), better suited for large scale disease monitoring especially in Africa, where PCR-based methods are not always practical (Nicholas *et al.,* 2000). Serological tests recommended for CBPP screening and eradication programmes are the complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA) (OIE, 2014). Other serological tests employed in CBPP diagnosis include the immunoblotting test (IBT) and the rapid latex agglutination tests (LATs). The IBT is highly specific and the most sensitive serological test so far described for CBPP, although not suitable for mass screening (OIE, 2014). The LAT gives results in two minutes using serum or whole blood, and has been used in the on-field diagnosis of CBPP in Nigeria (Okaiyeto *et al.,* 2011).

Although CBPP has been recognized in Nigeria for many years, and the incidence reported to be very high in the North-Eastern parts of the country (Aliyu *et al.,* 2003), sufficient information is needed on the epizootiology of the disease in other parts of the country (Fadiga *et al.,* 2011). Reports on the prevalence of CBPP in Plateau State are not thorough thus the aim of this study, which is to establish the seroprevalence of CBPP in the State.

Materials and Methods

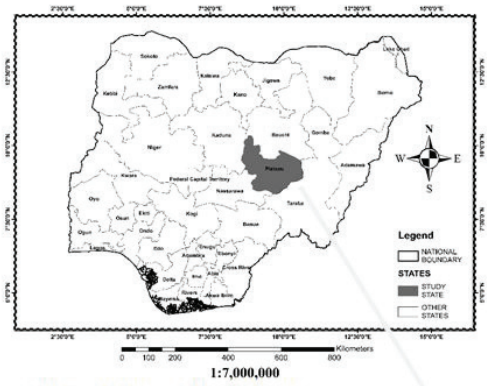
Study area

Plateau State is located in North-Central Nigeria and lies between Latitudes 8° and 10°N and Longitudes 7° and 11°E (Table 1). The State occupies 26,899 square kilometres

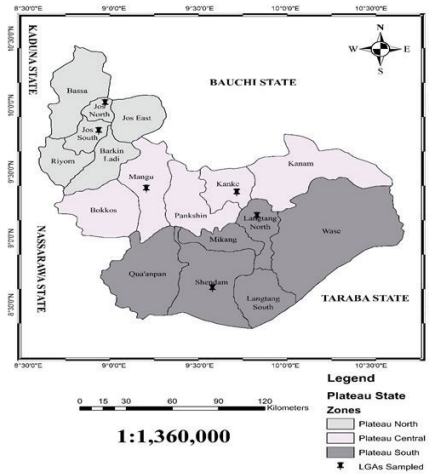
and shares common boundaries with four of the thirty-six States of the Federation. The State has 17 Local Government Areas (LGAs) spread across 3 zones; North with 6 LGAs, Central with 5 LGAs, and the South with 6 LGAs. There are two seasons, the dry (October to April) and wet (May to September). Cattle are a major source of meat supply in the State, which has an estimated cattle population of 1.07 million (www.plateaustate.gov.ng).

Sample size determination, sampling method and serum collection

A sample size of 146 sera was determined based on the formula by Thrusfield (2009) for simple random sampling method at 95% CI and 5% absolute precision with expected prevalence of 10.65% (Olabode *et al.,* 2013). A total of 528 samples were collected to allow for reasonable distribution of samples across the State and to also increase the precision of the study. Two (2) local government areas (LGAs) were randomly selected from each of the three (3) zones of the State, giving a total of six (6) LGAs sampled. One slaughter slab and cattle market in each LGA were randomly selected for this study. Additionally, one agro-pastoralist herd was also randomly selected per LGA for each visit. Only cattle above or at least a year old were included in this study and a tenth (10%) of this population, in each sample collection point, were randomly selected per visit.



MAP OF NIGERIA: STATES AND THE FCT
Source: GIS Laboratory, Department of Geography and Planning, University of Jos, Nigeria



MAP OF PLATEAU STATE SHOWING STUDY AREAS
Source: GIS Laboratory, Department of Geography and Planning, University of Jos, Nigeria.

Each LGA was visited at least four (n=4) times during the study period. The date, location, sex, and vaccination history (where available) of cattle were recorded during sample collection. For serum collection in cattle markets and agro-pastoralist herds, five (5) ml of blood was aseptically collected from the jugular vein using sterile 10 ml syringe and 18G hypodermic needle. In slaughter slabs, blood samples (5 ml each) were collected at slaughter into tubes, plugged and kept slanted. The blood samples were allowed to clot and sera obtained were decanted into serum bottles, transported to the laboratory and stored at -20°C until serological testing was carried out.

Serological examination

Serum samples (n=528) were assayed using the CBPP serum competition ELISA Kit® (Institut Pourquier, Montpellier, France). The test was carried out according to the protocol and recommendations of the manufacturer. The c-ELISA measures the percentage of inhibition (PI) of immunoglobulin G (IgG) in serum put into competition with a monoclonal antibody (MAb) that is a very specific epitope to Mmm (Séry *et al.,* 2014). The tests were interpreted thus:

- Sera with percentage inhibition (PI) equal to or lower than 40% were considered negative
- Sera with PI between 40 and 50% were considered doubtful and repeated. If they still remained doubtful, they were then considered negative.
- Sera with PI equal to or greater than 50% were considered positive

Statistical Analysis

Data obtained were presented as tables. Chi-square was used to test for association between CBPP prevalence and LGAs, seasons, sampling sites, sex with the aid of Epi-Info (version 7.0.) and values of $P<0.05$ were considered significant. Prevalence was determined using the equation below:

$$\text{Prevalence} = \frac{\text{Positive samples}}{\text{Total number examined}} \times 100$$

Results

The detection of Mmm antibodies using c-ELISA revealed 76 of the 528 serum samples positive giving an overall CBPP seroprevalence of 14.39%. The seroprevalence of CBPP based on LGAs were as follows; Jos North had the highest prevalence of 31.58% (18/57), followed by Shendam with 27.85% (22/79), Langtang North with 10.34% (6/58), Jos South with 9.84% (18/183), Mangu 8.45% (6/71). The least seroprevalence, 7.50% (6/80) was recorded in Kanke. There was a significant association ($P < 0.05$) between seroprevalence of CBPP and the LGAs sampled.

The seroprevalence of CBPP in cattle was found to be higher in the dry season with 23.74% (61/257) than the wet season with 6.64% (18/271) and the association was statistically significant ($P < 0.05$).

The seroprevalence of CBPP varied across different sample collection sites. The highest seroprevalence was recorded in slaughter slabs with 19.40% (39/201), followed by cattle markets with 14.96% (19/127) and lowest in cattle herds with 9.00% (18/200). The association between CBPP seroprevalence and sampling sites was statistically significant ($P < 0.05$).

Sex specific prevalence showed that bulls had higher seroprevalence of 15.43% (48/311) compared to cows with 12.90% (28/217). There was however, no significant association ($P > 0.05$) between the sex of cattle and the seroprevalence of CBPP (Table 1).

Discussion

Contagious bovine pleuropneumonia causes high morbidity and mortality losses, the financial implications which are of great significance to the cattle industry of affected nations (Tambi *et al.,* 2006). Even though CBPP is present in Plateau State (Chima *et al.,* 1999; Molokwu *et al.,* 2004; Aliyu and Kyari, 2005; Nwankpa *et al.,* 2010; Ankeli *et al.,* 2016), there has been no comprehensive study devoted to establish its serological prevalence in the State. From available reports, previous

Table 1: Prevalence and association of CBPP in cattle from Plateau State using c-ELISA

Variables	LGA	Number examined	Number positive	Prevalence (%)	Odds ratio	95% confidence interval	P-value	$\chi 2$
LGAs	Jos North	57	18	31.58	4.23	2.02-8.87	0.0001	34.24
	Jos South	183	18	9.84	1.00	0.50-1.99		
	Kanke	80	6	7.50	0.74	0.28-1.95		
	Mangu	71	6	8.45	0.85	0.32-2.23		
	Langtang North	58	6	10.34	1.06	0.40-2.80		
	Shendam	79	22	27.85	3.54	1.77-7.07		
Season	Dry	257	61	23.74	5.31	2.93-9.63	0.0001	34.00
	Wet	271	15	5.54				
Sampling site	Slaughter slabs	201	39	19.40	2.43	1.34-4.42	0.0120	8.848
	Cattle markets	127	19	14.96	1.78	0.89-3.54		
	Cattle farms	200	18	9.00	1.00	0.50-1.98		
Sex	Bulls	311	48	15.43	0.82	0.49-1.34	0.49	0.47
	Cows	217	28	12.90				
Overall		528	76	14.39				

serological studies on CBPP in the State have been based on monitoring of antibody levels in challenged and vaccinated cattle, clinical specimens submitted for routine diagnosis, and investigation of suspected CBPP outbreaks from which a 14.11% serological prevalence was estimated (Nwankpa *et al.,* 2004).

Our study revealed a 14.39% CBPP seroprevalence for Plateau State and also detected Mmm antibodies in cattle from all six LGAs sampled, indicating that CBPP is endemic in the State. The high seroprevalence recorded in this study is probably the result of breakdown in veterinary services, antibiotic usage by farmers to control the disease leading to the establishment of chronic carriers, coupled with transhumance leading to the spread of the disease, as well as vaccination failures (Vilei and Frey, 2010; Tambuwal *et al.,* 2011). It should be stated that although vaccination history was not available in all visited sites (especially in slaughter slabs), the Mmm antibodies detected in this study are likely due to natural infection as vaccination with T1/44 strain does not always induce detectable antibody responses. When produced, T1/44 antibodies do not persist after 3 months, thus the reason why c-ELISA is capable of detecting field infections even in areas where vaccination is practiced (Le Goff and Thiaucourt, 1998).

The seroprevalence of 14.0% obtained in our study is similar to that reported by Alhaji and Babalobi (2016) in Niger State, North-Central Nigeria. However, contrary to expectations, the figure obtained in this study also equalled that of Nwankpa et al. (2004) which may be attributed to delays in investigating disease outbreaks due to lack of mobilisation of funds and resources. In such cases, affected cattle would have been salvaged before samples are collected.

The finding in this study is higher than the 10.65% seroprevalence reported by Olabode et al. (2013), and earlier reports on prevalence rates for condemned lungs in abattoirs due to CBPP (Fayomi and Aliyu, 1997;

Halle *et al.,* 1998; Onu, 2004) in other parts of Nigeria. The higher prevalence reported in this study may indicate the endemicity of CBPP in Nigeria and Plateau State in particular. Nwanta and Umoh (1992), Aliyu et al. (2000) and Egwu et al. (2012) made similar observations in other states of Northern Nigeria. This finding also supports the observations of Dupuy et al. (2012) and Séry et al. (2014) on the spread of CBPP in other endemic areas of Africa. On the other hand, the reports of Aliyu et al. (2003) and Suleiman et al. (2015) observed much higher seroprevalences of 32% and 30.2% respectively, based on c-ELISA. The higher prevalence reported by Aliyu et al. (2003) could be attributed to the endemicity of CBPP in North-East Nigeria and the non-random nature of sample selection in abattoirs and slaughter slabs such that, the high estimates could be influenced by sick animals being sent for slaughter. The higher estimates reported by Suleiman et al. (2015), on the other hand, could probably be from bias as posited by the authors.

The findings in this study strengthen previous reports from several African countries which showed the presence of Mmm infection in cattle. Wade et al. (2015) reported a 3.4% Mmm positivity in slaughtered cattle in Northern Cameroun using polymerase chain reaction (PCR). In Ethiopia, Teklue et al. (2015) reported a CBPP seroprevalence of 11.9% in the north using CFT, while herd seroprevalence of 4.6% in the Highlands using c-ELISA was reported by Bonnet et al. (2005). In Kenya, prevalences were 11.2% and 4.2% using c-ELISA and CFT, respectively (Mtui-Malamsha, 2009). Recent studies in the West African sub-region have reported national seroprevalences of 0.54 % for Senegal (Mbengue *et al.,* 2013) and 18.11% for Mali (Séry *et al.,* 2014), respectively.

Based on LGAs sampled, the seroprevalence of CBPP in cattle was highest in Jos North and Shendam compared to other LGAs. This might be due to the high demand of their large populations for beef, and the commercial and administrative activities in these areas. Jos North also has a boundary with Bauchi State, which is regarded as CBPP-endemic (Onu, 2004), and boasts of a very vibrant cattle market that receives a large number of cattle from most States of North-East Nigeria. Shendam LGA recorded the second highest seroprevalence which could be attributed to it having the third largest cattle market in the State that receives cattle from most of the southern LGAs of the State and from neighbouring States of the CBPP-endemic North-East Nigeria. During the period of this study, there were incessant crises between farmers and nomadic herdsmen, which could have influenced migration of animals from the areas that showed low seroprevalence of the disease.

