ISSN 0378 – 9721

Volume 65 No 1

January / Janvier 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

January
2017 Volume 65 No. 1
Janvier

AFRICAN UNION
UNION AFRICAINE

IBAR PUBLICATION
PUBLICATION DU BIRA

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

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

Annual subcription: US$ 100.00

ISSN 0378-9721

Abonnement pour un an : 100$

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

VOL. 65 NO. 1 **CONTENTS** **JANUARY, 2017**

FACTORS ASSOCIATED WITH ACQUISITION OF ENTERIC EPISODES IN CATTLE WASTE HANDLERS IN MOROGORO, TANZANIA.

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Abstract

The study explored risk factors for acquiring enteric disease in animal waste handlers as occupational hazards. A qualitative survey of 124 of respondents from Morogoro peri-urban and urban areas was done. Eight four respondents had experienced enteric episodes as compared to forty who didn't mention, and among those who mentioned the episodes, 55.95% had reported this to a health facility while 44.05% had consulted a nearby pharmacy or drug shop. Heaping in living plots was mostly practiced storage method within living plots (70.97 %). The percentage of handlers who were aware of risks for acquiring enteric pathogens from cattle after handling their wastes was 43.55%. There was limited awareness of government guideline on handling such wastes (3.23%) and washing hands without soap was found to be the most common health measures taken after handling animal wastes (70.16%). Statistically significant difference was observed in knowledge on occupational hazards ($p = 0.001$), zoonotic pathogens awareness ($p=0.05$), experience of participants ($p=0.05$) and protective gears ($p = 0.022$). The animal wastes handlers could be constantly exposed to risks associated with enteropathogens due to the lack of training on proper measures to handle animal wastes as occupational hazard, government interventions of formulating laws and by-laws which are user friendly. This could protect the attendants themselves against the zoonotic enteropathogens, the public in general and the environment.

Keywords: Peri-urban; Occupational hazards; Episodes; Urban Livestock

FACTEURS DE RISQUE D'EPISODES DE MALADIES ENTERIQUES CAUSEES PAR LA MANIPULATION D'EXCREMENTS BOVINS EN TANZANIE

Résumé

La présente étude a examiné les facteurs de risque associés aux épisodes de maladies entériques chez des exploitants manipulant des déchets animaux en Tanzanie, dans le cadre des risques professionnels. Une enquête qualitative a été menée auprès de 124 exploitants manipulant des déchets animaux dans les zones urbaines et périurbaines de Morogoro. Un grand nombre de répondants (84) ont indiqué qu'ils avaient connu des épisodes de maladies entériques ; un nombre inférieur (40) ne l'a pas mentionné au cours de l'enquête. Parmi les répondants qui ont mentionné des épisodes, 55,95% l'avaient signalé à un établissement de santé, tandis que 44,05% avaient consulté une pharmacie ou une droguerie à proximité. L'entassement était la méthode généralement pratiquée pour le stockage des excréments bovins (52,42%) : la plupart des exploitants entassaient ces déchets à l'intérieur des parcelles où ils vivaient (70,97%). Le pourcentage d'exploitants conscients des risques de contracter des agents pathogènes présents dans les excréments bovins à la suite de la manipulation de ces déchets était de 43,55%. L'enquête a révélé que les exploitants n'étaient pas suffisamment sensibilisés aux directives publiques sur le traitement de ces

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déchets (3,23%). L'étude a également fait ressortir que le lavage des mains sans savon était la « mesure sanitaire » généralement appliquée après la manipulation des déchets animaux (70,16%). Les manipulateurs de déchets exposés au risque de contracter des maladies entériques étaient ceux qui méconnaissaient les risques du métier (p=0,000 et OR=20,5), qui avaient une connaissance limitée des pathogènes zoonotiques entériques (p=0,019 et OR = 8.62), tandis que l'expérience en manipulation d'excréments bovins était statistiquement significative vis-à-vis des épisodes de maladies entériques (p=0,000 et OR = 13,5). Les manipulateurs de déchets animaux sont constamment exposés à des entéropathogènes en raison du manque de formation aux méthodes appropriées à utiliser pour manipuler les déchets animaux, se protéger et protéger l'environnement, cette lacune étant à l'origine d'une accumulation de pathogènes zoonotiques.

Mots-clés : péri-urbain ; professionnel ; risques ; épisodes ; urbain ; bovins

Introduction

Animal wastes are secretions from animals which include urine, faeces, natural openings as well as effluents from animal houses (Pell, 1997). These often harbour enteropathogens that are transmissible between livestock and human (Cleaveland *et al.,* 2001; Makita *et al.,* 2011). Concurrent with the rise in urban and peri-urban populations in developing countries; an increase in both livestock farming and informal trade of livestock has hiked (Adam, 2001) to increase the likelihood of humans acquiring pathogens from livestock. Keeping animals under these conditions in urban environments often affects water quality due to waste run-off into local streams, and as many urban areas lack consistent supply of safe water and therefore use contaminated, untreated water as source of irrigation water and home consumption which increases the exposure of the public to enteropathogens (Hutchison *et al.,* 2005b). Despite these pin pointed flaws guidelines for farmers to follow are rarely in place in low-income countries (DFID, 2002; Adam, 2001).

Urban and peri-urban agriculture (UPA) is an assimilation of crop, livestock production practices and promotion of these products (DFID, 2002). UPA is practiced in low-income countries as pro-poor survival strategy for the urban and peri-urban dwellers to provide employment and income generation (DFID 2002). The practices of UPA is believed to increase dissemination of zoonotic agents from livestock to humans, especially cattle due to their co-existence (McDaniel *et al.,* 2014). Previous studies in relation to animal waste handling in Tanzania (Lupindu *et al.,* 2012; Kusiluka *et al.,* 2012) were mainly concerned with waste handling practices, hygiene and perceptions of the farmers to health and environmental risks from animal wastes especially cattle manure. However these studies did not earmark the waste handlers as key players in enteropathogens transmission because of the frequent contact with animal wastes which are described to contain enteric bacteria, parasites and viruses. Hence the aim of this study was to assess awareness and establish the attributing risk factors for recurrence of enteric episodes among cattle waste handlers in urban and peri-urban areas as compared to those who don't keep cattle.

Materials and Methods

Study area and design

Morogoro urban and peri-urban area is located at latitude 5°58" and 10° 0" and longitude 35° 25" and 35° 30"E with an ambient temperature ranging 18°C - 30°C and rainfall ranging 600mm - 1200mm per annual. It has a population of 315,866 people (URT 2012). The study area represents fast growing townships with an expansion of livestock sector as side activity for income supplementation, source of animal proteins or permanent employment for informal employed dwellers. A Cross sectional survey was carried out from January to October 2014 involving a random sample of 124 cattle waste attendants from a sampling frame of 398 cattle owners. A list of farmers was obtained from Morogoro Municipal Livestock Development office, and allocation of respondents was done using the

random number generation. The study involved 12 wards which were referred to as either urban, due to population density with indoor cattle husbandry, or peri–urban, which are areas transcribing from rural to urban status adopted by (Makita *et al.,* 2011).

Farm Management questionnaire

The attendants were interviewed using a structured questionnaire with an in depth interviews carried out with key informants who included Livestock Field officers, cattle owners and neighbours who did not keep cattle. The questionnaire were pretested and revised to eradicate enumerator mistakes. Attendants were asked on episodes of enteric disease, the time spent by an individual per day handling animal wastes and experience on that occupation, characteristics of cattle rearing and manure management practices, distance from the farm or animal house to the disposal site, equipment applied during animal waste collection, knowledge on manure disposal, government concerns on consequences of waste disposal and the by-laws or guidelines produced by government, awareness on enteric disease in cattle and health measures taken after handling animal wastes. In addition neighbours who did not keep were also included in the study as a control group for comparison with those who keep cattle. Most of the questions were close-ended and few of them had the provision to respond as "others, please specify". The District Livestock field officers were requested to provide evidence of existing guidelines for monitoring animal waste management in their respective Wards and on animal waste management practices. The attendants who reported to have enteric episodes were furthermore requested to fill a consent forms so that their medical records for the last three months could be retrieved from the health centres where they attended.

Data processing and analysis

Data were primarily entered into Microsoft Excel for initial analysis and were further analysed using Statistical Package for the Social Sciences (SPSS VERISON 17). The Chi – square test was used for initial analysis to show relatedness of the associated factors for enteropathogens acquisition. The factors inferred by Chi-square test as statistical significant were further assessed using multivariate analysis to compare the cluster of families who keep cattle and those who do not. The inference was done basing on the odds ratio at 95% confidence intervals and $p \leq 0.05$ to determine the statistical significance.

Results

In total 124 respondents were interviewed (Table 1) to obtain information

Table 1: Socio-demography of respondents

Location	**Male**	**Female**
Peri-urban	59	09
Urban	55	01
Education level of the respondents	**Number**	**Percentage**
Informal education	22	17.7%
Primary school	75	60.5
Secondary school	19	15.3
Tertiary education	05	4.1
Graduates	03	2.4
Experience on handling cattle animal wastes		
Less than 1 year	50	40.4
1 year and more	74	59.7

on associated risk factors, which could be associated to acquisition of enteropathogens from cattle or their wastes. The participants were mainly causal labourers paid to manage cattle in terms of milking, animal house cleansing and fetching grasses in the cut and carry zero grazing system. The socio-demographic records showed that most of the employees in the study areas were men with primary education (60.48 %), and a number of them had experience of more than two years (59.7 %) either on the same or different farms, but performing the same duties. However neither location nor education level could be associated with enteric episodes in contrast to experience. The attendants who worked for more than s year were likely to acquire enteric episodes (OR 13.5, $p < 0.05$) when compared to those who work less than a year.