The seroprevalence of CBPP in cattle in the dry season was higher compared to the rainy season. This corroborates the observations of Baluka et al. (2013) who suggested the dry season as an important risk factor associated with CBPP and other animal diseases outbreaks. Dry seasons could probably be the cause of the increased and uncontrolled cattle movements that precede many CBPP outbreaks. Consequently, outbreaks of infectious animal diseases occur regularly as a result of increased movements (Fevre *et al.,* 2006), with deleterious outcome. Of the over 17 million cattle in Nigeria, 90 percent are traditionally managed and it is generally accepted that animals kept under pastoral systems are continuously exposed to the risk of contracting infectious diseases such as CBPP, especially during the dry season because of extensive mixing in grazing and watering areas (Mariner *et al.,* 2006; Mtui-Malamsha, 2009; Alhaji and Babalobi, 2016). Also, the shortage of forage and water, coupled with the extra distances cattle cover to get to grazing and watering points during the dry season may perhaps induce stress thus predisposing cattle to CBPP and other diseases.

The highest seroprevalence in slaughter slabs, compared to cattle markets and herds in this study could also be influenced by sick animals being sent for slaughter. This too is in agreement with Aliyu et al. (2003) and Egwu et al. (2012) who reported sero- and lesion-based prevalences respectively higher in slaughter

slabs compared to that obtained from this present study which, in addition to slaughter slabs, included cattle markets and herds. The higher seroprevalence of CBPP in cattle markets compared to herds could also be as a result of the tendency of herd owners to sell off sick and unproductive cattle to minimize losses and meet financial demands. It can also be argued that cattle retained in herds for breeding, fattening, dairy and other production purposes get better veterinary care compared to those intended for immediate sale or slaughter.

The findings of this study also revealed a higher seroprevalence in bulls compared to cows however, there was no significant association within sex of cattle. This is in agreement with the reports of Egwu et al. (2012) who observed no significant sex predisposition to the development of CBPP lesions, and Teklue et al. (2015) who suggested similar exposure of both sexes to CBPP as reasons, respectively. Séry et al. (2014) and Gumel et al. (2015), on the contrary, have reported significantly higher prevalences of CBPP in cows than in bulls. This difference was attributed by the former to the fact that bulls are sold off while young compared to females that are retained in herds for production purposes and in enzootic areas, older animals (more of them cows) are more likely to have had repeated exposure to the pathogen resulting in infection. Gumel et al. (2015), in contrast, ascribed their finding to reproductive stresses that predispose cows to infectious diseases.

Conclusion

This serological study further confirms the continued presence of CBPP in Plateau State. The results also indicate that CBPP is widespread, and cattle across the State are at risk of being infected with the organism. This serological survey determined the prevalence of CBPP in the State and the findings will provide valuable information upon which policy framework on the control and prevention strategies of the disease could be adopted.

Acknowledgement

The authors would like to thank the National Veterinary Research Institute, Vom for funding this project, and Chimezie Ogbonnah, Sunday Kigbu and Rosemary Goji for their technical support.

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FARM ANIMAL HEALTH MANAGEMENTS AND TREATMENT PRACTICES AT DIGA DISTRICT, EAST WOLLEGA ZONE OF OROMIA REGIONAL STATE

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Abstract

Farm animal health problems with respective management, local knowledge and treatment options with prognosis were assessed in Diga district, Wollega-Oromia, Ethiopia from November 2014. Randomly selected farm animals with clinical cases being cattle (n=175), sheep (n=74), equine (n=38), poultry (n=27) and goat (n=3) presented at the clinic were studied. Animal sex, age, species and body condition were considered. Cases were characterized and determined using tentative diagnosis and patho-gnomonic signs. The owners were also interviewed for local name of the disease. The respective therapeutic drugs used were also assessed. Male cattle (57.7%), poultry (76.3%) and goat (66.7) were frequently presented to the Veterinary clinic than respective female while the reverse is true in sheep and equine. In total, the cases of gastrointestinal (GIT) (29.7%), skin and integuments (22.1%), cardiovascular (18.0%), respiratory diseases (14.2%), production problems (5.0%), musculoskeletal (4.7%), neuromuscular (3.8%) and reproductive system (2.5%) were observed in increasing order. With regards to specific diseases, trypanosomes (23.4%), lumpy skin disease (13.7%), black-leg (8.6%), babesiosis (7.4%), mangemites (6.9%), fasciolosis (5.7%), mastitis (4.6%), GIT parasites (4.6%), CBPP (4.0%) and tick infestation (3.4%) in cattle; fasciolosis (21.6%), pneumonia(13.5%), orf and pasteurellosis (8.1%), and mangemites, GIT parasites, CCPP, hypocalcemia each (6.8%) in sheep; orf (33.3%) and CCPP (33.3%) in goat were observed. Colic (31.6%), tetanus (26.9%), wound (18.4%) and pneumonic (10.5%) were frequent in equines while coccidian (55.6%) and NCD (40.7%) in poultry. Most of the cases and diseases are also known with local language. Oxyteteracycline, penstreap, deminazin aceturat and albendazole were frequently used drugs for the treatment of the aforementioned farm animal health problems with good (86.8%) but 13.2% poor prognoses. Discussion of the finding on health problems in this article was made for respective farm animal. The survey indicated infectious diseases, parasitic cases, and nutritional deficiency and management problems as major heath constraints for livestock development in the area. Thus, a need for expansion of quality Veterinary services and improvement in the feeding system with detail study on specific disease on each groups of farm animal in the area.

Key Words: Animal health, Diga, Veterinary Clinic, Prognosis

PRATIQUES DE GESTION DE LA SANTÉ ANIMALE ET DE TRAITEMENT DANS LE DISTRICT DE DIGA DE LA RÉGION DE WOLLEGA EST DANS L'ÉTAT RÉGIONAL D'OROMIA

Résumé

Les problèmes de santé des animaux d'élevage, ensemble avec les pratiques de gestion, les connaissances locales et les options de traitement avec pronostic ont fait l'objet d'évaluation dans le district de Diga de la région Wollega-Oromia en Éthiopie, à partir de novembre 2014. Des animaux de ferme sélectionnés de manière aléatoire, dont les cas cliniques comprenaient des bovins (n = 175), des ovins (n = 74), des équidés (n = 38), des volailles (n = 27) et des caprins (n = 3), présentés à la clinique, ont fait l'objet d'une étude. Le sexe, l'âge, l'espèce et l'état corporel des animaux ont été pris en considération. Les cas ont été caractérisés et déterminés grâce au diagnostic présomptif et aux signes pathognomoniques. Les propriétaires ont également été interviewés afin d'identifier le nom local de la maladie. Les divers médicaments thérapeutiques utilisés ont également été évalués. Les bovins mâles (57,7%), les volailles

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(76,3%) et les caprins (66,7) ont été fréquemment présentés à la clinique vétérinaire par rapport aux femelles respectives, tandis que l'inverse était vrai chez les ovins et les équidés. Au total, les cas de maladies gastro-intestinales (GIT) (29,7%), de la peau et des téguments (22,1%), de maladies cardiovasculaires (18,0%), de maladies respiratoires (14,2%), les problèmes de production (5,0%), du système musculo-squelettique (4,7%), neuromusculaire (3,8%) et reproducteur (2,5%) ont été observés dans l'ordre croissant. En ce qui concerne les maladies spécifiques, les trypanosomes (23,4%), la dermatose nodulaire contagieuse (13,7%), le charbon symptomatique (8,6%), la babésiose (7,4%), les acariens de la gale (6,9%), la fasciolose (5,7%), la mastite (4,6%), les parasites GIT (4,6%), la PPCB (4,0%) et l'infestation par les tiques (3,4%) chez les bovins ; la fasciolose (21,6%), la pneumonie (13,5%), orf et la pasteurellose (8,1%), les acariens de la gale, les parasites GIT, la PPCC, l'hypocalcémie (6,8%) chez les moutons ; orf (33,3%) et la PPCC (33,3%) chez les caprins ont été observés. Les coliques (31,6%), le tétanos (26,9%), les plaies (18,4%) et la pneumonie (10,5%) étaient fréquentes chez les équidés, tandis que les coccidiens (55,6%) et la MNC (40,7%) étaient fréquents chez les volailles. La plupart des cas et des maladies sont également connus dans la langue locale. L'oxytétracicine, le penstreap, l'acétate de déminazine et l'albendazole étaient les médicaments fréquemment utilisés pour le traitement des problèmes de santé des animaux de ferme susmentionnés avec 86,8% de bons pronostic et 13,2% de mauvais pronostic. La discussion des résultats sur les problèmes de santé dans cet article a été faite pour les divers animaux de ferme. L'enquête a révélé des maladies infectieuses, des cas parasitiques et des problèmes de déficience nutritionnelle et de gestion en tant que principales contraintes sanitaires au développement de l'élevage dans la région. Ainsi, il y a un besoin d'expansion des services vétérinaires de bonne qualité et d'amélioration du système d'alimentation avec des études détaillées sur les maladies spécifiques pour chaque groupe d'animaux de ferme dans la région.

Mots-clés : santé animale, Diga, clinique vétérinaire, pronostic

Introduction

Animal production is the main component of agricultural development in most parts of sub-Saharan Africa including Ethiopian rural, peri urban and urban community being the first in Africa and 10th in the world with an estimates of 44.3 million heads of cattle, 23.6 million sheep and 23.3 million goats (CSA, 2004). They constitute for traction power and income as well as act as source of milk and meat (Mekonnen *et al.,* 1989). Despite huge number of livestock, the sector could not impart the expected significant role in the country economy development and food self-sufficiency (Mukasa-Mugerwa, 1998) either due to the low genetic potential of indigenous, poor nutrition and reproductive performance, inadequate management, high disease incidence and parasite burden (Assegid, 2000; Ndikima *et al.,* 2000).

The diseases have numerous impacts on productivity; fertility and performance which resulted in morbidity, poor fertility and decrease power output, in sever case, mortality (CACC, 2003). The major predisposing factors for the diseases in tropical areas are environmental factors like temperature and humidity, topography of the area for easy occurrence of soil born diseases, stress conditions and drought which resulted in limited feed availability with low vegetation coverage. Palling and Dwinger (1993) and (Bennett and Ljpelar (2005) showed parasitic cases as the major constraints to livestock production in the humid and sub-humid portions. Poor, even absence of high quality animal health services with insufficient area coverage aggravated the situation in the country (Assegid, 2000).

Knowing the type and extent of major health problems is very important to the livestock owners, veterinarians and researchers which can assist in the development of heard health strategies and with selection of possible interventions (Radostitis *et al.,* 1994; Ndikima, 2000). Clinical diseases of farm animals were assessed by Alemu and Zegey (2011) at Gondar and Haftu et al. (2014) at Eastern Zone of Tigray, Ethiopia. However, such livestock health information with the knowledge of local community about the cases was not yet assesses in the Diga District, East Wollega

Zone of Oromia Regional State, Ethiopia. The study was aimed to assess farm animal health problems with the respective management, local knowledge and treatment options with prognosis as well as the prevailing indigenous knowledge of local community on farm animals' health problems at Diga District.

Materials and Method

Description of study area

Study was carried out at Diga district located at 343 km from Addis Ababa, the Capital City, Eest Wollega Zone of Oromia Regional state, Ethiopia. The study area has an altitude range of 1380-2300 meters above sea level and receiving an average annual rain fall of 1416 mm. The temperature range is 14.6-30.4°C with average of 22.5°c. With regards to agro-climate, 58% and 42% of district are low land and mid-altitude respectively. It have a mixed (crop-livestock production) farming systems. According to Central Statistic Authority (2009), livestock were kept by individual farmers and with estimated population of 57,586 cattle, 11, 220 sheep, 6,091 goats, 136 horses, 46 mules, 2,948 donkeys and 31, 241 poultry.

Study animal

Study was conducted on randomly selected farm animals of all type presented for health problems at Diga Veterinary Clinic. The sex, age, breed and treatment given to them were registered. Accordingly, most frequently presented animals for health cases at the clinic were cattle, sheep, goat, equines and poultry.