The study showed that livestock keepers in the study areas deposit animal wastes within 20 meters radius (71.0%) in their living plots. The frequency of waste collection from animal houses was found to be high in urban areas as compared to those in peri-urban areas, and the common method of manure management was heaping (52.4 %) as a key method of either storage before taken into crop fields or storage before selling or gifting to friends (Table 2). These factors were compared statistically in which neither the distance where the wastes were thrown, the frequency of waste collection from cattle houses nor treatments strategies were associated with acquisition of enteric episodes in the waste attendants.

The results showed that most of the respondents had limited understanding on the that enteric pathogens infecting cattle could also affect human (54.5 %), while 41.1 % were aware of occupational hazards in their work. It was also revealed that there were no government campaigns (96.8 %) or guidelines in terms of training farmers or livestock field officers on the best approach to dispose manure, where and only 3.2% mentioned to know the guidelines. The respondents had various schedule of activities where most of them first did cleaning, milking and lastly feeding the animals. With respect to health measures considered after handling manure, 37.9% washed hands without soap and some did nothing (16.1%). Most of the farmers had cattle houses which were roofed and cemented floor (68.6 %) which was more common in urban than in peri-urban areas (Table 3).

The families who were not keeping cattle reported fewer cases of enteric episodes where only 8.1% of the respondents mentioned the episodes. In spite of some respondents in the exposure group being aware of the occupational hazards when handling cattle wastes physical injuries were ranked as the most feared effect one could acquire (38.7 %) when attending cattle as compared to enteric episodes (12.1 %) as shown in Table 4.

Table 2: Animal waste management practices in the study areas

Waste treatment strategies	Frequency (%)	
Distance waste thrown		
Within 20 meter	88	71.0
More than 20 meter	36	29.0
Frequency of waste collection		
Everyday	85	68.5
More than one day	39	31.5
Storage methods		
Heaping on plots	65	52.4
Direct spreading in fields	23	18.6
Plastic bags	06	4.8
Throwing	30	24.2

Table 3: Factors associated with enteric episodes in cattle waste handlers

Factor considered	Responses (%)
Attendant's knowledge of enteric disease in cattle	
Yes	54 (43.6)
No	70 (56.5)
Knowledge on occupational hazards	
Yes	51 (41.1)
No	73 (58.9)
Government campaigns	
No	120 (96.8)
Yes	04 (3.2%)
Awareness on contamination when or after handling wastes	
No	80 (64.5)
Yes	44 (35.5)
Health measures taken after handling cattle wastes	
Washing hands without soap	47 (37.9)
Taking bath	13 (10.5)
Washing hands with soap	44 (35.5)
Do Nothing	20 (16.1)
Schedule of activities of the animal attendant	
Cleaning, milking and feeding	54 (43.6)
Milking, cleaning and grazing	35 (0.06)
Cleaning and milking only	13 (10.5)
Non - specific schedule	22 (17.7)

Table 4: Health risks associated with handling animals and their wastes

Associated health risk with cattle handling	Frequency (%)	
Respiratory conditions	08	06.5
Helminthosis	28	22.6
Enteric diseases	15	12.1
Skin infections	25	20.2
Physical injuries	48	38.7

Table 5: The list of significant factors associated with enteric episodes in animal waste handlers

Factors for enteric episodes	O. R	P value
Experience on the same occupation	13.50	0.05
Knowledge on cattle occupational hazards	20.5	0.006
Knowledge of enteric disease in cattle	8.62	0.05
Proper protective gears on the attendants	-2.45	0.022

Animal waste handlers who mentioned to have an episodes of enteric disease within six months, and a follow up was undertaken at medical centres to validate the information. Eighty seven responded that had experienced enteric episodes of which 53 of them were diagnosed at a health facility with the condition as per physician prescriptions.

The knowledge of the attendants on the enteric disease in cattle (OR 8.62. $p < 0.05$), occupational hazards associated with enteric pathogens in cattle (OR 20.5, $p < 0.006$) and protective gears during work (OR -2.45, $p < 0.022$) whereas the other factors were statistically not associated with enteric episodes (Table 5). Furthermore the means of the exposure (animal waste attendants) and control (family members who do not keep cattle) groups were compared as clusters using the paired mean t – test analysis where the means were shown to be statistically significant different ($p < 0.000$).

Discussion

The study aimed at determining the awareness among cattle waste handlers on factors associated with enteric episodes acquisition when and after handling cattle wastes, and to estimate influencing risk factors. There are studies which have been conducted to determine the zoonotic diseases in human and cattle ecosystems and those which could be due to animal waste management (Hutchison *et al.,* 2005b; Klein *et al.,* 2010; Makita *et al.,* 2011), but information on the qualitative description on the factors contributing to enteropathogens transmission in lowly developed countries is limited. In this study the collection of wastes from animal houses was mostly done using tools as previously described (Lupindu *et al.,* 2012; Kusiluka *et al.,* 2012) but most of the attendants did not put on proper protective gears. Our analysis revealed a trend that attendants who wore proper protective gears had lower likelihood of acquiring episodes of enteric disease as compared to those who did no put on proper protective gears. These findings are in accordance to some studies which have shown that pathogens, such as pathogenic E. coli strains, are associated with animal wastes and can be transferred if necessary measures are seriously neglected (Lipp et al, 2001; Kang'ethe *et al.,* 2007).

The results furthermore revealed that most livestock keepers heap the animal wastes along their residential plots, as previously reported (Lupindu *et al.,* 2012). This may be protective approach towards enteropathogens spreading, since it has been shown that storing wastes for a relatively long time leads to significant reduction of enteropathogens (Turner, 2002). On the other hand, despite this merit, animal wastes from such heaps could flush enteropathogens into the main water streams as reported by (Graham and Nachman, 2010). The attendants in this study were not well informed on the fact that handling of animal wastes exposed them to a risk of acquiring enteropathogens that is why most of them ranked physical injuries (38.71 %) to be most feared as compared to enteric infections (12.10%), this could apparently be true since they mostly encounter butts and any physical ailments from cattle, and enteric episodes could be associated with other etiological factors. In addition the results also showed that some livestock keepers did directly spreading of animal wastes in crop fields a finding which concurs with (Lupindu *et al.,* 2012); this has been reported to present a hygienic risk as enteropathogens compromise food security, environmental degradation, water quality and it may aid in spread of emerging diseases such as Avian influenza (Makita *et al.,* 2011; Woolhouse, 2005a).

The multivariate logistic regression analysis (Table 5) depicted that attendant who had served longer on the same occupation with less knowledge on existence of zoonotic pathogens were relatively at higher risk of enteric episodes. This finding concurs with Mołocznik (2004) and Martinez et al. (2009) who described exposure of various biological agents to workers in agricultural sector including livestock to be associated with ailment in such attendants. The reason for this surprising observation is currently unknown

and deserving attention, however it might be hypothesised that working for long periods as cattle waste attendant brings an attendance of acquiring bad working habits to ignore the standard operating procedures although if the working tools and gears are properly utilised the risk could be substantially mitigated.

The lack of knowledge regarding occupational hazards following cattle and their waste handling is supported by several authors whose findings reported enteropathogens or their traits acquisition in cattle waste attendants (Kang'ethe *et al.,* 2007a; Lupindu *et al.,* 2014) where both authors isolated verotoxigenic E coli O157:H7 from humans who were in contact with animal wastes while (Yaita *et al.,* 2014) reported E. coli of animal origin in human stools who had travellers' diarrhoea. Furthermore the duration for working in agricultural sector has been associated with likelihood of acquiring farm related risks in workers (Grace *et al.,* 2008). Ellis et al. (1998) and Graham and Nachman (2010) reported the accumulation of animal wastes around living plots to facilitate flushing of enteropathogens into water systems. In general, animal waste management needs to be improved so as to outweigh the adverse impacts of their disposal which compromises their benefits. The existence of the E. coli pathogenic factors were also confirmed by our previous work which sampled the respondents who reported the enteric episodes (Madoshi *et al.,* 2016).

Animal wastes in Tanzania are handled as other house hold wastes where they are either directly spread once freshly voided in crop fields, heaped under open air, selling or gifting to friends for free. Furthermore the practiced method to handle manure is based on the easiness to perform, habits and expediency (Mlozi, 1997a; Nasinyama *et al.,* 2006; Lupindu *et al.,* 2012). Apparently the study has shown that there are no guidelines from the state organs on proper treatment and disposal of animal waste, and only 3.23% of the respondents had awareness on guidelines a circumstance which gives an opportunity to farmers to dispose wastes at their vicinity and convenience.

This study was limited by the case definition of enteropathogens as an outcome factor since it was not specific enough to define the true aetiology of enteric episodes. Nevertheless it has provided an insight of the factors which could be regulated by either formal or informal training of animal waste handlers, local authorities and livestock personnel to properly manage the wastes. The results of this study imply that animal waste handlers have a high likelihood of enteric episodes compared to the family members where cattle are not kept. Since animal waste handlers including the family members where cattle are kept are not properly trained on occupation hazards and acquisition of enteropathogens that are transmissible between human and cattle, causing them to underrate the protective measures. Thus it is advised that proper formal training of handler, designing of good manure handling practices and formulation of practicable guidelines by local government can be tools for mitigating enteric episode scenarios.

Acknowledgements

The authors appreciate the Ministry of Foreign affairs of Denmark through the Danish International Development Agency (DANIDA) for financial support under "Opportunities and Challenges in Peri-urban Livestock Farming in Tanzania' project, coordinated at the College of Veterinary and Medical Sciences (SUA). The National Institute for Medical Research (NIMR) for research permit using human subjects (NIMR/HQ/R.8a/Vol.IX/1883), the participants who willingly participated and Executive Directors for Morogoro rural and municipal councils.

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PERFORMANCE AND ILEAL CHARACTERISTICS OF FINISHING BROILERS FED DIETS SUPPLEMENTED WITH PREBIOTICS

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Abstract

An experiment was conducted to evaluate the effect of prebiotics supplemented diets on performance characteristics and gut morphology of broiler chickens. The study involved 320 day-old Anak broiler chicks, used to assess the utilization of prebiotics [*Mannose oligosaccharides* (MOS) and *Lactose oligosaccharides* (LOS)] by broiler chicks. The chicks were alloted to eight treatment groups of 40 birds each and four replicates of 10 birds each in a 2 x 4 factorial arrangement. The additives (MOS and LOS) were added to the diets at four levels (0, 250, 500 and 750ppm) per additive. The birds were fed for 56days, after which data on feed intake, body weight gain, feed conversion ratio and ileal morphology were collected. The data was subjected to analysis of variance and significant means were separated using Duncan's Multiple Range Test. Results showed that final weight values significantly ($p<0.05$) ranged from 1276.67 g – 1503.38 g. The ileum morphology of the finishers broilers showed that villi height, lamina propria depth, basal width and apical width were significantly ($p<0.05$) influenced by prebiotic sources and levels. The villi height of 2250.00µm was highest at 500ppm MOS level of inclusion, while the least value of 538.30µm was obtained in birds fed 0ppm Lactose inclusion. It was therefore, concluded that, prebiotic MOS at 500ppm could be used in feed to obtain the better weight gain and FCR, with normal gut morphology of broiler chickens.

Keywords: Performance, ileal morphology, broilers, *mannan oligosaccharide*, *lactose oligosaccharide*

PERFORMANCE ET CARACTERISTIQUES DE L'ILEON DES POULETS DE CHAIR EN PHASE DE FINITION RECEVANT UNE ALIMENTATION SUPPLEMENTEE EN PREBIOTIQUES

Résumé

Une expérience a été menée dans le but d'évaluer l'effet des régimes alimentaires supplémentés en prébiotiques sur la performance, les caractéristiques et la morphologie intestinale des poulets de chair. L'étude a utilisé des poussins de poulet Anak âgés de 320 jours pour évaluer les effets de prébiotiques (mannose-oligosaccharides ou « MOS » et lactose-oligosaccharides ou « LOS ») chez les poussins de chair. Les poussins ont été répartis en huit groupes de traitement de 40 oiseaux chacun avec quatre répétitions de 10 oiseaux chacune, selon un dispositif factoriel 2 x 4. Les additifs (MOS et LOS) ont été incorporés aux régimes à quatre niveaux (0 ; 250 ; 500 ; et 750 ppm) par additif. Les oiseaux ont reçu cette alimentation pendant 56 jours, après quoi les données sur la quantité d'aliments ingérés, le gain de poids corporel, l'indice de consommation et la morphologie de l'iléon ont été recueillies. Ces données ont été soumises à une analyse de variance, et les moyennes significatives ont été séparées en utilisant le test à gammes multiples de Duncan. Les résultats ont montré une variation significative ($p <0,05$) des valeurs pondérales finales : de 1276,67 g à 1503,38 g. La morphologie de l'iléon des poulets de chair a révélé que la hauteur des villosités, la profondeur de la lamina propria, la largeur basale et la largeur apicale ont été significativement ($p <0,05$) influencées par les sources et les niveaux des prébiotiques. La hauteur des villosités de 2250,00µm était la plus élevée au niveau d'inclusion 500ppm de MOS, tandis que la valeur la plus faible de 538,30µm a été

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obtenue chez les oiseaux soumis à l'inclusion de lactose à 0ppm. Il a donc été conclu que les prébiotiques MOS à 500 ppm pouvaient être utilisés dans l'alimentation pour obtenir les meilleurs résultats en termes de gain pondéral et d'indice de consommation, avec une morphologie intestinale normale des poulets de chair.

Mots-clés : performance, morphologie de l'iléon, poulets de chair, mannose-oligosaccharide, lactose-oligosaccharide

Introduction

Antibiotics have been used in agriculture to promote growth and welfare of animals for the past 80 years in the United States and other countries (Dibner and Richards, 2005). The long term and extensive use of antibiotics in human and veterinary medicine have resulted in selection of resistant bacterial strains. Resistance among gram-negative bacteria, like *E. coli* and *Salmonella spp*. has generated the strongest objection to antibiotic use (Gustafon and Bowen, 1997). As an illustration of many other surveys in the literature conducted to show antimicrobial resistance, Nayak and Kenney (2002) showed that 25% of the Salmonella isolates from turkey flocks in West Virginia were resistant to one or more antibiotics, including gentamycin, spectinomycin, streptomycin, tetracycline, tobramycin and trimethoprim. The European Union ban on the use of most of the sub-therapeutic antibiotics in animal feed was based on the fears of antibiotic resistance being transferred via the food chain and proposed the precautionary principle since 1997 (Cervantes, 2005). Cervantes (2005) reported that, the ban of antibiotic feed additives has resulted to significant decrease of antibiotic resistance among bacteria isolated from raw meat products. Additionally, associated with the ban on feed-additive antibiotic was a rise in the incidence of colibacillosis and necrotic enteritis in poultry (Truscott and Alsheikhly, 1997; Ferket, 2003) and pigs (Casewell *et al.,* 2003; Cervantes, 2005).

However, with the limitation of antibiotic growth promoters (AGPs), the consequent need for their total withdrawal becomes necessary. Hence, the need to find alternative feed supplements that have probiotic and prebiotic effects and promote growth of broiler chickens, thus achieving both enhanced performance and good health without the use of antibiotics. In order to find better alternatives to AGPs, research has focused on utilization of feed additives such as enzymes, probiotics, prebiotics, symbiotic products and even nutrient to enhance gut health and prevent or limit production losses due to enteric infections.

Prebiotics are dietary components that are not digested by the host, but they benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gastro-intestinal tract (GIT), predominantly those that produce short chain fatty acids (SCFA). However, dietary supplementation of prebiotics has been shown to stimulate these unculturable bacteria in humans (Rastall *et al.,* 2005), and pigs (Konstantinov *et al.,* 2003).

Lactose is a major type of sugar found in milk and milk products, including human milk. It constitutes less than 80% of the solid in milk, in animals. Lactase and enzymes produced by the small intestine break down lactose so it can be absorbed into the blood stream. Lactose is one of the prebiotics and it functions by lowering the gut pH through lactic acid production, inhibiting/preventing colonization of pathogens, modifying metabolic activities of normal intestinal flora and stimulation of the immune system (Gomez and Angels, 2011).

*Mannan oligosaccharide*s are derived from yeast cell wall and work slightly differently from fructo-oligosaccharides. The major difference between fructo-oligosaccharides and mannan-oligosaccharides (MOS) is that MOS products do not selectively enrich for beneficial bacteria. The binding and removing of pathogens while stimulating the immune system are the primary mode of action for MOS products (Patterson and Burkholder,

2003). Therefore, this study was designed to evaluate the effects of prebiotic supplemented diets on performance and gut morphology of finishing broiler chickens.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry Unit of the Directorate of the University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. It falls within the rainforest vegetation zone of South-Western Nigeria at latitude 7o13` 49.46"N, longitude 3o26` 11.98"E and altitude of 76 meters above sea level. The climate is humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity is 34.7oC and 82% respectively.

Experimental Diets

A basal diet was formulated to conform to the nutrient requirements of broiler chickens in the Tropics according to NRC (1994) as shown in Table 1. Diets were prepared by adding *Lactose oligosaccharide* or *Mannan oligosaccharide* at 0ppm, 250ppm, 500ppm and 750ppm), and this gives a total of eight diets.

Experimental birds, design and management

A total of 320 day-old broiler chicks of commercial strain (Anak 2000) were purchased from Nubreed Farms Ltd., Abeokuta, Ogun State, Nigeria. The birds were fed ad libitum on broiler starter for a period of 28 days (Table 1) and finisher diet (Table 2) from week 5 to 8. They were allocated randomly to eight dietary treatment groups of 40 birds each. Each treatment was further divided into four replicate groups of ten birds each. The birds were assigned randomly to eight dietary treatments in a 2 × 4 factorial arrangement. Prebiotics (Lactose and Mannose) were included in a 2 x 4 factorial arrangement of 4 inclusion levels (0ppm, 250ppm, 500ppm and 750ppm) and two prebiotic sources (Lactose and Manose). Fresh and clean water was supplied ad libitum. All the routine and occasional management practices were carried out as at when due using standard practice.

Data Collection

Growth parameter

Data were collected on growth indices such as: weight gain, feed intake and feed conversion ratio.

Feed conversion ratio (FCR) was calculated as the ratio (kg/kg) of average daily DM intake to average daily BW gain.

Gut morphology of broiler chickens

This analysis was carried out at the Veterinary Pathology Laboratory of Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. At the end of the experiment (56 days), one bird per replicate was slaughtered. The small intestine (ileum, duodenum and jejunum) was excised immediately and fixed in formalin for the measurement of villus height (VH), Apical width (AW), Lamina Propria Depth (LPD) and Basal width (BW). They were determined at a magnification of x10.

Proximate Analysis

The proximate composition of the diets were determined according to the methods described by AOAC (2000).

Statistical Analysis

All data collected were analysed using the General Linear Model of SAS (1999), and means were subjected to analysis of variance (ANOVA) in a 2 x 4 factorial design (SAS, 1999), while significant ($P<0.05$) means were compared using Duncan's Multiple Range Test (Duncan, 1955).