Animal health services at Diga district

The district have 10 veterinary clinics grouped into three groups based on the size, facilities, cervices plan, number of animal handled, diagnostic facilities and treatment drug availability criteria. These are two "types-C", three "type D", and five "health posts" clinics according to descending order of aforementioned criteria. There are 17 veterinarians in this district which include one Doctor of Veterinary Medicine and 16 animal health assistant. History taking and do physically-clinical examination for general health parameters (heart beat and temperature and respiratory rate) are major diagnostic techniques used. All animals treated were recorded with respective case history, clinical signs, clinical findings and the

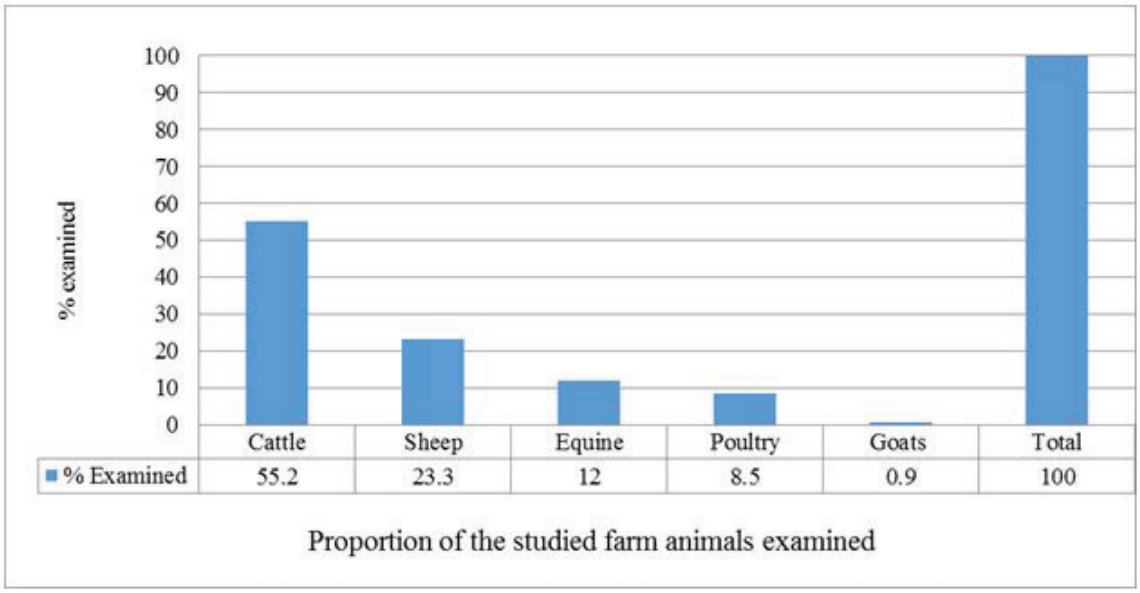

Fig. 1: Frequency of studied farm animals presented at study clinic

provided treatment. Diminazene aceturate, oxtetracycline, penstipe, pencilline, albendazole, and ivermectin are the most frequently available and used drugs in that clinic. Follow-up of the treated animal were made to asses prognosis of the cases.

Study design and Sample size calculation

The study sample size was calculated using 29.16% expected diseases occurrence (MoA, 2012) at 95% confidence interval with 0.05 precision using formulas set by Thrusfield (2007). Thus, a total of 317 animals presented for clinical cases including 175 cattle, 74 sheep, 3 goats, 38 equines and 27 poultry were examined from November 2014 –March 2015. While data collection, types of farm animals with respective sex, age, breed body conditions and months of the cases were considered. Using case history and physical-clinical examination for general health parameters, the health problems were categorized in to the animal system affected, possible general causes, and specific common cause with respective treatment used. The owners were immediately interviewed for local name of the respective case. Prognoses of all treated study cases were also assessed.

Data Analysis

Considering all variables of study, data was stored in to excel 2007© spread sheet (MS Excel) and imputed to be analyzed by IBM SPSS 20 and WinPepi Version 11.35 software Using ANOVA, Odds ratio (OR) and 95% OR confidence interval (CI) were calculated for $p < 0.05$ computing significance of association.

Results

The frequency of major farm animals with health problems, and presented at study clinical were shown in Fig. 1 bellow in the decreasing order which cattle are most (55.2%) but goats are less (0.9%) frequent.

As indicated in Table 1, the male cattle (57.7%), poultry (76.3%) and goat (66.7%) were frequently presented to the clinic than respective female while the reverse is true in sheep and equine. Adult and local breed farm animals were frequently present for health

Table 1: Studied animals presented to clinic by sex, breed, body condition and month of the year

Parameters of study		Cattle (n=175)	Sheep (n=74)	Equine (n=38)	Poultry (n=27)	Goats (n=3)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Sex	Female	74 (42.3)	46 (62.2)	22 (81.5)	9 (23.7)	1 (33.3)
	Male	101 (57.7)	28 (37.8)	5 (18.5)	29 (76.3)	2 (66.7)
Age	<6 month	13 (7.4)	6 (8.1)	12 (44.4)	-	-
	6 month -2 years	148 (84.6)	63 (85.1)	15 (55.6)	35 (92.1)	3 (100)
	>2 years	14 (8.0)	5 (6.8)	-	3 (7.9)	-
Breed	Cross*	12 (6.9)	1 (1.4)	17 (63.0)	-	-
	Local	163 (93.1)	73 (98.6)	10 (37.0)	38 (100)	3 (100)
Body condition	Good	19 (10.9)	10 (13.5)	-	-	2 (66.7)
	Moderate	98 (56.0)	35 (47.3)	25 (92.6)	25 (65.8)	1 (33.3)
	Poor	58 (33.1)	29 (39.2)	2 (7.4)	13 (34.2)	-
Month	November	25 (14.3)	10 (13.5)	3 (11.1)	6 (15.8)	1 (33.3)
	December	33 (18.9)	9 (12.2)	1 (3.7)	3 (7.9)	-
	January	41 (23.4)	22 (29.7)	7 (25.9)	11 (28.9)	2 (66.7)
	February	47 (26.9)	17 (23.0)	9 (33.3)	13 (34.2)	-
	March	29 (16.6)	16 (21.6)	7 (25.9)	5 (13.2)	-

*n= number of animal with cases presented to the clinic; *exotic for poultry*

Table 2: Major cases encountered in examined 317 farm animals by system affected and general causes of the disease in the studied area

	Health problems encountered	No. (%)	OR	95% OR CI*
System affected	GIT Diseases	94 (29.7)	16.3	7.7-34.1[A]
	Cardiovascular cases	57 (18.0)	8.5	3.9-18.0
	Musculoskeletal	15 (4.7)	1.8	0.8-4.3[a,b]
	Neuromuscular	12 (3.8)	1.5	0.6-3.7[a,b]
	Production diseases	16 (5.0)	2.1	0.8-5.6[a]
	Reproductive system cases	8 (2.5)	1	0.3-3.1[a,b]
	Respiratory Disease	45 (14.2)	6.4	3.2-15.9
	Skin and integuments	70 (22.1)	10.9	5.1-26.8[B]
General causes of disease	Farm animal management problems	5 (1.6)	1	0.2-4.4[c]
	Bacterial causes	56 (17.7)	13.4	5.3-43.3[C]
	Ectoparasitic	31 (9.8)	6.7	2.5-22.5
	Fetal oversize	1 (0.3)	1	0.01-78.7
	Fungal causes	1 (0.3)	1	0.01-78.7
	GIT parasites / helminthes	39 (12.3)	8.7	3.4-28.8
	Metabolic diseases	16 (5.0)	1	0.2-4.4[c]
	Miscellaneous	53 (16.7)	12.5	4.9-40.6[C]
	Protozoal causes	72 (22.7)	14.4	5.9-35.1[C]
	Viral causes	43 (13.6)	8.6	3.4-20.9

**OR= Odds ratio; A is significantly higher than a; B is significantly higher than b; C is significantly higher than c*

Table 3: Studied animals with systemic diseases and the general causes

System affected and possible causes of disease		Cattle (n=175)	Sheep (n=74)	Equine (n=38)	Poultry (n=27)	Goats (n=3)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Animal system affected	GIT Diseases	28 (16.0)	27 (36.5)	12 (31.6)	26 (96.3)	1 (33.3)
	Cardiovascular cases	54 (30.9)	3 (4.1)	-	-	-
	Musculoskeletal	15 (8.6)	-	11 (28.9)		-
	Neuromuscular	1 (0.6)	-	-	-	-
	Production cases	11 (6.3)	5 (6.8)	-	-	1 (33.3)
	Reproductive system cases	6 (3.4)	1 (1.4)	-	-	-
	Respiratory Diseases	13 (7.4)	23 (31.1)	7 (18.4)	1 (3.7)	1 (33.3)
	Skin and integuments	47 (26.9)	15 (20.3)	8 (21.1)	-	-

System affected and possible causes of disease		Cattle (n=175)	Sheep (n=74)	Equine (n=38)	Poultry (n=27)	Goats (n=3)
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
General causes of disease	Animal Management	3 (1.7)	1 (1.4)	-	-	1 (33.3)
	Bacterial causes	28 (16.0)	11 (14.9)	16 (42.1)	-	1 (33.3)
	Ectoparasitic	20 (11.4)	11 (14.9)	-	-	-
	Fetal over size	1 (0.6)	-	-	-	-
	Fungal causes	1 (0.6)	-	-	-	-
	GIT parasite	18 (10.3)	21 (28.4)	-	-	-
	Metabolic diseases	11 (6.3)	5 (6.8)	-	-	-
	Miscellaneous	15 (8.6)	15 (20.3)	22 (57.9)	1 (3.7)	-
	Protozoal causes*	54 (30.9)	3 (4.1)	-	15 (55.6)	-
	Viral causes	24 (13.7)	7 (9.5)	-	11 (40.7)	1 (33.3)

**coccidian in cases of poultry*

cases. During the months of November and January, cases of all farm animals are area presented to the clinic.

Based upon the animal system affected and general causes of the disease condition, animal health problems encountered were categorized and classification (Table 2). The GIT diseases (29.7%) and skin and integuments cases (22.2%), cardiovascular (18.0%), neuromuscular (3.8%) and reproductive (2.5%) systems cases were observed in descending orders. With regards to classes of the diseases, protozoal cases were leading (22.7%) followed by bacterial diseases (17.7%) and viral (13.6%). Fungal (0.3%) and fetal oversize (0.3%) were also observed (Table 2).

Of farm animal system affected, the GIT and respiratory diseases were commonly observed among all studied farm animals. Cardiovascular cases (30.9%) were frequent in cattle while GIT diseases in sheep (36.5%), equine (31.6%) and poultry (96.3%). With regards to major causes of health problems, protozoal cases (30.9%) in cattle and (55.6%) in poultry, and miscellaneous causes (20.3%) in sheep and (57.9%) in equine were frequently observed (Table 3).

Regarding specific health problem, trypanosomoses (23.4%), lumpy skin disease (13.7%), blackleg (8.6%) and babesiosis (7.4%) in cattle, Contagious Caprine Pleuropnemonia (CCPP) and orf each 33.3% in goats, and pneumonia (13.5%), fasciolosis (21.2%) and wound (8.15%) in sheep were commonly observed. Colic (31.6%) and tetanus (28.9%) were frequently encountered in equine while coccidiosis (55.6%) and Newcastle Disease (NCD) (40.7%) were commonly observed in poultry (Table 4). Others than actinobacillosis, babesiosis, Pes des Petites Ruminantum (PPR), coccidian, hypocalcemia, tetanus and strangels, almost all of encountered livestock health problems are known by local language the "Afaan Oromo".

Discussion

Observation of wide ranges of in farm animal health problem at Diga town Veterinary Clinic showed the challenges facing livestock's in the district. The health cases of cattle (52.5%), sheep (23.3%), equines (12%), poultry (8.5%) and goat (0.9%) presented for a number of clinical cases during the study period indicate the indirect effects such as low output in meat, milk, and draft power, poor growth and fertility, and treatment cost. Those animal health problems hamper the rural small holder farmers' livelihood development. Some of those cases would risk for public health (Assegid, 2000). Similarly, Haftu et al. (2014) reported 55.6% cattle, 32% shoat, and 12.9%

Table 4: Specification of major cases with naming by local language, the "Afaan Oromo" and respective treatments in studied farm animal

Name of the encountered diseases			Cattle (n=175)	Goat (n=3)	Sheep (n=74)	Equine (n=38)	Poultry (n=27)
Common name	**Local name "Afaan Oromo"**	**Drugs used for treatment**	**No. (%)**	**No. %)**	**No. (%)**	**No. (%)**	**No. (%)**
Castration	Kolaasuu	NA	3 (1.7)	1 (33.3)	1 (1.4)	-	-
Mastitis	Dhukuba mucha	Penstrip, Pencellin	8 (4.6)	-	-	-	-
Pasteurellosis / pleuropnemonia	Gororsa	OxyTTC	3 (1.7)	-	6 (8.1)	4 (10.5)	-
Pneumonia	Qufaa	OxyTTC	2 (1.1)	-	10 (13.5)	-	1 (3.7)
Retained fetal membrane	Dillun hafuu	MR, OxyTTC	2 (1.1)	-	-	-	-
Actinobacillosis	NK	Penstrep	2 (1.1)	-	-	-	-
Aspiration pneumonia	Fe'amuu	Penstrep	1 (0.6)		1 (1.4)	2 (5.3)	-
Babesiosis	NK	deminezene aceturate	13 (7.4)	-	-	-	-
Black leg/ PPR/colic / coccidian**	Abagorba/ NK/ Cininna/ NK	Pencellin/OxyTTC*/ASA/ Amperoleum	15 (8.6)	-	1 (1.4)	12 (31.6)	15 (55.6)
Bloat	Bokoka	Natural oil with Fomy sop	3 (1.7)	-	-	-	-
CBPP/CCPP*	Sombee	Penstrip, Pencellin	7 (4.0)	1 (33.3)	5 (6.8)	-	-
Dermatophytosis	Barule	Penstrip, OxyTTC	1 (0.6)	-	-	-	-
Dystocia	Dhaluu dadhabu	MR, Penstrip	1 (0.6)	-	-	-	-
Fasciolosis	Ramo Tiru	Albendazole	10 (5.7)	-	16 (21.6)	-	-
GIT parasites	Maxantu garaa	Albendazole, ivermetin	8 (4.6)	-	5 (6.8)	-	-
Grain over load	Callan garaa qabaa	Indigestion powder,	5 (2.9)	-	-	-	-
Hypocalcemia	NK	calcium borogluconate	3 (1.7)	-	5 (6.8)	-	-
Leechen/lice/ lamenes ***	Dhulandhula/Injire/ Okolu	MR/ amitraz/ASA, Pencellin	2 (1.1)	-	5 (6.8)	1 (2.6)	-
Lumpy skin disease	Gororsitu	OxyTTC	24 (13.7)	-	-	-	-
Mange mites	Cittoo	ivermetin	12 (6.9)	-	3 (4.1)	-	-
Orf	Borcoqa	OxyTTC*	-	1 (33.3)	6 (8.1)	-	-