Result and Discussion

The interaction effect for prebiotic sources and levels on the performance of broiler finisher is shown in Table 3. Total feed intake and FCR were affected ($P<0.05$) by sources of prebiotic supplemented diets. All birds fed with lactose and 500ppm mannose

Table 1: Gross composition of Experimental Diets to be fed to broiler chicks (0-4weeks) (g/kg)

	Lactose				Mannose			
Ingredients	**0ppm**	**250ppm**	**500ppm**	**750ppm**	**0ppm**	**250ppm**	**500ppm**	**750ppm**
Maize	460.00	460.00	460.00	460.00	460.00	460.00	460.00	460.00
Soyabean meal	330.00	330.00	330.00	330.00	330.00	330.00	330.00	330.00
Fish meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wheat offal	100.00	100.00	100.00	100.00.	100.00	100.00	100.00	100.00
LOS	+	++	+++	++++	-	-	-	-
MOS	-	-	-	-	+	++	+++	++++
Oyster shell	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Bone meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Methionine	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Analysis								
M.E (Mjg-1)	2,743.30	2,743.30	2,743.30	2,743.30	2,743.30	2,743.30	2,743.30	2,743.30
Crude Protein	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Crude Fibre	3.78	3.78	3.78	3.78	3.78	3.78	3.78	3.78
Ether Extract	3.69	3.69	3.69	3.69	3.69	3.69	3.69	3.69
Ca (%)	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78
AV Phos (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Methionine (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65

Vitamin and mineral premix contained the following per kg diet:Vit;4,000,000Iu,Vit D:80000,VitB12:25mg, Niacin:6000mg,Vit E40000,Vit k3 800mg,VitB3 1000mg,Vit B26000mg,Vit B6:5000mg panthotenic Acid:20000, Folics Acid:200mg, Biotine 8mg, Maganese:30000, Iron8000mg, Zinc:2000mg, Copper: nill, Cobalt: 80mg, Iodine:400mg, Selenium:40mg, Choline:80000mg

*** Lactose: -: 0ppmL.S, +:250ppm L.S ++:500ppm L.S +++:750ppm L.S,*

****Mannose: -: 0ppmB.C, +:250ppmB.C, ++:500ppmB.C, +++:750ppmB.C*

had higher (P<0.05) values of final live weight and total weight gain. Improved (P<0.05) feed conversion ratio was observed on birds fed with mannose-supplemented diet at 500ppm while other dietary treatments recorded lower values. This was in line with findings of Hooge (2004) who concluded that, birds fed MOS showed improved growth performance and feed conversion ratio compared to birds fed diets containing antibiotic growth promoters. Also, Blake et al,. (2006), indicated that, the addition of MOS to broiler diets showed a positive influence in promoting body weight gain with values far above birds fed control diet. Higher final live weight, weight gain and improved FCR recorded for broilers fed 500ppm mannan oligossacharides (MOS) suggested the superiority of MOS over lactose (LOS) in supporting growth and feed utilization. The result of the interaction effect of prebiotic sources and levels on the ileum morphology (Table 4), showed that villi height, lamina propia

Table 1: Gross composition of Experimental Diets to be fed to broiler chicks (0-4weeks) (g/kg)

	Lactose				Mannose			
Ingredients	**0ppm**	**250ppm**	**500ppm**	**750ppm**	**0ppm**	**250ppm**	**500ppm**	**750ppm**
Maize	470.00	470.00	470.00	470.00	470.00	470.00	470.00	470.00
Soyabean meal	260.00	260.00	260.00	260.00	260.00	260.00	260.00	260.00
Vegetable oil	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Fish meal (72%)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Wheat offal	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00
Bone meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Oyster shell	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Methionine	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
*Premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
**Lactose	-	+	++	+++	-	-	-	-
***Mannose	-	-	-	-	-	+	++	+++
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Analysis								
ME (MJKg-1)	12.80	12.80	12.80	12.80	12.80	12.80	12.80	12.80
Crude Fibre	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.88
Crude Protein	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Fat	3.51	3.51	3.51	3.59	3.51	3.51	3.51	3.51
Ca (%)	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47
AV phos (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Methionine (%)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

Vitamin and mineral premix contained the following per kg diet:Vit;4,000,000Iu,Vit D:80000,VitB12:25mg, Niacin:6000mg,Vit E40000,Vit k3 800mg,VitB3 1000mg,Vit B26000mg,Vit B6:5000mg panthotenic Acid:20000, Folics Acid:200mg, Biotine 8mg, Maganese:30000, Iron8000mg, Zinc:2000mg, Copper: nill, Cobalt: 80mg, Iodine:400mg, Selenium:40mg, Choline:80000mg

*** Lactose:-: 0ppmL.S, +:250ppm L.S ++:500ppm L.S +++:750ppm L.S,*

****Mannose: -: 0ppmB.C, +:250ppmB.C, ++:500ppmB.C, +++:750ppmB.C*

depth, basal width and apical width of broiler finishers were influenced (P<0.05) by prebiotic sources and levels. The villi height (P<0.05) was highest at 500ppm mannose level of inclusion (2250.00um), while it was least (583.30um) at 750ppm mannose inclusion. Higher (P<0.05) values in BW of birds fed 500ppm mannose was recorded, and the least (P<0.05) value of 50.00um in birds fed 500ppm levels of lactose inclusion. The trend observed in width (apical) of villus showed that birds fed 0ppm lactose, 250ppm mannose and 500ppm mannose recorded higher values, and those on 500ppm lactose had least (P<0.05) values. The report

Table 3: Interaction effect of prebiotic sources and prebiotic levels of inclusion on the performance characteristics of finishing broilers

Parameters	Lactose				Mannose				
	0ppm	250ppm	500ppm	750ppm	0ppm	250ppm	500ppm	7500ppm	SEM
Average initial weight (g)	666.67	640.0	660.0	640.0	660.0	660.0	680.0	600.0	19.33
Average final weight (g)	1370.0^{ab}	1356.67^{ab}	1383.33^{ab}	1386.67^{ab}	1280.0^{b}	1280.0^{b}	1503.33^{a}	1276.67^{b}	66.58
Total weight gain (g)	703.33^{ab}	716.67^{ab}	723.33^{ab}	766.67^{ab}	620.0^{b}	620.0^{b}	783.33^{a}	656.67^{b}	66.38
Total feed intake (g)	1356.67^{ab}	1356.67^{ab}	1383.33^{a}	1453.33^{ab}	1280.0^{ab}	1280.0^{ab}	1273.0^{ab}	1276.0^{b}	66.58
Feed conversion ratio	1.93^{ab}	1.89^{b}	1.91^{b}	1.89^{b}	2.06^{a}	2.06^{a}	1.62^{c}	1.94a^{b}	0.06

^{abc} *Mean on the same row having different superscripts are significantly different (P<0.05)*

Table 4: Interaction effect of prebiotic sources and prebiotic levels of inclusion on gut morphology (μm) of finishing broilers

Parameters	Lactose				Mannose				
	0ppm	250ppm	500ppm	750ppm	0ppm	250ppm	500ppm	7500ppm	SEM
Villi height	538.30^{d}	550.00^{d}	750.00^{d}	922.70^{cd}	975.00^{cd}	1372.30^{bc}	2250.00^{a}	1800.00^{ab}	129.44
Lamina propia depth	725.00^{a}	512.67^{bc}	437.67^{cde}	462.67^{cde}	262.67^{f}	562.67^{b}	337.67^{ef}	375.00^{def}	30.21
Basal width	325.00^{bc}	427.70^{b}	100.^{cd}	425.00^{b}	127.70^{cd}	162.70^{cd}	750.00^{a}	100.00^{cd}	59.59
Apical width	125.00^{a}	75.00^{c}	100.00^{b}	75.00^{a}	57.67^{d}	112.67^{a}	125.00^{a}	60.00^{cd}	8.35

^{abcdef} Mean on the same row having different superscripts are significantly different (P<0.05)

of intestinal morphology of the broiler finisher in the present study supported the works of Savage et al. (1996) and Iji et al. (2001) that MOS influenced the physical properties of the epithelial lining by increasing the number of globlet cells with an inclusion level of mannose.

Conclusion

Higher final live weight, weight gain, improved FCR and villi height recorded for broilers fed 500ppm mannan oligossacharides (MOS) suggested the superiority of MOS over lactose (LOS) in supporting growth and gut morphology of broiler chickens.

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SEASONAL PREVALENCE AND COMPARISON BETWEEN SENSITVITY OF CONVENTIONAL AND SEROLOGICAL DETECTION OF *FASCIOLOSIS* IN RUMINANTS SLAUGHTERED IN MAIDUGURI ABATTOIR, NORTHEAST NIGERIA

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Abstract

The study was conducted to determined seasonal prevalence of *fasciolosis* and compare between its conventional diagnosis and serological identification in ruminants slaughtered at Maiduguri abattoir, northeastern Nigeria. Nine hundred samples each of faeces and blood; that is 300 each from cattle, sheep and goats was collected for the analysis. The prevalence of Fasciola in cattle, sheep and goats during the rainy season were: 12.0% (95% confidence interval <CI>: 8.8-16.2), 9.0% (95% CI: 6.3-12.8) and 7.3% (95% CI: 4.9-10.9) respectively and during the dry season, the prevalence were 15.3% (95% CI: 11.7-19.8), 10.3% (95% CI: 7.4-14.3) and 11.0% (95% CI: 7.9-15.1) respectively. The odds of the likely hood of infestation with fasciola during the rainy season was higher in goats (odd ratio<OR>: 1.72; 95% CI: 0.99-3.05), followed by sheep (OR: 1.38; 95% CI: 0.82-2.35) and least in cattle (OR: 0.73; 95% CI: 0.33-1.01). While during the dry season, the odds was higher in sheep (OR: 1.57; 95% CI: 0.97-2.58), followed by goats (OR: 1.47: 95% CI: 0.91-2.38) and then cattle (OR: 0.68; 95% CI: 0.42-1.10), there was no significant association between the species in both season (P>0.05). The result of the comparative evaluation of Fasciola revealed that *ELISA* was more sensitive than the sedimentation method (AUC for *ELISA* = 0.512–0.521) while the (AUC for sedimentation method = 0.500–0.521) during both rainy and dry seasons. Fasciola infestation was prevalent in ruminants seasonally and endemic in the study area. Serological method was found to be the diagnostic technique of choice for the diagnosis of *fasciolosis*. Awareness needs to be created on *fasciolosis* in the study area and sensitive diagnostic tool like *ELISA* should be use for the early detection of the parasite.