Name of the encountered diseases			Cattle (n=175)	Goat (n=3)	Sheep (n=74)	Equine (n=38)	Poultry (n=27)
Common name	Local name "Afaan Oromo"	Drugs used for treatment	No. (%)	No. %)	No. (%)	No. (%)	No. (%)
Tetanus	NK	Penicillin	1 (0.6)	-	-	11 (28.9)	-
Tick infestation	Silmi	amitraz	6 (3.4)	-	2 (2.7)	-	-
Trypanosomosis	Gandi	deminezene aceturate	41 (23.4)	-	3 (4.1)	-	-
Tunga/ strangels/NCD#	Mujale/NK/Fingal	MR/ Penstrip, Pencellin/ OxyTTC	-	-	1 (1.4)	1 (2.6)	11 (40.7)
Wound	Madaa	Penstrip, Pencellin, OxyTTC	2 (1.1)		4 (5.4)	7 (18.4)	-
Post therapy prognosis	Good	154 (88.0)	3 (100)	70 (94.6)	35 (92.1)	13 (48.1)	
	Poor	21 (12.0)	0	4 (5.4)	3 (7.9)	14 (51.9)	

*CBPP in cases of cattle, CCPP in cases of goat;
**Black leg in cases of cattle, PPR in cases sheep, Colic for equine, coccidia for poultry
***Leechen in cases of cattle, lice in cases sheep; lameness for equine
# Tunga for sheep/ strangles for equine/NCD for poultry***
NK=not known; pasteurelossis for cattle, pleuropneumonia for equine;
MR=Manual Removal; OxyTTC=Oxytetracycline; NA=not applicable

poultry cases in selected district of Tigray, Ethiopia. Unlike Haftu et al. (2014) which did not include equines, different cases equines.

Cattle health problem

Bacterial and viral infectious diseases: Current study revealed, lumpy skin disease (13.7%) higher than 5.60%, blackleg (8.6%) similar with 9.56%, mastitis (4.6%) similar with 2.14% and pasteurellosis (1.7%) lower than 7.91% reports of Moges and Bogale (2012) in northern Ethiopia observed and were among the infectious disease frequently occurred in cattle at study area. Contagious Bovine Pleuropneumonia was as 4.0% and Zerihun (2004) also reported similar LSD prevalence in Ada'a Liben district, central Ethiopia. This could be due to high incidence of mechanical vector, the biting flies that favor fast transmission in the form of outbreak. It has a very high morbidity and moderate mortality (Radostits et al. 1994) effect resulting in economic losses from cattle production performance and hides quality. Several studies reported that blackleg were claimed to be the leading cattle health problem in Ginchi water shed area (Belayneh, 2002). Blackleg was reported to be the most important infectious disease with prevalence rate of 20% in the northern part of Ethiopia which was greater than this study it may be because of season of study or climatic condition difference. Legesse (1996) and Zerihun (2004) also mentioned that it was common infectious disease of cattle in Ada Liben district.

Parasitic diseases: Among the parasitic diseases trypanosomes was the leading (23.4%) and higher than 2.31% reports of by Moges and Bogale (2012) in cattle from northern Ethiopia. Cases of gastrointestinal tract parasite (16.0%) were found the next frequently and similar with 14.7% reported by Moges and Bogale (2012). Cattle fasciolosis (5.7%) was lower than the 32.45% report of Moges and Bogale (2012), 33.8% report of Regassa (1985) and 47 % report of Ameni (2001) from Ethiopia. The high occurrence of parasitic diseases in the study area could be associated with difference in the study area where the present study is located in western Ethiopia tsetse belt, absence of

strategic deworming practices and the humid weather condition of area promoting survival of the parasite and their intermediate host in mixed farming system. Belayneh (2002) at Ginchi watershed and Zarihun (2004) at Ada'a Liben district also reported gastro intestinal helmenthiasis as a common disease affecting cattle in crop-livestock production system areas of Ethiopia. This study also revealed occurrence of ectoparasites including mange mites (6.9%) at similar rate with 7.4% (Assegied, 1991) but higher than 3.1% (Moges and Bogale 2012) and 1.86% (Chalachew, 2001) from different parts of Ethiopia. An animal that is affected by mange mites shows with clinical signs like rubbing, itching, emaciated body condition and loss of hair in the field area observed. Relatively low (3.4%) of cattle were also infested by tick in the area.

Production and reproductive system cases: The present findings of 6.3% production and 3.4% reproductive system cases were similar with 3.1% (Haftu et al. 2014) and 4.8% (Alemu and Zegeye, 2011) reports from selected areas in Ethiopia. Present finding of 1.7% hypocalcaemia was lower than the 5.8% metabolic disorders reported by Alemu and Zegeye (2011) showing the health problem livestock in Ethiopia.

Small Ruminants health problems

Bacterial and viral infectious diseases: Pasteurollosis was found to be the most economically important bacterial infectious disease of sheep in the Woreda. This is in agreement with the result of (Ayeleti et al. 2004) which indicated that pasteurollosis is a major concern in north Shoa, central high lands of Ethiopia. The causative agent of pasteurollosis is known to be a normal habitat of the upper respiratory tract. When there is stress, the bacteria multiply rapidly and invade the lower respiratory tract where infection is initiated. Due to its short incubation period, stressed animals become diseased and die immediately even without showing clinical signs. The main reason for the frequent occurrence of the disease in the study area could be due to several predisposing factors like high temperature and aridity of the area. The other most probable reason may be low level of implementation of prevention strategies. The next most important small ruminant infectious disease is orf which is a viral disease affect mainly young animals and occurred with 8.1% in an area. The result was greater than the findings of (Woldemeskel and Ashenafi, 2003) that orf was a common viral disease with occurrence rate of 3% in northern part of Ethiopia. This difference can be due to climatic condition and management. The other most common bacterial disease found at the area was contagious caprine pleuro-pneumonia.

Parasitic diseases: Gastrointestinal parasitic diseases in small ruminants were found high which was studied during cross sectional study was conducted. This could be attributed to overgrazing of infested pasture and low use of antihelmenthics. This result 6.8% is in lower with previous findings in Ethiopia high land sheep (Tekalye et al. 1992; ILCA, 1996). Greater result has also been described by (Belay, 1998) in western part of Ethiopia (15%). Fasciolosis was also the major health problems of sheep mentioned during the survey protocol procedures in the study area. This result agrees with (Tembley, 1997) that described fasciolosis as a very important disease of sheep in the high lands of Ethiopia. Its importance in the present study area may be explained by wide range land marshy area and water holes in which wet up the dry season and good opportunity for the survival of the intermediate host, water snails and consequently gives high chance for the development of the fasciola parasite

Production and reproductive system cases: Although hypocalcemia was observe at 6.8% only in goats as production health problem, no other respective conditions were observed in the area. In other area dystocia and retained fetal membrane was reported at 3.35% (Haftu et al. 2014) in small ruminate. Alemu and Zegeye (2011) also reported reproductive tract health problem in sheep (4.3%) and goat (18.2%) as well as metabolic cases of 2.4% in sheep and 6.1% in goats in Ethiopia. The present finding and previous reports showed prevalence of production and reproductive system cases of small ruminants in Ethiopia.

Equine health problems

Present finding showed 49.15% strangle 32.20% epizootic lymphangitis and 18.64% eye infection in equines. Haftu et al. (2014) did not included study on equine health. Tetanus is fatal bacterial disease in about 80 % of infected horses (Rodriquez, 1991). It was about 30% in present study showing the most frequently seen bacterial disease in equine in the area. Lameness and hyena bite were also identified as a traumatic health problems. These diseases related to the housing systems, ragged, swampy and open fencing systems, which predisposing. Unsuitable topography and improper loading style of donkeys, which expose donkeys remarkably to lameness (Rodriquez, 1991). Colic was one of a considerable abnormality at incidence rate that could be attributed from heavy infestation with the red worms, restricted access to water, poor teeth status, access of fermentable feed staff, torsion of intestine miscellaneous cases had and others (Radostitis et al. 1994). Pneumonic conditions are also most important equines as it is described by 10.5% of the studied cases and getting the second position and from the diseases affecting adult equines it has got third rank. Foals are particularly prone to pneumonic condition (Alemnesh, 2004) could be due to the low resistance to microorganism causing the infection. The higher (31.6%) cases equine colic observed from present than the 3.4% (Alemu and Zegeye, 2011) from Gonder Veterinary Clinic, Ethiopia showed the incidences of the cases in Diga area.

Health problems in poultry

Gastrointestinal tract disease (93.6%) were common in poultry with coccidiosis was 55.6% but lower than the 11.7% reported by Haftu et al. (2014) be due to humid weather condition of the present study area which fevers sporulation of coccidian oocyst. This health problem could results in reduction of poultry industry in the studies area. Presence of an overall the world poultry disease mainly coccidiosis were also reported by Nematollahi et al. (2009). Effects of coccidiosis mainly include loss of weight, retarded growth, and drop in egg production and mortality of affected chickens (McDougald et al. 1990; Calnek et al. 1991; Rodreguez et al. 1997).

The study also revealed high (40.7%) New castle Disease (NCD) which was lower than the 60.0% (Haftu et al. 2014) from Northern Ethiopia were observed, indicating its economically significant infectious viral disease of chickens. This presence of NCD at high frequency in the district could be due to absence of control and prevention methods to reduce its economical impact. It was also reported by (Dessie and Jobre, 2004) that NCD as a major poultry health constraint, which cause heavy mortality and morbidity to village chicken and affects productivity of the system in the country. Haftu et al. (2014) reported foul pox (18.3%) in poultry but not observed in the present study area.

Frequently used therapeutic drug and prognoses of the cases

Uses of antimicrobial drugs at high rates Oxytetracycline (23.0%) and penstripe (20.8%) are indicative and concomitant with prevalence of infectious diseases in the area. Use of diminazene aceturate at 18.0% was for treatments of trypanosome cases. As high prevalence of GIT parasite were observed in the area, albendazole was a drug of choices at high rate (12.3%). On the other hand, DACA (2009) has been reported presence some drug resistance cases in Ethiopia. But, observation of significantly high good prognoses (86.8%) shows diagnostic efficiency of the local veterinarian and the therapeutic efficacy of the drugs used in the area. However, frequent use of drugs could results in the developments of resistance (WHO, 2001).

Indigenous knowledge of farm animal health problems

Most of science and technologies were developed from local indigenous knowledge (Moonga and Chitambo, 2010). Present finding shows most of livestock health problems were known by local name. This indicates, the intimate linkage of farmer livelihood with livestock share them an observational skill on health

conditions which allowed for characterizing and differentiating each of the health cases. Application of indigenous methods of control of pests and diseases (Adekunle et al. 2002) was also reported from Nigeria. Thus, the present interviewer almost indicated, they knew farm animal health cases by local language as an indigenous knowledge. The presence of similar indigenous skill on livestock health problem by other Ethiopian language were indicated by Haftu et al. (2014).

Conclusion

Farm animals' health problems at Diga town Veterinary Clinic with high prevalence of trypanosomes, LSD, black leg, babesiosis, mange mites, fasciolosis and GIT parasites were the most important diseases in the area. In addition, frequent presence of poultry NCD and coccidian infection showed health problems as a leading causes of mortality, production losses, reduce growth rate, reduce reproduction ability of animals, down grade of livestock products and food borne diseases. Intensified livestock health delivery system with supply of different types of drugs under drug resistance control was recommended. Further epidemiological survey on the associated risk factor for cases with mitigation strategies shall also be implemented to increases farmers livelihood from the sector. .

Acknowledgements

We would like to appreciate the collaboration of Diga district Veterinary Clinic in West Wollega, Oromia Regional State for creating smooth and suitable working environment for this study. Cooperation of interviewees' community for providing detailed information on their skill of indigenous livestock health problems was highly appreciated.

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SURVEY OF PASTURALISTS' KNOWLEDGE, ATTITUDE AND PRACTICES WITH REGARD TO TUBERCULOSIS IN KATSINA STATE, NIGERIA

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Abstract

In a study to determine the Knowledge, Attitude and Practices (KAP) of pastoralists in Katsina State of Nigeria in relation to tuberculosis, 592 pastoralists were selected from two Local Government Areas (LGAs) from each of the three senatorial districts of the State. Two Wards were also selected from each of the LGAs. The study showed that 96.76% of the respondents knew 5 animal diseases while 8.27% and 4.05% knew 10 and 15 animal diseases respectively. Also, 71.60% of them knew tuberculosis in animals and man and also its Treatment Centres. Their sources of knowledge of these diseases included Veterinarians, (28.50%), hospitals (6.70%), friends (4.56%), fellow herdsmen (3.37%), and electronic media (31.25%). Signs of tuberculosis they knew in cattle included coughing (61.48%), weight loss 18.90%, death 16.80%, and others (6.70%). They indicated its methods of transmission in animals to included ingestion 15.20%, drinking contaminated water 36.60% and closeness with other animals (10.49%). In man they included drinking contaminated milk 36.48% in addition to consumption of contaminated meat 33.10% and closeness to infected individuals30.40%. With regard to their attitude to tuberculosis, 87.80% of the respondents were eating roasted meat and drinking milk daily along with taking milk directly from the udder of their cattle (35.10%) and consuming sheep and goats' milk (30.40%). Practices of respondents' indicated54.50% of them managing their cattle under extensive system and used community ponds to water their animals. Consequently, there is need for public enlightenment towards the dangers of tuberculosis

Key words: Knowledge, Attitude, Practices, Tuberculosis, Katsina State.

ETUDE DES CONNAISSANCES, ATTITUDES ET PRATIQUES DES ELEVEURS EN RAPPORT AVEC LA TUBERCULOSE DANS L'ÉTAT DE KATSINA AU NIGERIA

Résumé

Dans une étude visant à déterminer les connaissances, les attitudes et les pratiques des éleveurs en rapport avec la tuberculose dans l'État de Katsina au Nigéria, 592 éleveurs ont été sélectionnés dans deux zones de gouvernement local (LGA : Local Government Area) de chacun des trois districts sénatoriaux de l'État. Deux équipes ont également été choisies dans chacune des LGA. L'étude a montré que 96,76% des répondants connaissaient 5 maladies animales tandis que 8,27% et 4,05% connaissaient respectivement 10 et 15 maladies animales. En outre, 71,60% d'entre eux connaissaient la tuberculose chez les animaux et l'homme et aussi ses centres de traitement. Leurs sources de connaissance de ces maladies comprenaient les vétérinaires, (28,50%), les hôpitaux (6,70%), les amis (4,56%), les écoliers (3,37%) et les médias électroniques (31,25%). Les signes de tuberculose qu'ils connaissaient chez les bovins étaient notamment la toux (61,48%), la perte de poids 18,90%, la mortalité 16,80% et autres (6,70%). Ils ont indiqué que les méthodes de transmission chez les animaux comprenaient l'ingestion 15,20%, la consommation d'eau contaminée 36.60% et la proximité d'autres animaux (10.49%). Chez l'homme, les méthodes de transmission comprenaient la consommation de lait contaminé 36,48% en plus de la consommation de viande contaminée 33,10% et la proximité des personnes infectées30,40%. En ce qui concerne leurs attitudes par rapport à la tuberculose, 87.80% des répondants mangeaient de la viande rôtie et buvaient quotidiennement du lait y compris le lait directement trait du pis de leur vache (35,10%) et la consommation de lait de mouton et de chèvre

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(30,40%). Les pratiques des répondants ont indiqué que 54,50% d'entre eux élevaient leur bétail en système extensif et utilisaient des étangs communautaires pour l'abreuvage de leurs animaux. Par conséquent, il est nécessaire d'éclairer le public sur les dangers de la tuberculose.

Mots-clés : connaissances, attitudes, pratiques, tuberculose, Etat de Katsina.

Introduction

Livestock production in Nigeria is characterized by extensive husbandry and management systems with the pastoralists holding greater than 70% of the National cattle herd (Saidu *et al.,* 1991). The animals have also been shown to be infected with a number of diseases as there is no much attention given to the animals by way of routine herd health management programmes (Kaltungo, 2013; Buhari, 2014; Ibrahim, 2016). Hitherto, Government used to provide annual vaccination against the major vaccinatable animal diseases. With the coming of the economic down turn, this practice has soon given way and it is expected that diseases would find their way into the national herd. The level of education of most of the pastoralists is also low and may result in their lower attempt to look for Veterinary care. It is for this reason that this study was undertaken to determine the pastoralists' knowledge, attitude and practices with regard to tuberculosis in Katsina State, Nigeria as doing so would help a lot in our understanding on how to go about organizing control majors against the disease.

Materials and Methods

The study was undertaken in Katsina State which is one of the 36 States in Nigeria. It has 34 Local Government Areas (LGAs) and is in the North Western ecological zone of Nigeria. The State is divided into three Senatorial districts and is located between latitudes 11°1' and 13°221 North and longitudes 6°221 East and 9°21 North (MANR, 2011). It is boarded by Niger Republic to the North, Sokoto and Zamfara States to the West, Kano State to the North West, Jigawa State to the North East and Kaduna State to the South (NPC, 2006). Katsina State is endowed with a human population of 5,801,584 and cattle population of 1,109,729 (NPC, 2006; MANR, 2011). There are also an estimated 50,000 pastoralists in the State (NPC, 2006). Majority of the people in the State depend on Agriculture producing subsistence and cash crops like sorghum, millet, cowpea and groundnuts (MANR, 2011).

Two LGAs were selected from each of the three Senatorial districts using random sampling without replacement and that two Wards were further selected from each of the selected LGAs. An estimated 10% of the population of the pastoralists in the State was selected such that a total of 50 pastoralists were selected from each Ward after which further selection was based on convenience and willingness of the selected pastoralists to participate in the study.

The study was conducted between January and September 2016and that an inclusion criterion was used to involve only pastoral cattle herd owners domicile in the areas where these pastoral cattle were found in the State during the survey period while an exclusion criterion was pastoral cattle herders that were less than 15years of age.

The study was designed to collect data from the pastoralists using a closed ended semi-questionnaires. The questionnaires were administered to the pastoralists using the 'Hausa' language which was understood by all in the area. Information required from the pastoralists included their educational standard, knowledge on animal and zoonotic diseases, their attitude on eating and drinking habits, animal husbandry and management practices among others.

Results

One hundred respondents from each of Kankara, Bindawa, Mashi, Kaita and Batagarawa LGAs and 92 from Kafur LGA participated in

the study. (Table1). Of the 592 respondents, 32 (5.40%) were of the age group of 15 to 30 years old while 227 (38.34%), 286 (48.31%) and 52 (8.78%) were of the age groups of 31 to 46, 47 to 60 and above 60 years of age respectively (Table 1). There was statistically significant difference in the age groups that participated in the study (X^2=51.224; df=10; p=0.0000). Furthermore, 404 (68.24%) of the respondents had primary education while 162 (27.36%) and 24 (4.05%) had secondary and post-secondary education respectively and this is statistically significant with those with Primary education being the most respondents in the study (X^2=14.060; df=6; p=0.029) (Table I).

Of the 592 respondents that participated in the study, 510 (96.76%) reported that they could recognize five animal diseases while 49 (8.27%) and 24 (4.05%) others indicated knowing 10 and 15 animal diseases respectively and this is statistically significant with those knowing five diseases only being the majority (X^2=77.088; df=10; p=0.0001) (Table II). Among the animal diseases they could recognize, 259 (43.70%) respondents indicated their ability to recognize Contagious Bovine Pleuropneumonia in their cattle while 130 (21,90%), 118 (19.90%), 52 (8.70%) and 23 (3.80%) others said they could recognize fasciolosis, helminthosis, Black leg and Foot and Mouth Disease respectively (Table II). Their ability to recognize these diseases is statistically significant (X^2=90.865; df=20; p=0.001). As to their knowledge of tuberculosis, 423 (71.60%) reported knowing the disease while the remaining 169 (28.50%) did not know the disease and this is statistically highly significant (X^2=258.117; df=5; p=0.0002). Similarly, 442 (74.60%) of the respondents were aware of Tuberculosis treatment centres while 150(23.30%) others were not aware of them (Table III). This is statistically significant (X^2=223.103; df=5; p=0.0000)

As to their sources of knowledge of these diseases, 169 (28.50%) of the respondents indicated the veterinarian while 40 (6.70%), 27 (4.56%), 20 (3.37%) and 185 (31.25%) others indicated hospitals, friends, fellow herdsmen and the electronic media to be respectively as their sources of knowledge on livestock diseases (Table III). The sources of knowledge for these respondents are statistically highly significant (X^2=64.450; df=20; p=0.0001) As of the duration of their knowledge of Tb, 99 (18.41%) of the respondents indicated knowing the disease for the last 2 years while 203 (34.29%) of them reported knowing the disease since the last 5 years (Table III). This is statistically significant (X^2=70.190; df=5; p=0.000).

Four hundred and fifty (76.01%) of the respondents indicated knowing cattle to come down with tuberculosis while 112 (20.60%) of them were not aware whether cattle could come down with the disease or not (Table IV). Their knowledge of the disease is statistically significant (X^2=102.52; df=5; p=0.0001). As to the signs of Tb in cattle, 364 (61.48%), 122 (18.90%), 100 (16.80%) and 16 (2.70%) others reported signs of Tb in cattle to be coughing, weight loss, death and others respectively and this is statistically highly significant (X^2=100.182; df=15; p=0.001). On enquiring on the means of acquiring the disease by cattle, 90 (15.20%) of the respondents indicated ingestion as a means of acquiring the disease while 217 (36.60%), 213 (35.90%) and 62 (10.49%) others indicated drinking contaminated water, closeness to infected animals and infected humans respectively to be other means of acquiring infection by cattle (Table IV). Their knowledge of means of transmission of the disease is highly significant (X^2=78.774; df=15; p=0.0001).

As for their knowledge of how humans acquire infection with Tb, 484 (81.70%) of the respondents agreed that humans could acquire Tb infection and that signs of the disease in man included coughing 281 (47.46%), weight loss 132 (22.29%), fever 110 (18.50%), and death 52 (8.78%) (Table V). The knowledge of clinical signs of tuberculosis in man by these respondents is highly significant (X^2=60.047; df=15; p=0.000). With regard to how humans acquire Tb infection, 196 (33.10%) of the respondents indicated contaminated meat while 216 (36.48%) and 180 (30.40%) others respectively indicated contaminated milk and closeness with infected humans (Table V).

TABLE I: Respondents' age and educational levels in selected Local Government Areas of Katina State, Nigeria

LGA	Number of respondents	Age[a]				Educational Level[b]		
		15-30 years old	31-46 years old	47 – 60 years old	>60 years old	Primary	Secondary	Post-Secondary
Kankara	100	8	47	33	12	87	12	1
Bindawa	100	1	26	68	5	67	29	4
Kafur	92	0	30	50	12	66	23	3
Mashi	100	12	35	50	8	70	24	6
Kaita	100	5	25	55	15	49	42	7
Batagarawa	100	6	64	30	0	65	32	3
Total	**592**	**32 (5.40%)**	**227 (38.34%)**	**286 (48.31%)**	**52 (8.78%)**	**404 (68.24%)**	**162 (27.36%)**	**24 (4.05%)**

(a) $X^2 = 51.224; df = 10, p = 0.000$
(b) $X^2 = 14.060; df = 6, p = 0.029$

TABLE II: Respondents' knowledge on animal diseases in selected Local Government Areas of Katina State, Nigeria

LGA	Number of respondents	No. animals diseases known(a)			Names of diseases known(b)				
		5	10	15	CBPP	Fasciolosis	Helminthosis	Black leg	Foot & Mouth Disease
Kankara	100	81	7	3	45	30	17	5	3
Bindawa	100	57	22	21	30	28	18	19	5
Kafur	92	92	0	0	40	10	14	15	3
Mashi	100	90	10	0	24	30	35	5	6
Kaita	100	90	10	0	58	10	20	8	4
Batagarawa	100	100	0	0	62	22	14	0	2
Total	**592**	**510 (96.76%)**	**49 (8.27%)**	**24 (4.05%)**	**259 (43.70%)**	**130 (21.90%)**	**118 (19.90%)**	**52 (8.70)**	**23 (3.80%)**

(a) $X^2 = 77.088; df = 10, p = 0.0001$
(b) $X^2 = 90.865; df = 20, p = 0.001$

TABLE III: Respondents' Knowledge and sources of knowledge on tuberculosis and other diseases in selected Local Government res of Katsina State Nigeria