Keywords: *Fasciolosis*, prevalence, abattoir, *ELISA*, Maiduguri

PREVALENCE SAISONNIERE ET COMPARAISON ENTRE LA SENSIBILITE DU DIAGNOSTIC CONVENTIONNEL ET DE L'IDENTIFICATION SEROLOGIQUE DE LA FASCIOLOSE CHEZ LES RUMINANTS ABATTUS A L'ABATTOIR DE MAIDUGURI DANS LE NORD-EST DU NIGERIA

Résumé

Cette étude a été menée dans le but de déterminer la prévalence saisonnière de la fasciolose et d'établir une comparaison entre le diagnostic conventionnel et l'identification sérologique de la fasciolose chez les ruminants abattus à l'abattoir de Maiduguri, dans le nord-est du Nigeria. Neuf cent échantillons d'excréments et de sang, soit 300 provenant de chacune des trois espèces (bovines, ovines, et caprines), ont été recueillis pour analyse. La prévalence de Fasciola chez les bovins, les ovins et les caprins pendant la saison des pluies était respectivement de 12,0% (IC 95% <IC> : 8,8-16,2), 9,0% (IC 95% : 6,3-12,8) et 7,3% (IC 95% : 4,9-10,9) ; et pendant la saison sèche, elle était respectivement de 15,3% (IC 95% : 11,7-19,8), 10,3% (IC 95% : 7,4-14,3) et 11,0% (IC 95% : 7,9-15,1). Les probabilités d'une infestation par Fasciola pendant la saison des pluies étaient plus élevées chez l'espèce caprine (rapport de cotes « odd ratio » <OR> : 1,72 ; IC 95% : 0,99-3,05), suivie de l'espèce ovine (OR : 1,38 ; IC 95% : 0,82 ; 2,35), et enfin l'espèce bovine (OR : 0,73 ; IC 95% : 0,33-1,01). Si pendant la saison sèche, les probabilités étaient plus

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élevées chez les ovins (OR : 1,57 ; IC 95% : 0,97-2,58), suivis des caprins (OR : 1,47 : IC 95% : 0,91-2,38) et des bovins (OR : 0,68 ; IC 95% : 0,42-1,10), on n'a pas relevé d'association significative entre les espèces dans les deux saisons (P> 0,05). Le résultat de l'évaluation comparative de Fasciola a révélé que le test ELISA était plus sensible que la méthode de sédimentation (l'AUC pour ELISA = 0,512-0,521 ; l'AUC pour la méthode de sédimentation = 0,500-0,521) pendant les deux saisons, pluvieuse et sèche. L'infestation de Fasciola était répandue saisonnièrement chez les ruminants et endémique dans la zone d'étude. La méthode sérologique s'est révélée être la technique de choix pour le diagnostic de la fasciolose. Il faudrait mener une sensibilisation sur la fasciolose dans la zone d'étude, et utiliser ELISA comme outil de diagnostic sensible pour la détection précoce du parasite.

Mots-clés : fasciolose, prévalence, abattoir, ELISA, Maiduguri

Introduction

Fasciolosis also known as fascioliasis, liver rot and distomatosis is an important parasitic disease in tropical and subtropical countries which limited the productivity of ruminants (Keyyu *et al.,* 2005). It is a serious disease of cattle, sheep, goats, buffalo and other ruminants cause by trematodes usually Fasciola gigantica and rarely Fasciola hepatica in the tropics and the life cycle of which involves snails as an intermediate host (Magaji *et al.,* 2014). Ruminants provide animal protein for humans in general (Ikeme and Obioha, 1973). In Nigeria, they serve as a major source of income, animal protein, hide and skin for industrial raw materials and contribute significantly to agricultural gross domestic product GDP and hence economy of the nation (Biu and Babangida, 2004). They also play an important role in the social and cultural life of most of the communities in the northern part of Nigeria (Tewe, 1997). Borno State is the largest livestock producing area in Nigeria and accounts for about 30% and 10% of the cattle and small ruminant populations in the country, respectively (Nwosu and Srivastava 1993). However, the productivity of cattle has been limited by parasitic infections including *fasciolosis* (Keyyu *et al.,* 2005) and this threatens the food security of the population and its social balance. The disease is usually characterized by a chronic, sometimes acute or subacute inflammation of the liver and bile ducts, accompanied by submandibular oedema, anaemia, anorexia, general intoxication, and death (Ogunrinade and Ogunrinade, 1980).

Apart from its veterinary and economic importance throughout the world, *fasciolosis* is also of great public health importance which affects people all over the world (Mas-Coma *et al.,* 1999). According to the World Health Organization (WHO), Fasciola affects nearly 2.4 million people, from 61 countries. About 180 million people worldwide are at the risk of infection (Keiser and Untzinger, 2005; WHO, 2007). Therefore, *fasciolosis* is not only considered secondary zoonotic disease but also recognized as an emerging or re-emerging human disease in several countries of the world (Mas-Coma *et al.,* 1999). *Fasciolosis* is devastating parasitic trematodes that is recently considered to be attributed to climate change. Climate has an impact on the parasite and its intermediate host, Galba truncatula, where rainfall and temperature are the greatest influence on the transmission efficacy of the disease (Fox *et al.,* 2006).

Fasciolosis is an endemic disease of both human and animals in Nigeria (Biu *et al.,* 2013; Ejeh *et al.,* 2015). *Fasciolosis* is better known as a veterinary disease, human *fasciolosis* cases have been steadily rising since the 1970s, primarily in rural areas, and has been severely neglected by the medical community especially in Nigeria where confirmatory diagnosis of specific parasitic diseases are often not been carried out before treatment due to lack of infrastructural facility, increased spread and chronic nature, it is now a disease of global human concern (WHO, 2013).

In animals, *fasciolosis* is usually diagnosed with coprological examination and by using serological testing most commonly enzyme-linked immunosorbent assay (*ELISA*). The faecal examination is most commonly used technique which detects fluke eggs in faeces 8-12 weeks

Figure 1: Map of Borno State Nigeria showing the study area

of post-infection (Torgerson and Claxton, 1999). However the technique is not useful for early detection of infection. The biochemical and haematological profiling is also taken into account (Torgerson and Claxton, 1999; Graczyk and Fried, 1999). In most countries the incidence of *fasciolosis* are recorded at abattoir by meat inspection (Khaitsa *et al.,* 1994). The serological techniques are applied for early diagnosis of infection after 2-4 week post-infestation (Dumenigo *et al.,* 2000). Serologic diagnoses have been developed as an alternative approach to faecal egg detection which can test a large number of sera at a time and also detect infection earlier than faecal egg examination. There are evidences to show that serodiagnosis can detect the presence of infection as early as 2 weeks after infection (Fagbemi and Guobadia, 1995). The study was aimed to determined seasonal prevalence of *fasciolosis* and compare between its conventional diagnosis and serological identification in ruminants slaughtered at Maiduguri abattoir, northeastern Nigeria.

Materials and Methods

Study Area

The study was conducted in Maiduguri abattoir in Borno State. The state is situated within the semi-arid zone of West Africa. It lies on latitude 11˚05'N and longitude 13˚05'E and at an altitude of 354m above sea level. It has a total area of 72,609 square kilometre. The state shares boundaries with Adamawa State to the south, Gombe State to the West, Yobe State to the North – west, Cameroon to the East, Republic of Niger to the North and Chad Republic to the North – east. Borno State falls within the Sahel region of West Africa an area noted for great climatic and seasonal variations. The rainy season is characterized by a very short period which lasts for 3 – 4 months (June – September). It is followed by a cool dry period (harmattan) (October – March) and a dry period (April – June). The livestock population

within the state are: cattle (4,800,000), sheep (9,900,000) and goats (15,720,000). The types of animals slaughtered in the abattoir daily and their approximate number are: cattle (85 – 100), sheep (80 – 100) and goats (90 – 110).

Sample Size

To determine the sample size, the expected prevalence of fascioliasis in Maiduguri, Borno State will be taken into consideration by 42.7% (Nwosu and Srivastava 1993). According to (Thrusfield 2005), the formula for sample size determination with 95% confidence interval and at 5% absolute precision is as follows:

$$N = \frac{1.96^2 \times Pexp\ (1\text{-}Pexp)}{D2}$$

Where N=required sample size
1.96 = the value of Z at 95% confidence interval (C.I)

Pexp = expected prevalence

D = desired absolute precision (5%)

Therefore, at 42.7% expected average prevalence, the sample size will be

$$N = \frac{(1.96)^2 \times 0.427\ (1\text{-}0.427)}{(0.05)^2}$$

N = 376

But 900 of each samples (faeces and blood), 300 each from cattle, sheep and goats was collected for the analysis.

Sample Collection

Samples was collected for a period of one year covering two seasons from June to October, 2015 marking the rainy season and November to May, 2016 marking the dry season. Convenient sampling technique was used for the sampling and about 2g of faecal sample was collected directly from the rectum of each animal using hand (manually) with disposable hand gloves into a sample bottle for analysis. For bile collection, the whole gallbladder was removed from each animal species through gentle excision from the liver using scalpel blade. Care was taken to prevent spilling of the bile from the gallbladder to the thoracic cavity of the slaughtered ruminant. The bile was then emptied into a suitable container and 10% formalin solution was added for preservation prior to laboratory analysis. The adult liver flukes used in this study were collected from naturally infected ruminants which were brought to the abattoirs, by incising the liver along the biliary tracts taking all necessary precautions to avoid any damage to the parasite. The infected livers were squeezed manually to macerate the parenchyma and the flukes were carefully removed and placed into sterile sample bottles containing formalin and transported to laboratory for analysis.

Faecal, bile and adult parasites samples collected were placed into a clean and sterile universal bottles containing 10% formaldehyde solution for preservation and were taken to the University of Maiduguri Veterinary Parasitology Laboratory in a cool box for analysis and identification.

For serology, 10mls of blood was collected from each animal at the point of slaughter into a sterile universal bottle using 10ml syringes. The collected samples were kept in a slanting position for coagulation to take place. The clear serum were decanted into a clean sterile container then stored in a cool box before transporting to a laboratory for analysis. In the laboratory, the samples were further centrifuged at 150 revolutions per minute for further serum purification.

Laboratory Analysis

Two grams of faecal sample was weighed and grounded with pestle and mortar and mixed with 10% formal saline to make a solution. The mixture was sieved to separate it from the debris. Diethyl ether was added to the sieved mixture about one third of the test tube and centrifuged at 1500rpm for 5minute. Four layers where observed which are: diethyl ether, faecal debris, 10% formal saline and the sediment at the bottom of the test tube. The sediment was picked using pipette and

was dropped on a clean slide and covered with cover slip which was observed under a microscope using low magnification for egg identification.

Bile was then centrifuged at 1500rpm for 5minutes. The sediment was picked with a pipette, dropped on a clean glass slid, covered with a cover slip and observed under a microscope using low magnification for eggs identification

Each of the adult Parasites (Fasciola) was washed thoroughly 2 to 3 times in a 0.9% normal saline solution to remove debris. The adult parasites were pressed between two glass slides to facilitate visualization of the internal structures and fixed in 70% methanol (Langeron, 1949). The parasites were stained with Borexcamine and mounted on slides using DPX to dry for 24 to 48 hours and observed under stereo microscope for morphological identification for speciation.

Fasciola Antibodies Detection

Blood collected was centrifuged at 1500rpm for 5 minutes to obtain the serum. The serum was collected for Fasciola antibodies detection using DRG Fasciola gigantica IgG *ELISA* EIA-4503 (DRG Diagnostics International Inc, USA).

Principle of the Test

The test uses 96-well microtitration plates sensitised by a monoclonal antibody specific to one protein of Fasciola. This antibody is used to trap the protein as well as to purify it from lysate of the parasite. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the specific protein, whereas the even columns (2, 4, 6, 8, 10 and 12) contain only the monoclonal antibody.

Procedure of the Test

All components were brought to 21 °C ± 3 °C before use. The micro plate was removed from its wrapper. The blood sera were prepared and samples were diluted at 1:100. 990 µL aliquots of dilution buffer, prepared as instructed was distributed to 10 mL tubes. 10 µL aliquots of the samples were added to each of the tubes and mixed briefly on a mechanical stirrer. 20 µL aliquots of each of the samples were distributed to the microwells of a dilution plate. 180 µL of dilution buffer was added, mixed five times by orbital agitation. 90 µL aliquots of dilution buffer was distributed to the wells of the kit's microplate and 10 µL of the 1:10 was transferred prediluted samples, mixed five times by orbital agitation

The positive and negative sera were diluted at1:100. The sample were distributed at the rate of 100 µL per well and incubated at 21 °C ± 3 °C for one hour, covered with a lid. The plate was rinsed with the washing solution prepared as instructed by the manufacturer, 300 µL of the washing solution was added. The entire operation was repeated two times, taking particular care to avoid bubble formation in the wells using manual washer, however, the depth of the needles' was carefully immersed to the bottom of each well.

The conjugate was diluted 1:50 in the dilution buffer and100 µL of the diluted conjugate solution was added to each well, incubated for 1 hour at 21 °C ± 3 °C, covered the plate with a lid, washed and 100 µL of the chromogen solution was added to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells then incubated for 10 minutes at 21 °C ± 3 °C, protected from the light and uncovered. 50 µL of stop solution was added per microwell. The blue colour was changed into a yellow colour and read the optical densities in the microwells using a plate reader and a 450 nm filter.

$$OD = \frac{\text{(Delta OD Sample)}}{\text{(Delta OD Positive)}} \times 100$$

The following table was to determine serum degree of positivity.

0	+/-	+	++	+++
Val<10%	<= Val<15%	<= Val<45%	<= Val<75%	<= Val

The degree of positivity was interpreted as follows:
0: No Fasciola gigantica infestation, +/-: Dubious

outcome, Redo the test in a month, +: Low-grade infestation, ++: Moderate infestation, +++: Heavy infestation

The test revealed 8 cattle, 5 sheep and 7 goats were heavily infested; 7 cattle, 11 sheep and 8 goats were infested with a low grade while the rest were moderately infested with Fasciola parasite

Statistical Analyses

Data obtained from the study were analyzed using descriptive statistics, Chi-square, ROC and odd ratios with JMP version 11 software (SAS Institute Inc., Cary, NC, USA). Analysis was considered significant at $p<0.05$.

Results

Table 1 shows the seasonal prevalence of *fasciolosis* in three ruminants species (Cattle, Sheep and Goats) slaughtered in Maiduguri abattoir. The analysis revealed the prevalence of Fasciola in cattle, sheep and goats during the rainy season in the present study were: 12.0% (CI: 8.8-16.2), 9.0% (CI: 6.3-12.8) and 7.3% (CI: 4.9-10.9) respectively although there was no significant statistical association ($p>0.05$) between infestation with *fasciolosis* in each of the ruminant species slaughtered in Maiduguri abattoir during the rainy season ($\chi^2 = 3.923$, df = 2, p = 0.1406).

Also during the dry season, the prevalence were 15.3% (CI: 11.7-19.8), 10.3% (CI: 7.4-14.3) and 11.0% (CI: 7.9-15.1) respectively in cattle sheep and goats, no significant statistical association ($p>0.05$) between the infestation with fasciolo0.1273).

Table 2 showed the odds of the likelihood of infestation with *fasciolosis* during the rainy season was higher in the goats (1.72) than in the cattle (0.58) and the association was not significant $p>0.05$. The odds was also higher in the sheep (1.38) than the cattle (0.73) with no significant association ($p>0.05$). The odd of the likelihood of infestation with *fasciolosis* was higher in the goats (1.25) than the sheep (0.80) and the association was not significant ($p>0.05$).

During the dry season, the odd of the likelihood of infestation with *fasciolosis* was higher in the goats (1.47) than in the cattle (0.68) and the association was not significant ($p>0.05$). It was also higher in the sheep (1.57) than in the cattle (0.63) with no significant association ($p>0.05$), while in the sheep, it was higher (1.07) than in the goats (0.93) with no significant association ($p>0.05$).

Figure 2 showed the seasonal comparison of the sensitivity between conventional and serological methods in cattle. For each curve, area under the curve (AUC) showed the sensitivity of each method as employed for the analysis. The sensitivity of the conventional method was higher during the dry season (0.512) than during the rainy season (0.500) as compared to serological method with same sensitivity of 0.512 in both dry and rainy seasons.

Figure 3 showed the seasonal comparison of the sensitivity between conventional and serological methods in sheep. For each curve, area under the curve (AUC) showed the sensitivity of each method as employed for the analysis. The sensitivity of the conventional method was higher during the dry season (0.517) than during the rainy season (0.500) as compared to serological method with higher sensitivity of 0.519 in rainy seasons than in the dry season of 0.517.

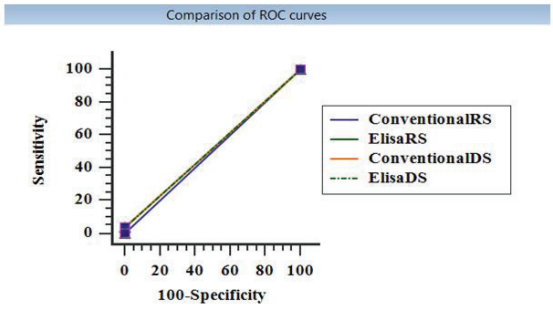

Figure 2: Receiver Operating Curve (ROC) showing Comparison of Sensitivity between Conventional methods and Serological method (ELISA) in the detection of Fascioliasis in Cattle Slaughtered in Maiduguri Abattoir in Rainy and Dry Seasons

Table I: Seasonal Prevalence of *Fasciolosis* in three Ruminant species (Cattle, Sheep and Goats) Slaughtered in Maiduguri Abattoir

	Rainy Season, 2015							Dry Season, 2016					
Species	**Number Examined**	**No. +ve**	**Prevalence (%)**	**95% CI**	**χ^2**	**p-value**	**df**	**No. +ve**	**Prevalence (%)**	**95% CI**	**χ^2**	**p-value**	**df**
Cattle	300	36	12.0	8.8 - 16.2	3.923	0.1406	2	46	15.3	11.7 - 19.8	4.122	0.1273	2
Sheep	300	27	9.0	6.3 - 12.8				31	10.3	7.4 - 14.3			
Goats	300	22	7.3	4.9 - 10.9				33	11.0	7.9 - 15.1			

where df = degree of freedom; No. ex = number examined;
No. +ve = Number positive