LGA	Number of respondents	Knowledge of TB Treatment Centres(a)		Knowledge of Tuberculosis(b)		Source of Knowledge(c)					Duration of knowledge(d)	
		Yes	No	Yes	No	The veterinarian	Hospital friends	Friends	Fellow herdsmen	Media	2 years	5 years
Kankara	100	14	86	59	41	10	8	2	5	34	34	12
Bindawa	100	54	46	78	22	35	5	8	2	28	15	35
Kafur	92	92	0	81	11	50	11	7	4	20	13	38
Mashi	100	100	0	93	7	46	2	5	7	40	1	66
Kaita	100	82	18	100	0	26	14	5	2	53	30	47
Batagarawa	100	100	0	12	88	2	0	0	0	10	6	5
Total	592	442 (74.60%)	150 (23.30%)	423 (71.45%)	169 (28.50%)	169 (28.50%)	40 (6.70%)	27 (4.56%)	20 (3.37%)	185 (31.25%)	99 (18.41%)	203 (34.29%)

(a) $X^2 = 2223.103; df = 5, p = 0.000$ *(b)* $X^2 = 258.117; df = 5, p = 0.0002$ *(c)* $X^2 = 64.450; df = 20; 0p = 0.0001$ *(d)* $X^2 = 70.190; df = 5; p = 0.00$

The knowledge of means of transmission of the disease in humans by these respondents is highly significant (X^2=43.805; df=10; p=0.0001). A further 472 (79.70%) of the respondents reported knowing that cattle could acquire the disease from man while 439 (74.15%) others reported man acquiring the disease from cattle and this is highly statistically significant with the significance being greater for those that indicated man acquiring the disease from cattle (X^2=105.602; df= 5; p=0.000; X^2=137.078; df=5; p=0.000) (Table VI). Further enquiry showed that, 91 (15.37%) of the respondents knew Tb patients and that 43 (7.29%) of them said they knew how the people acquired Tb while the remaining 501 (84.60%) respondents said they did not know anybody with Tb (Table VI). Their knowledge of people with Tb is statically significant (X^2=14.947; df=5; p=0.011).Enquiring on whether their family members had Tb indicated that 5 (0.8%) had Tb in the family while the remaining 587 (99.15%) reported not having TB in their families (Table VI).This knowledge is not significant (X^2=10.481; df=5; p=0.063).

Enquiry on their attitude towards eating showed that 520 (87.80%) of the respondents were in a habit of eating roasted meat ('Suya') daily while 60 (10.13%) and 25 (3.70%) of them indicated eating 'Suya' once a week and once a month respectively.The eating of 'Suya' by these respondents is statistically significant in exposing them to tuberculosis should the meat be contaminated with tubercle bacilli (X^2=30.586; df=4; p=0.0001). In addition, 25 (4.22%) of them said they were staying with known Tb patients in their families.This practice was also found to be significant (X^2=11.317; df = 5; p= 0.045) (Table VII).

With regard to drinking fermented milk ('Nono'), 520 (87.80%) respondents indicated drinking it daily while 49 (8.27%) and 22 (22 (3.70%) reported taking 'Nono' only once a week and once a month respectively (Table VIII). The taking of 'Nono' by these respondents is statistically highly significant as a possible means of acquiring tuberculosis (X^2=36.854; df= 8; p=0.000). As for drinking milk directly from the udder of their cattle, 391

TABLE IV: Respondents' Knowledge of tuberculosis in animals in selected Local Government res of Katsina State Nigeria

LGA	Number of respondents	Cattle acquire Tb[(a)]		Signs of tuberculosis in animals(b)					Means of acquiring TB by cattle(c)		
		Yes	No	Coughing	Weight loss	Death	Others	Ingestion	drinking contaminated water	Closeness to other animals	From infected human
Kankara	100	34	46	46	23	18	13	4	45	50	1
Bindawa	100	86	14	50	35	12	3	16	40	38	6
Kafur	92	83	9	66	20	6	0	20	34	19	19
Mashi	100	64	36	67	10	13	0	8	36	46	10
Kaita	100	93	7	80	9	11	0	31	38	20	11
Batagarawa	100	90	10	55	15	30	0	21	24	40	15
Total	592	450 (76.01%)	122 (20.60%)	364 (61.48%)	112 (18.90%)	100 (16.80%)	16 (2.70%)	90 (15.20%)	217 (36.60%)	213 (35.90%)	62 (10.49%)

(a) $X^2 = 102.52; df = 5, p = 0.0001$ *(b)* $X^2 = 100.182; df = 15, p = 0.001$ *(c)* $X^2 = 78.774; df = 15, p = 0.0001$

TABLE V: Respondents' knowledge of tuberculosis in man in selected Local Government areas of Katsina State Nigeria

LGA	Number of respondents	Disease in Humans(a)		Signs of Tuberculosis in man(b)				Method of acquiring tuberculosis by man(c)		
		Yes	No	Coughing	Weight loss	Fever	Death	Contaminated meat	Contaminated milk	Closeness with infected humans
Kankara	100	52	48	41	23	21	5	15	46	39
Bindawa	100	75	20	35	40	29	6	45	20	35
Kafur	92	68	24	44	29	20	9	33	26	33
Mashi	100	100	0	73	12	5	10	25	42	33
Kaita	100	96	4	38	15	15	12	40	42	18
Batagarawa	100	93	7	50	13	20	10	38	40	22
Total	592	484 (81.70%)	103 (17.30%)	281 (47.46%)	132 (22.29%)	110 (18.50%)	52 (8.78%)	196 (33.10%)	216 (36.48%)	180 (30.40%)

(a) $X^2 = 111.199; df = 5, p = 0.000$ *(b)* $X^2 = 60.047; df = 5, p = 0.000$ *(c)* $X^2 = 43.805; df = 10, p = 0.0001$

TABLE VI: Respondents' knowledge on tuberculosis being zoonotic in selected Local Government Areas of Katsina State, Nigeria

LGA	Number of respondents	Cattle acquiring Tb from man(a)		Humans acquiring Tb from cattle(b)		Knowledge of anybody with Tb(c)		Knowledge how he acquired Tb(d)		Member of Family with Tb(e)	
		Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Kankara	100	14	86	52	48	14	86	2	98	0	100
Bindawa	100	95	5	44	56	7	93	0	100	3	97
Kafur	92	86	6	90	2	16	76	5	87	0	92
Mashi	100	100	0	68	32	26	74	16	84	0	100
Kaita	100	89	11	92	8	13	87	10	90	2	98
Batagarawa	100	88	12	93	7	15	85	10	90	0	100
Total	592	472 (79.70%)	120 (20.27%)	439 (74.15%)	153 (25.37%)	91 (15.37%)	501 (84.60%)	43 (7.29%)	549 (92.70%)	5 (0.80%)	587 (99.15%)

(a) $X^2 = 105.602$; $df = 5$, $p = 0.000$ *(b)* $X^2 = 137.078$; $df = 5$, $p = 0.000$ *(c)* $X^2 = 14.947$; $df = 5$, $p = 0.011$ *(d)* $X^2 = 25.957$; $df = 5$, $p = 0.001$

(e) $X^2 = 10.481$; $df = 5$, $p = 0.063$

(66.04%) indicated doing so while the remaining 201 (33.70%) reported not doing so and this is statistically very significant (X^2=42.335; df=5; p=0.001). When enquired on the effect of taking milk directly from the udder of their cattle, 208 (35.10%)respondents reported that there was adverse effect in taking milk from the udder while the remaining 384 (64.80%) said there was no adverse effect at all (Table VIII). The consideration of some of the respondents that there was no adverse effect in taking milk from the udder is highly significant (X^2=179.064; df=5; p=0.0001). With regard to taking sheep and goats milk 180 (30.40%) respondents reported doing so while 412 (69.50%) others did not (Table VIII). The taking of sheep and goats milk is statistically highly significant as a possible means of acquiring tuberculosis, should the animals be infected with tubercle bacilli (X2=51.258; df=5; p=0.0001).

Enquiring on the habit of milking and eating simultaneously, 115 (19.42 %) reported they were in the habit of milking and eating simultaneously while 477 (80.50%) of them indicated not doing so and this is statistically highly significant (X^2=45.358; df=5; p=0.0002). When asked why they milked and ate simultaneously, those that were in this habit {62 (10.97%)} indicated that there was no adverse effect in doing so and that 59 (9.96%) of them said the milk was safe (Table X). The eating and milking simultaneously has very high significant effect in the acquiring tuberculosis should the cow be infected with BTb (X^2=45.528; df=5; p=0.0001). For those that were not in the habit of milking and eating simultaneously {240 (40.50%)}, they observed that this could result in diseases transmission while 258 (43.50%) of them observed that they would rather want to milk first before eating (Table IX).

Their attitude towards disease control indicated that 468 (79.05%) of the respondents reported accepting childhood vaccination while 77 (13.00%) of them indicated not vaccinating their children and this is statistically significant for disease control (X^2=274.871; df=5; 0.0001). Furthermore, 261 (44.08%) of the respondents reported vaccinating their children against Tb while 149 (25.16%), 96 (16.21%) and 56 (9.45%)

TABLE VII: Eating habits of Respondents in selected Local Government Areas of Katsina State, Nigeria

LGA	Number of Respondents	Frequency of eating 'Suya'(a)			Staying with TB Patient(b)	
		Daily	Once a week	Once a Month	Yes	No
Kankara	100	83	11	6	7	93
Bindawa	100	89	20	1	5	95
Kafur	92	88	4	0	2	90
Mashi	100	16	10	3	8	92
Kaita	100	5	2	10	3	97
Batagarawa	100	60	25	2	0	100
Total	592	520 (87.80%)	60 (10.13%)	25 (3.70%)	25 (4.22%)	567 (98.70%)

(a) $X^2 = 30.586$; $df = 4$, $p = 0.0001$ *(b)* $X^2 = 11.317$; $df = 5$, $p = 0.045$

TABLE VIII: Drinking habits of Respondents with respect to milk in selected Local Government Areas of Katsina State, Nigeria

LGA	Number of Respond-ents	Frequency of drinking "Nono"(a)			Drinking milk directly from cattle Udder(b)		Effects of taking milk from animals(c)		Drinking milk from Sheep and Goats(d)	
		Daily	Once a week	Once a Month	Yes	No	Yes	No	Yes	No
Kankara	100	83	11	6	81	19	36	64	25	75
Bindawa	100	89	10	1	75	25	16	84	10	90
Kafur	92	88	4	0	50	42	83	9	30	62
Mashi	100	93	4	3	83	17	43	57	56	44
Kaita	100	74	4	10	52	48	20	80	28	72
Batagarawa	100	93	16	2	50	50	10	90	31	69
Total	**592**	**520 (87.80%)**	**49 (8.28%)**	**22 (3.70%)**	**391 (66.04%)**	**201 (33.70%)**	**208 (35.10%)**	**384 (64.80%)**	**180 (30.4%)**	**412 (69.50%)**

(a) $X^2 = 36.854$; $df = 8$, $p = 0.000$ *(b)* $X^2 = 42.335$; $df = 5$, $p = 0.001$ *(c)* $X^2 = 179.064$; $df = 5$, $p = 0.0001$ *(d)* $X^2 = 51.258$; $df = 5$, $p = 0.000$

TABLE IX: Respondents' eating and drinking Practices in relation to Tuberculosis in selected Local Government Areas of Katina State, Nigeria

LGA	Number of Respondents	Milking and eating simultaneously		Reason for drinking while milking		Why no drinking while milking	
		Yes	No	No adverse effect	Milk is safe	Fear disease transmission	Milking of cattle first
Kankara	100	12	88	10	12	15	78
Bindawa	100	10	90	0	10	24	76
Kafur	92	20	72	11	9	51	21
Mashi	100	42	58	28	10	40	18
Kaita	100	20	80	10	10	30	50
Batagarawa	100	11	89	3	8	80	20
Total	**592**	**115 (19.42%)**	**477 (80.50%)**	**62 (10.97%)**	**59 (9.96%)**	**240 (40.50%)**	**263 (43.50%)**

(a) $X^2 = 45.358; df = 5, p = 0.0002$ *(b)* $X^2 = 45.528; df = 5, p = 0.0001$ *(c)* $X^2 = 130.884; df = 5, p = 0.0001$

TABLE X: Acceptance of Childhood Vaccination programmes in selected Local Government Areas of Katina State, Nigeria

LGA	Number of Respondents	Acceptance of Childhood vaccination(a)		Vaccines Routinely taken(b)		
		Yes	No	BCG	Poliomyelitis	Measles
Kankara	100	95	5	43	17	18
Bindawa	100	87	13	30	20	27
Kafur	92	2	43	43	29	10
Mashi	100	100	0	45	38	10
Kaita	100	91	9	50	30	16
Batagarawa	100	93	7	50	15	15
Total	**592**	**468 (79.05%)**	**77 (13.00%)**	**261 (44.08%)**	**149 (25.16%)**	**96 (16.21%)**

(a) $X^2 = 274.871; df = 5, p = 0.0001$ *(b)* $X^2 = 38.225; df = 15, p = 0.000$

of them reported vaccinating their children against Poliomyelitis, Measles and Small pox respectively (Table X).Their vaccinating their children was significant (X^2=38.225; df=15; p=0.000).