Table II: Comparative Seasonal Association of Infestation with Fasciola in species of Cattle, Sheep and Goats Slaughtered in Maiduguri Abattoir

	Rainy season 2015						Dry season 2016			
Species	**No. Examined**	**No. +ve**	**No. –ve**	**Odd ratio**	**95% CI**	**P-value**	**No. +ve**	**Odd ratio**	**95% CI**	**P-value**
GT by CT	300/300	22/36	278/264	1.72	0.9953, 3.0459	0.0520	33/46	1.47	0.9106, 2.3807	0.1158
CT by GT	300/300	36/22	264/278	0.58	0.3283, 1.0048		46/33	0.68	0.4200, 1.0982	
SH by CT	300/300	27/36	273/264	1.38	0.8166, 2.3530	0.2300	31/46	1.57	0.9701, 2.5762	0.0663
CT by SH	300/300	36/27	264/273	0.73	0.4249, 1.2245		46/31	0.63	0.3882, 1.0308	
SH by GT	300/300	27/22	273/278	0.80	0.4411, 1.4369	0.4557	31/33	1.07	0.6379, 1.8073	0.7914
GT by SH	300/300	22/27	278/273	1.25	0.6959, 2.2669		33/31	0.93	0.5533, 1.5677	

Key: GT = Goats; CT = Cattle; SH = Sheep

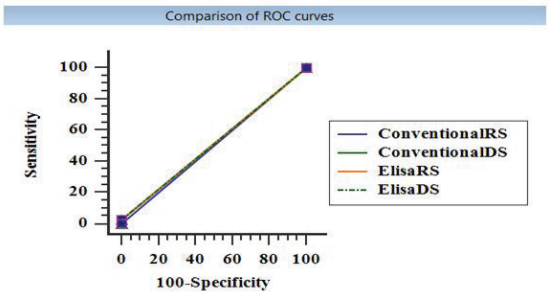

Figure 3: Receiver Operating Curve (ROC) showing Comparison of Sensitivity between Conventional methods and Serological method (ELISA) in the detection of Fascioliasis in Sheep Slaughtered in Maiduguri Abattoir in Rainy and Dry Seasons.

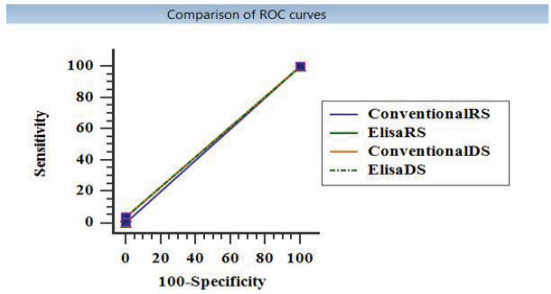

Figure 4: Receiver Operating Curve (ROC) showing Comparison of Sensitivity between Conventional methods and Serological method (ELISA) in the detection of Fascioliasis in Goats Slaughtered in Maiduguri Abattoir in Rainy and Dry Seasons.

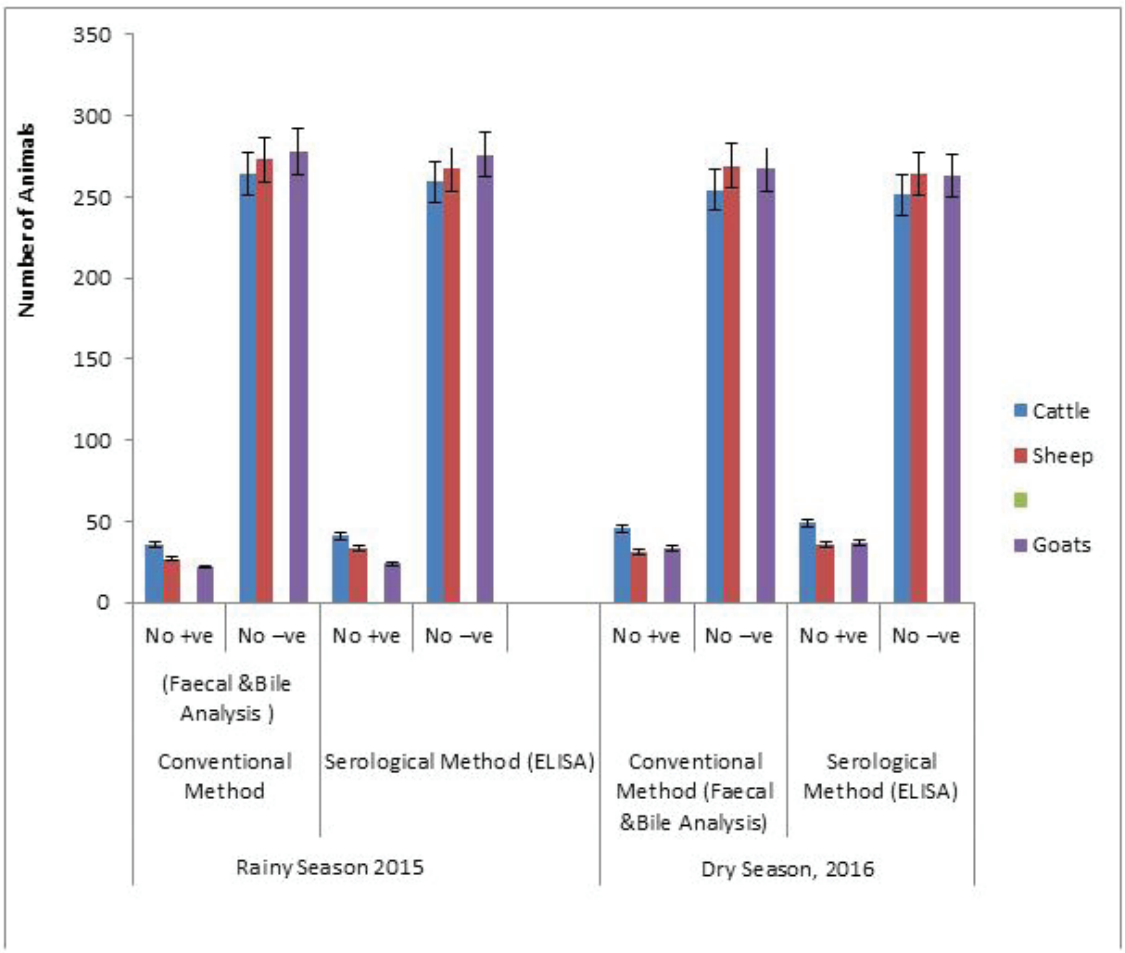

Figure 5: Seasonal Comparison of Sensitivity between Conventional method and Serological detection of *Fasciolosis* in Cattle, Sheep and Goat Slaughtered in Maiduguri Abattoir

Figure 4 showed the seasonal comparison of the sensitivity between conventional and serological methods in goats. For each curve, area under the curve (AUC) showed the sensitivity of each method as employed for the analysis. The sensitivity of the conventional method was higher during the dry season (0.521) than during the rainy season (0.500) as compared to serological method with same sensitivity of 0.521 in both dry and rainy seasons.

Figure 5 Showed the seasonal comparison of sensitivity between Conventional (faecal and bile analysis) and serological (*ELISA*) methods for Fasciola detection in cattle, sheep and goats slaughtered in Maiduguri abattoir. Serological (*ELISA*) method was more sensitive than conventional (faecal and bile analysis) method because it was able to depict all positive samples and some negative samples by conventional method in the rainy and dry seasons.

Discussions

To our knowledge, this is the first time comparism was made between conventional and serological method in detection of fasciola infestation in ruminants in Maiduguri abattoir, Northeastern Nigeria. The seasonal prevalence of *fasciolosis* in these ruminants was found to be higher in dry season than rainy season. This agreed with the previous report of (Ogunrinade *et al.,* 1981 and Ulayi *et al.,* 2005) both in Nigeria. We attributed this to the fact that during dry season irrigation activities are high and cattle tends to concentrate in such areas for pasture, these serve as sources of infection to animals grazing in such areas contaminated with metacercaria. Also older cattle which are in poor condition during dry season because of scarcity of forages may not be able to withstand the effect of relatively small number of flukes. The prevalence of *fasciolosis* was higher in cattle than in Sheep and goats in both seasons. This is in agreement with the reports of (Henok, and Mekonnen, 2011). During the dry season, the prevalence was slightly higher in goats than in sheep. The prevalence rate obtained from this study contrasted with that reported by (Nwosu and Srivastava, 1993) who reported 42.7% prevalence in cattle in the same study area. This is higher than reported in the current study. This could be as a result of slaughtering animals imported from Chad republic, Niger and Cameroun where they predominantly graze on river banks. During the current study, however, animals were not imported to the state from the neighbouring countries due to the activities of the insurgence. Only the indigenous animals were sampled for this study. It is also possibly that farmers are getting aware of the need to control the disease and are making efforts, resulting in the decrease in prevalence.