With regard to control of tuberculosis in their herds, 322 (54.39%) respondents said they reported such cases to the veterinarian while 138 (23.31%), 54 (9.12%), and 41 (6.92%) of the respondents reported either selling such infected animals, leaving them in the herd or doing nothing (Table XI).Their reporting to the veterinary authorities was statistically highly significant (X^2=330.215; df=15; p=0.0000). Two hundred and fifty (42.22%) of the respondents reported that they socialize with friends at the "ruga" at night while 75 (12.60%), 31 (5.23%) and 235 (39.60%) socialize with brothers, sisters wife/husbands respectively (Table XI). Socialization at night by the respondents was statistically significant (X^2 =149.405; df = 15, p = 0.0000). Also, 33 (5.50%) of respondents answered in the affirmative for presence of cough in their contacts while the remaining 569 (94.42%) had a no for an answer. This was also found to be statistically significant (X^2 =33.586; df = 5, p = 0.0001) (Table XI).

With regard to their practices, 323 (54.50%) of the respondents reported managing their cattle under extensive system while 52 (8.70%) and 217 (36.60%) of them reported keeping their animals under intensive and semi-intensive management system respectively (Table XII). The management system being practiced by these pastoralists was seen to be highly significant in the possibility of introduction of tuberculosis (X^2=182.068; df=10; p=0.0000).

Among the respondents 116 (19.50%) grazed their herds within one kilometre to their 'Ruga' while 280 (47.29%) and 196 (33.10%) of them grazed their herds two kilometres and 3 three kilometers away from their 'Ruga' respectively (Table XIII). Similarly, 185 (31.25%) of them used community pond to water their animals while 59 (9.90%) and 347 (58.61%) of them used earth dams where other people also used to fetch water for their household use and where other cattle herds

TABLE XI: Respondents' actions with regard to tuberculosis n selected Local Government areas of Katsina State, Nigeria

LGA	Number of Respondents	Action with cattle with Tb(a)				Socialization at night(b)				Presence of cough in contacts(c)	
		Report to the Veterinarian	Sell	Leave in herd	Do nothing	With friends at 'Ruga'	With brothers	With sisters	With wife/ husband	Yes	No
Kankara	100	18	12	43	27	1	13	6	10	7	93
Bindawa	100	35	50	10	5	66	20	2	12	16	94
Kafur	92	48	4	1	2	8	12	6	66	0	92
Mashi	100	70	30	0	0	31	13	5	50	0	100
Kaita	100	61	32	0	7	28	15	10	47	6	94
Batagarawa	100	90	10	0	0	46	2	2	50	4	96
Total	**592**	**322 (54.39%)**	**138 (23.31%)**	**54 (9.12%)**	**41 (6.92%)**	**250 (42.22%)**	**75 (12.60%)**	**31 (5.23%)**	**235 (39.60%)**	**33 (5.50%)**	**569 (94.42%)**

(a) X^2 *=330.215; df= 15, p = 0.0000 (b)* X^2 *=149.405; df = 15, p = 0.0000 (c)* X^2 *=33.586; df = 5, p = 0.0001*

TABLE XII: Livestock management systems by respondents in selected Local Government Areas of Katsina State, Nigeria

LGA	Number of Respondents	Animal Management Practices		
		Extensive	Intensive	Semi-intensive
Kankara	100	78	3	19
Bindawa	100	66	9	25
Kafur	92	28	28	36
Mashi	100	22	6	72
Kaita	100	82	3	15
Batagarawa	100	47	3	50
Total	**592**	**323 (54.50%)**	**52 (8.70%)**	**217 (36.60%)**

X^2 = 182.068; df = 10, p = 0.0000

TABLE XIII: Respondents' herding and grazing practices in selected Local Government Areas of Katsina State, Nigeria

LGA	No of respondents	Grazing distance from homestead ('Ruga')(a)			Watering of Animals(b)		
		1Km	2Km	3Km	Community pond	Earth dam where people fetch water	Facility where other cattle use
Kankara	100	18	36	46	44	9	47
Bindawa	100	4	20	76	38	34	28
Kafur	92	36	40	16	20	6	66
Mashi	100	9	83	8	28	0	72
Kaita	100	28	50	22	44	10	45
Batagarawa	100	21	51	28	11	0	89
Total	**592**	**116 (19.50%)**	**280 (47.29%)**	**196 (33.10%)**	**185 (31.25%)**	**59 (9.90%)**	**347 (58.61%)**

(a) *X^2 = 178.533; df = 10, p = 0.0000* *(b)* *X^2 = 149.853; df = 10, p = 0.0000*

TABLE XIV: Housing Management Practices by respondents in selected Local Government Areas of Katsina State, Nigeria

LGA	Number of Respondents	Distance of 'Buka' from Cattle Kraal			
		1 Metre	5 metre	10 Metre	>10 Metre
Kankara	100	40	30	12	18
Bindawa	100	15	50	25	10
Kafur	92	45	35	12	0
Mashi	100	29	30	15	26
Kaita	100	40	15	10	35
Batagarawa	100	70	20	10	0
Total	**592**	**239 (40.37%)**	**180 (30.40%)**	**84 (14.50%)**	**89 (15.03%)**

also used for watering respectively (Table XIII). The use of such places fir watering their cattle is statistically highly significant as a source of infection fir their animals (X^2=149.853; df=10; p=0.0000).

As to the location of their rooms with respect to the cattle kraals, 239 (40.37%) of the respondents reported that their rooms ('Buka') were one metre from the cattle kraal while 180 (30.40%),84 (14.18%) and 89 (15.03%) of them indicated that their houses were 5 metres, 10 metres away from their rooms (Table XIV).

Discussion

From the study, some of the pastoralists were found to acquire Western education, with some going up to post-secondary education level. Their imbibing Western education could be due to the introduction of the National Commission for Nomadic education (NCNE) Programme by the Federal Government in 1989. It could also be through the annual Livestock Rearers Workshops(LRWs) organized by the Department of The Veterinary Surgery and Medicine of the Ahmadu Bello University, Zaria from 1980 to 1990 (Saidu, 1980; Saidu, 1986).

The study has also shown that the pastoralists that participated in the study had knowledge on livestock diseases as they cloud recognize five to fifteen animal diseases. This could be as a result of the fact that parents train their children on the job. It could also be through the LRWs as stated by Saidu (1980; 1986) and even through the regular radio programmes on livestock diseases by some States Agricultural Development Projects (Kaltungo, 2013; Buhari, 2014). Their observations in this study have also confirmed this suggestion. Their ability to recognize these diseases by name further buttresses the suggestion that the sources of knowledge for these diseases could be as reflected by them in this study.

Their particular knowledge of tuberculosis and its treatment Centres could be as their family members were involved. Furthermore, their knowledge of the signs of the disease in animals and humans could be due to their educational levels, infection in family members and radio programmes on the disease in both animals and humans as stated earlier. The study has shown that the respondents were aware of the means of transmission of the disease in animals and humans and the disease even being zoonotic. However, their habits of eating 'Suya' and drinking raw milk, 'Nono' and even drinking milk directly from the udder does not indicate their full education on the dangers of the disease. Another reason that can be advanced for their habit of eating 'Suya' and unpasteurized milk could be the short period of training through LRW as mentioned above or lack of adequate technology transfer by the State's Agricultural Development Projects and the State Veterinary Services.

Their practice of milking and eating simultaneously is capable of endangering the

milkers' health and those of the family members should the cows being milked be infected with Mycobacteriumsp, especially as some of them considered that there was no adverse effect in doing so.

From this study it was shown that the pastoralists that participated in the study adopted childhood vaccination, including vaccination against tuberculosis. This could be due to the activities of the NCNE Extension Agents who are constantly with the pastoralists imparting health technology messages along with animal health and production technologies as indicated by Ardo (1998),Saidu (1996; 1997) and Saidu and Abdullahi, (1997). With regard to disease control, their practices of reporting to The veterinary Clinics or selling such infected animals is understandable as States are offering clinical services to livestock owners (Audi, 1990).As for their socialization with friends, there is the likelihood of any one of them to come down with the disease especially that they reported some of them to be coughing, should any of them be infected with Tb. Similarly, the fact that some of them reported some of their family members had Tb; the disease could be spread in the family and even friends. Thus this practice of socialization in the evening could result in the pastoralists coming down with the disease.

The extensive management practice as done by the respondents has been reported to be faced with possible transfer of diseases (Kaltungo, 2013; Billy, 2014). This is because animals of unknown health background could mix together and in the process transfer infection to others. Another possible means of transfer of infection is by the pastoralists mixing their animals with others at watering points as Buhari (2014) reported that mixing animals at watering points could facilitate spread of brucellosis in animals.

Acknowledgements

The researchers hereby acknowledge the support and cooperation of the pastoralists who participated in this study. We also want to extend our sincere appreciation to the Ardo who helped in the identification of the pastoralists and standing by the researchers during the course of the study.

We also extend our sincere thanks and appreciation to the Katsina State Government for allowing us to conduct this study. We hope that the results of this study will go a long way in improving livestock production in Nigeria and Katsina State in particular.

Conflict of interest

The authors hereby declare that there is no conflict of interest involved in this work.

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DETECTION OF PENCILLIN RESIDUE IN COW MILK AT KOMBOLCHA DAIRY FARMS, NORTHEASTERN ETHIOPIA

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Abstract

The use of antibiotics in dairy cattle for the treatment of diseases such as mastitis has contributed to the presence of residues in dairy products. Penicillin is commonly used veterinary drug to treat mastitis in dairy cattle. However, abundant use of it may be associated with the presence of its residues in milk at unsafe concentrations that can adversely affect public health. Therefore the present study was undertaken to detect penicillin residues in milk from six private dairy farms at Kombolcha North East Ethiopia during the time period between December 2015 and March 2016. A total of 100 milk samples were collected from healthy lactating cows of six dairy farms and were screened for Penicillin residues by using Delvotest SP NT. Moreover a questionnaire survey was carried out by personal interviews with all of the dairy farm owners from whom milk samples were taken, to assess their awareness about dairy farm management practices and antibiotic residues. Penicillin residues (at five parts per billion or higher) were found in 36% of the samples test. Prevalence of penicillin residues in different farms ranged from 25% to 50%. Questionnaire survey among the dairy farm owners established that not a single dairy farm owner ever used antibiotic test kit for detection of residues. Similarly post milking teat dipping, marking of milking equipment for treated cows and dry cow therapy for controlling mastitis was practised only in one dairy farm out of 6 farms. 4 farm owners (66.6%) knew the impact of residue in milk processing manufactures, however all of them were aware of antibiotic residues of having public health impact, drug withdrawal period, and all of them used different milking equipment and kept records for treated cows. The results of the present investigation indicate that penicillin residues exist within the milk of dairy farms in Kombolcha town. Co-ordinated nationwide surveillance of animals' by products for antibiotics residues is required together with determining their concentration, and initiating monitoring programmes and awareness campaigns to sensitize the populace on the dangers associated with residues in animal products in Ethiopia

Key words: Cow, Ethiopia, Delvotest, Penicillin-residues, raw-milk

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Introduction

Livestock ownership currently supports or sustains the livelihoods of an estimated 700 million rural poor, approximately 70% of the world's rural poor population (PPLPI, 2001). The dairy cow is one of the most important investments a farmer can make to improve their standing (ILRI, 2003) because of their inherent value, the nutritional valuable milk produced, the work they can perform, and the way it can help diversify farming activities. The importance of the dairy cow is expected to increase as food imports to sub-Saharan Africa (SSA) are projected to more than double by 2030 under a business as usual scenario (The World Bank, 2008). The World Bank classifies livestock as a high- value market and reports this market is the fastest-growing agricultural market in most developing countries (The World Bank, 2008).

Several measures have been initiated by the Ethiopian government to increase the productivity of livestock through various schemes and policies to improve breeding, feed and fodder availability and effective disease control which has resulted in increasing the milk production significantly. Small hold dairy farms has become an important secondary source of income for thousands of rural families and has assumed important role in providing employment and income generating opportunities Diseases are considered major obstacle for milk production in cows.Antibiotics have been used in the dairy industry for more than five decades to treat or prevent diseases like mastitis and to a lesser extent to increase milk production or improve feed efficiency. However, a wide and improper use of antibiotics like pencillin without veterinary control lead to presence of antibiotic residues in milk which give negative effects during the dairy productive sequences (Fonsesca *et al.,* 2009). Antibiotic residues above the MRL have different harmful effects on consumers like allergic reactions, disturbance of intestinal microflora (Dewdney *et al.,* 1991; Stoker *et al.,* 2007). Moreover, antibiotic residues beyond the MRL level can produce a great loss in fermented dairy products industry by inhibition of starter fermentation during cheese and yoghurt production (Molina *et al.,* 2003).

In Africa, in parallel to the incautious use of antibiotics in human medicine, agricultural sectors consume a large portion (50%) of antibiotics in animal farming to treat or to minimize potential outbreaks of diseases or to promote animal health (Miller *et al.,* 2003). In order to safeguard human health, the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have set standards for acceptable daily intake and maximum residue limits in foods (FAO and WHO, 1995).Regulatory limits for antimicrobial residues have been imposed on the dairy industry in many countries (Folly and Machado, 2001). However, there is no clear regulation controlling antibiotic contamination of feedstuffs in many African countries including Ethiopia. In a bid to reduce the cost of veterinary services, some farmers purchase drugs from the market without sound diagnostic advice leading to the abuse and misuse of drugs and also withdrawal periods are not being maintained. In Ethiopia, few research reports indicate the existence of chemical residues contamination in milk and meat such as Oxytetracycline and Penicillin G antibiotic residue in cow milk (Abebew, 2008). However no such study has been conducted on the residual levels of Penicillin in milk in South Wollo region of Ethiopia. Therefore the objectives of this work were to determine the prevalence of penicillin G residues in milk samples from small holder dairy farms in Kombolcha, and to assess the knowledge of the dairy farm owners about antibiotic residues in milk

Materials and Methods

Study Area

The study was conducted in six dairy farms of Kombolcha town. Kombolcha is located 376km northeast of Addis Ababa 11°84' N and 0.39°46' E at an altitude of 1500-18400 meters above sea level. The area receives an average rainfall and temperature of 750 mm - 900 mm

and 25°C - 30°C, respectively. About 33.6% of the district's area is under crop production, and 1.47% is serving as a grazing land. According to CSA (2006), the animal population of the study area comprises of 90,664 cattle, 12,975 sheep, 31,043 goats, 489 horses, 7,758 donkeys, 866 camel and 43,010 poultry.

Study Population

The study animals are lactating cows found in six dairy farms of Kombolcha town. These private dairy farms have a capacity ranging from 50 to 120 cows with the milk production of around per day. The all lactating cows from these farms belong to cross breeds. The milk produced from these dairy farms is not only supplied for local consumption in Kombolcha town but also to Kemise, Harbo, and different areas of Afar region.

Study Design and Sampling Method

Six dairy farms were selected using stratified random sampling method. Animals were selected for sampling using simple random sampling techniques. A total of 100 milk samples were collected randomly from the cows in six farms during the period between Decemebr to June, 2016.

Sample size was determined based on the previously reported values of antibiotic contaminated milk samples in Ethiopia (11.5 %) (Desalegn, 2008) by using the formula given for simple random sampling methods (Thrusfield, 2005).

$$n = 1.962\ [pexp\ (1\text{-}pexp)]/d2$$

Where: n = Required sample size Pexp = Expected prevalence of brucellosis (11.5% by Desalegn, 2008))

d = Desired absolute precision level at 95% confidence level (5%) 1.96 = The value of Z at 95% confidence level.

Thus the desired sample size for Pexp = 0.115 is n = 156, however, only 100 samples have been included in the study due to shortage of Delvo test kits.

Milk samples were collected from individual lactating cows from six dairy farms by standard milk sampling techniques (Sears *et al.,* 1993; Quinn *et al.,* 1994). Briefly, before sampling, teat ends were immersed in iodine solution (0.5%), and after approximately 20 seconds, teats were dried with disposable towels. Each teat end was scrubbed with a cotton pledget saturated in ethyl alcohol (70%). The first 3-4 streams of milk were discarded. The collecting vial were held as near horizontal as possible, and by turning the teat to a near horizontal position, approximately 20 ml of milk was collected in sterile caped tubes and numbered. Each sample was labelled legibly and accompanied by necessary identification information, which included date of sampling, type of samples and identification code. All milk samples were transported under chilled conditions to the laboratory of School of Veterinary Medicine, Wollo University and stored at –20 0C for approximately one week and thawed at room temperature for eight hours before analysis.

Questionaire survey

A questionnaire survey was administered to six dairy farm owners whose lactating cows were included in the study. The farms were visited to conduct personal interviews with the dairy farm owners in order to assess the awareness of farm owners about dairy farm management practices and antibiotic residues. Moreover, knowledge assessment and observations were made about the use of post-milking teat dips and training on dairy management, professional qualification of person who administers antibiotics to cows, record keeping, use of dry cow therapy and number of milking cows, marking of treated cows, milking of treated cows using separated equipment, and knowledge of withdrawal periods of antibiotics was also collected.

Screening test

Screening tests were conducted using a commercial Delvotest SP NP kit (The DSM Food Specialities B.V. The Netherlands). Delvotest is a broad spectrum screening

test for the detection of different antibiotic residues in milk. The test is made of an agar gel containing a standard number of bacterial spores (Bacillus stearothermophilus) and a pH indicator bromocresol purple. The test is based on the diffusion of antimicrobial residues that may be present in milk in to agar. The residues decrease or prevent the growth of bacteria. So, delay or prevent colour changing from purple to yellow. According to the manufacturing company, the sensitivity of the test for Penicillin is 0.003-0.004 IU/ml. The procedure was done according to the manufacturer's instructions. Briefly, 100 tubes containing agar containing nutrient and indicator were set up, followed by the addition of 0.1 ml milk samples from each of the 100 milk samples. For the positive and negative controls, we set up control milk solutions with and without antimicrobials respectively. The tubes were sealed and incubated at 64 °C for 3 hours. The color changes in each tube, including the positive and negative controls, were recorded. Orange/ yellow and purple colors indicated negative and positive results, respectively. The changes in the color of the agar in each tube were assessed by three people.

The distilled water was used as negative control for which the same above procedure was applied except that instead of the milk samples, the negative control (distilled water) was used. Pencillin-G purchased from local market was used as the positive control as per the kit guidelines.

Data Management and Analysis

The data collected was entered and managed in MS Excel. And will be analyzed by using SPSS Microsoft war version 20.0. Prevalence estimation of penicillin residue was determined using standard formula (i.e. the number of positive samples divided by the total number of samples tested). Descriptive statistics such as percentages and frequency distributions were used to describe the nature and the characteristics of the data.

Results

Out of 100 milk samples screened for the presence of penicillin residue using the Delvo SP-NT Test, 36 samples (36%) were positive for penicillin residues at levels above the defined MRL (indicated by purple color). Eleven of the total 100 milk samples (11%) were detected to have residues below the regulatory limit (or were partially positive as indicated by yellowish-purple mixture colour) and 53 milk samples (53%) negatively responded to the test. (Figure 1). Prevalence of Penicillin residues in milk samples among various dairy farms ranged from 25% to 50% as shown in Table 1.

Response to questionnaire survey in the dairy farm and their proportions of the dairy farms which applied different management practices were summarized in table 2. All the owners from six dairy farms showed willingness to participate in the survey and were interviewed. The average number of animals per farm was 55 and the mean number of milking cows was 2.1. All the owners revealed that that the animals routinely encountered many diseases and animals were being treated locally without consultation of veterinarian. Five out of six farmers had participated in any training of dairy farm management; however only one farmer reported that post milking teat dips and dry cow therapy for controlling mastitis was practiced. All of the interviewed dairy farm owners practised: markings for treated cows, different milking equipment for treated cows, record keeping practice for treated cows but none of them practised antibiotic kit use. Moreover all of them were aware of drug withdrawal periods, public health significance of drug residues, and of impact of residue in milk processing manufactures.

Discussions

Milk and dairy foods are considered healthy nutrient-rich foods especially for children because they serve as good sources of calcium and vitamin D as well as protein and other essential nutrients. It is therefore

important to provide a safe supply of milk in the society as regards chemicals as well as microbiological aspects. Several studies conducted in different parts of the world showed that antibiotic residues could be found in cow milk, which is the first choice of milk for human use worldwide. The present study was undertaken to determine the presence of penicillin G residues in milk samples from dairy farms in Kombolcha town, Ethiopia. The results showed a high residual presence of penicillin (36%), in dairy cows in six dairy farms of Kombolcha town. The high incidence of penicillin residues observed in the current study probably reflects widespread use of penicillin for treatment and prevention of cattle diseases and is possibly exacerbated by milk being sold for consumption whilst cattle are under a therapeutic or prophylactic regimen of penicillin or milking was practised before the end of the withdrawal period (Karimuribo et al. 2013). Since the milk from the six dairy farms under study is supplied to large community including Kombolcha, Dessie, Harbow, Kemise etc and some areas of Afar region, large section of society are exposed to small doses of antimicrobials. This was the first study on antibiotic residues in milk samples from Amhara region and 2nd in Ethiopia. The results in the present study were higher than 16.66% of penicillin residues observed in Nazareth dairy farms, Ethiopia (Abebew *et al.,* 2014). Also the results were higher than the prevalence of antibiotic residue in milk in other neighbouring countries [10.8 % in Kenya (Shitandi, 2001); 13 % in Uganda (Sasanya *et al.,* 2008); 23% in Nigeria (Shata *et al.,* 2015). This variation in the degree of contamination of milk with antibiotic residues could be explained by the level of legislation, differences in laboratory methods used for antibiotic residue detection and variation in farm management system in different regions (Ergin and Filazi, 2010). This could be indicative of the necessity to establish national standards based on the dietary intake patterns in Ethiopia.

In addition to the common adverse effects (toxicity, allergic reations, and disturbances in production of fermented milk by-products) associated with antibiotic residues, antibiotic resistance is considered to be a major threat to both humans and animals. Already antibiotic resistance against penicillin has been reported among the bacteria (Staphylococci) isolated from cow milk samples from the study area (Tassew *et al.,* 2016) which may result in treatment failures.

The questionnaire survey conducted during the study period included questions that were helpful to gain insights into farm management practices and knowledge of dairy farm owners associated with antibiotic usage and antibiotic residues in North East Ethiopia. Knowledge of antibiotic use is a crucial step in the prevention of antibiotic residue presence in animal by products. Despite huge efforts of the government and non-government institutions to promote and improve livestock management practices in the areas, our study highlighted that general knowledge of antibiotic residues among the dairy farm owners was so somehow encouraging, however farm management practices were not in line with their knowledge. For instance all the six dairy farm owners (100%) were aware of antibiotic residues of having public health impact, drug withdrawal period, and all of them used different milking equipment and kept records for treated cows. However not a single dairy farm owner ever used antibiotic test kit for detection of residues. Similarly post milking teat dipping, marking of milking equipment for treated cows and dry cow therapy for controlling mastitis which could have a great effect in protecting against new intramammary infections (Sanchez and Watts, 1999), was practised only in one dairy farm out of 6 owners. 4 farm owners (66.6%) knew the impact of residue in milk processing manufactures. Almost similar results were reported by Abebew *et al.,* (2014) in a questionnaire survey in Addis ababa. Pencillin was the most common drug used for different diseases and for mastitis, intramammary route was the favourite. Use of intramammary antibiotics in treatment of mastitis and waiting shorter than required time periods before milk collection are the major reasons for the presence of antibiotic residues in milk (Ruegg

and Tabone, 2000; Oliver *et al.,* 2011). All the dairy farm owners used contract laborers or part-time employee for milking activities, who were not giving much attention to the importance of hygienic conditions during milking. Health services were given mostly by the practitioners coming to the farms or sometimes by taking the animals to veterinary clinics. Regular health programs by professionals were not practiced. As the size of dairy farm increased, the tendency of antimicrobial residue on the farm also increased because large farms were more likely to take measures towards minimizing financial losses resulting from mortality on the farm such as prophylactic and therapeutic use of antibiotics. Easy access to antibiotics such as penicillin, together with a lack of awareness, insufficient extension activities and inadequate usage guidelines from manufacturers, may lead to misuse and overuse of the drug and possibly failure to observe withdrawal periods. These actions may contribute to the presence of high levels of antibiotic residues in milk. Indiscriminate and irrational use of antibiotics as observed in the present study without following standard procedures may result in unexpected residues in food supplies and could cause serious health hazards to consumers.

Conclusion

The present study although a preliminary work still highlights that problem of antibiotic residues exists in South wollo region which suggest that the public consuming milk and milk byproducts originating from the Kombolcha dairy farms may have been exposed to antimicrobial residues. Co-ordinated nationwide surveillance of animal byproducts for antibiotics residues is required together with determining their concentration, and initiating monitoring programmes and awareness campaigns to sensitize the populace on the dangers associated with residues in animal products in Ethiopia.

Acknowledgments

We Authors would like to express our special thanks to Wollo University Research and Community Sevice Office for funding of our research project. We would like also thank Mr. Mulubrihan Rahimeto for his kindness and invaluable help during our laboratory work conducted at Wollo University, School of Veterinary Medicine Microbiology Laboratory.

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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 2017

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

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Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

Letters to the Editor: the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

Key notes and special calls: The editor will, from time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

Conference Proceedings: Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/ conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

Submission Guidelines

Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, , title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-repetitive way. Subheadings may be accepted.
8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. . Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

Short Communications: Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
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The authors should be cited in a chronological order by year and then by a or b; in the reference list they should be listed alphabetically.

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

Examples of References

- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

Illustrations
Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

Abbreviations, Symbols and Nomenclature
All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

Ethical guidelines
BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
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When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

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