The comparison of conventional and serological (*ELISA*) methods for Fasciola detection showed slightly higher sensitivity for ELISA than the conventional method, but statistically there were no differences in rainy and dry seasons, though ELISA depicted all (100%) the positive samples obtained by conventional method. It is also 74% specific by detecting positive samples from the negative ones by conventional method. This is in agreement with Ardo et al. (2013) who reported 100% sensitivity and 81.7% specificity using ELISA kit in the diagnosis of Fasciola in cattle in Adamawa State. Palmer et al, (2014) reported 100% sensitivity and 88.80% specificity in cattle, sheep and horses in Australia. Afshan et al. (2013) in Pakistan also reported ELISA detection as more sensitive tool than conventional method. This is because ELISA test detected anti-Fasciola antibodies from the second week after infection and the advantage was to provide an earlier diagnosis than the coprological test which does not detect presence of Fasciola eggs until 8–10 weeks post infection when most of the pathology has occurred. However, the presence of antibodies does not always correlate with the existence of active infection; it may just show exposure to the parasite. The finding in this study is not in agreement with the previous study of Aliyu *et al.* (2014) in Zaria, Nigeria who reported higher prevalence in conventional method than serology (ELISA). We attributed this to the fact that the ELISA kit used in their study

was mainly to detect Fasciola gigantic than the one used in this study since the two species of fasciola might have marked dissimilarities in their antigenic epitopes.

Conclusion

Fasciola infestation was prevalent in Cattle, Sheep and Goats seasonally and was endemic in Maiduguri since the prevalence of the disease was recorded high in both seasons with an increase during the dry season. Serological (ELISA) method was found to be the diagnostic technique of choice for the diagnosis of Fasciola as it was more sensitive and specific than conventional method (faecal and bile analysis). Awareness needs to be created on the importance of prevention and control of animal diseases especially, *fasciolosis* in the study area. Grazing land should be provided for herdsmen and snail breeding should be control on such lands during rainy and dry season. Sensitive diagnostic tool like ELISA should be use for the early detection of the parasite in individual animal before slaughter and animals coming into the country should be properly screened for *fasciolosis*.

Acknowledgement

The authors would like to acknowledge the staff of Ministry of Agriculture and Forestry Resources and animal handlers in Maiduguri abattoir. The laboratory expertise of Mr. Yauba Alhaji Mohammed of Parasitology laboratory, University of Maiduguri and Mr. Peter of Polio laboratory, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Borno State is highly appreciated.

Authors' contribution

JWL and NNA (University of Maiduguri) designed, planned and coordinated the study. JWL (University of Maiduguri) carryout the abattoir sampling and laboratory analysis. MIF (ABU Zaria) participated in data interpretation and manuscript preparation. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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ANTIBIOGRAM OF BACTERIAL PATHOGENS ISOLATED FROM SUBCLINICAL MASTITIS IN KOMBOLCHA, SOUTH WOLLO, ETHIOPIA

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Abstract

The present investigation was carried out to study the prevalence of bacterial pathogens responsible for subclinical mastitis in cattle and their antibiogram pattern to selected antibiotics. The study was carried out on lactating cows in small holder dairy farms in and around Kombolcha, South Wollo, Amhara region, Ethiopia. Accordingly, 110 healthy lactating cows were randomly selected from different farms for screening subclinical mastitis by using California Mastitis Test (CMT). Milk samples with positive results (1, 2, 3) on CMT were processed for isolation and identification at Kombolcha Regional Veterinary laboratory by culturing and requisite biochemical tests. 62 out of 110 lactating cows were found positive for subclinical mastitis, affecting one or two quarters, giving a prevalence of 56%. Out of 62 positive milk samples, a total of 88 different bacterial isolates were isolated and the biochemical tests identified 8 bacterial species. The most predominant species were S.epidermidius (n=23; 26.1%), followed by S. aureus (n=16; 18.2%), *E. coli* (n=14 or 15.9%), S. dysagalactiae (n=10; 11.4%), S. faecalis (n=8; 9.1%), S. agalctiae (n=7; 8%), Entrobacter (n=5; 5.7%), and B. cerius (n=5; 5.7%). The antibiogram of the bacterial isolates to standard antibiotic discs determined by disc diffusion method revealed the highest sensitivity to gentamicin and ciprofloxacin followed by chloramphenicol, in decreasing order. However, Most of the isolates were resistant to routinely used antibiotcis like penicillin G, streptomycin and tetracycline. Present study highlights the higher prevalence rates of subclinical mastitis in the study area and resistance of bacterial isolates to routinely used antibiotics. Judicious use of antibiotics based on antibiotic sensitivity and pharmacokinetics in mastitis control program is recommended to reduce the chances of treatment failure and related economic losses.

Keywords: Antibiogram, Antibiotic Discs, Bovine subclinical mastitis, Ethiopia

ANTIBIOGRAMME DE PATHOGENES BACTERIENS ISOLES CHEZ LES VACHES ATTEINTES DE MAMMITE SUBCLINIQUE A KOMBOLCHA, DANS LE SUD DE WOLLO EN ÉTHIOPIE

Résumé

La présente étude a été réalisée dans le but d'examiner la prévalence de pathogènes bactériens responsables de la mammite subclinique chez les bovins et d'établir l'antibiogramme correspondant pour déterminer leur sensibilité à certains antibiotiques. L'étude a utilisé des vaches en lactation dans de petites exploitations laitières dans et autour de Kombolcha, au sud de Wollo, dans la région d'Amhara en Éthiopie. Ainsi, 110 vaches allaitantes en bonne santé ont été sélectionnées de manière aléatoire dans différentes exploitations, pour un dépistage de la mammite subclinique utilisant le California Mastitis Test (CMT). Les échantillons de lait avec des résultats positifs (1, 2, 3) selon le CMT ont été traités pour isolement et identification des pathogènes au Laboratoire vétérinaire régional de Kombolcha, qui a procédé à la culture et aux essais biochimiques nécessaires. Soixante-deux (62) des 110 vaches en lactation se sont révélées positives pour la mammite subclinique, avec un ou deux quartiers affectés, soit une prévalence de 56%. Des 62 échantillons de lait positifs, un total de 88 isolats bactériens différents ont été isolés, et les tests biochimiques ont identifié 8 espèces bactériennes. Les espèces prédominantes identifiées étaient, dans l'ordre décroissant, S.epidermidius (n=23; 26,1%), suivies de S. aureus (n=16; 18,2%), E. coli (n=14 or 15,9%), S. dysagalactiae (n=10; 11,4%), S. faecalis (n=8; 9,1%), S. agalctiae (n=7; 8%), Entrobacter (n=5; 5,7%), et B. cerius (n=5; 5,7%). L'antibiogramme des isolats bactériens sur disques antibiotiques classiques déterminés par la méthode de diffusion sur disque a révélé une forte sensibilité à la gentamicine, suivie de la ciprofloxacine et du chloramphénicol, dans l'ordre décroissant. Cependant, la plupart des isolats ont résisté

aux antibiotiques utilisés habituellement, comme la pénicilline G, la streptomycine et la tétracycline. La présente étude met en évidence des taux de prévalence très élevés de la mammite subclinique dans la zone d'étude et la résistance des isolats bactériens aux antibiotiques couramment utilisés. L'utilisation judicieuse d'antibiotiques sur base de la sensibilité des pathogènes aux antibiotiques et de la pharmacocinétique dans le programme de contrôle de la mammite est recommandée afin de réduire les risques d'échec thérapeutique et les pertes économiques connexes.

Mots-clés : antibiogramme, disques antibiotiques, mammite subclinique bovine, Éthiopie

Introduction

Despite having the largest livestock population than any African country, the per capita consumption of milk in Ethiopia is only 19 kg per year (Redda, 2001) which is far lower than other countries in the region. The annual milk production in Ethiopia ranges from 797, 9000 to 1,197, 500 metric tons (FAO, 2003) which is far below the national demand for milk and milk products in the country, given the considerable potential for small holder income and employment generation from high value dairy products. Of the factors impeding productivity, Bovine mastitis remains the most costly disease for the dairy industry worldwide.

Although clinical mastitis can be detected by visible changes in milk composition (clots, wateriness) and/or mammary glands (redness, pain, and/or swelling), subclinical form is difficult to detect due to the absence of any visible signs in udder or milk (Viguier *et al.,* 2009). Subclinical mastitis is 3–4 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds (Bachaya *et al.,* 2011). About 70 to 80% of the estimated $140 to $300 losses per cow per year from mastitis related to decreased milk production were attributed to subclinical mastitis (Leitner *et al.,* 2012). Such losses are of particular importance in Ethiopia, which has low economic output with a per capita income of less than one US dollar per day.

Information on the prevalence and antimicrobial susceptibility of mastitis pathogens becomes crucial for a successful and rational mastitis control program. Bovine mastitis is the single most common reason for antimicrobial use in lactating cows (Rajala-Schultz *et al.,* 2004). Therefore, antimicrobial resistance patterns of mastitis pathogens have received much interest in recent years. Over 137 infectious causes of bovine mastitis are known to date and the most common pathogens are staphylococci, streptococci, and Gram-negative bacilli (Sumathi *et al.,* 2008). Antimicrobial resistance reduces efficacy of current treatments in bovine mastitis (Owens *et al.,* 1997). Moreover, these resistant pathogens especially from subclinical cases can be easily transmitted to humans through food chain (Ungemach, 1999). In addition, antibiotics in milk interfere with the process of fermentation and therefore such milk is considered unsuitable for cheese or yoghurt production (Phillips, 2001). The sale of antibiotics is free in Ethiopia, while the mastitis treatment is usually performed by the farmers themselves, and the antimicrobial agents most commonly used are tetracyclines and betalactams (Nibret *et al.,* 2011)

Although there are number of previous reports from different parts of the country, however most of these were focused on clinical mastitis (; Ayano *et al.,* 2013 ; Nibret *et al.,* 2011; Sori *et al.,* 2005). Keeping in view the paucity of information related to subclinical mastitis in cows in Ethiopia, in general and in South Wollo, in particular, the present study was initiated to estimate the prevalence of subclinical mastitis, isolate the major bacterial pathogens, and to determine their antimicrobial susceptibility in smallholder dairy farms in the South Wollo zone., Ethiopia.

Material and Methods

Study area

The present study was conducted in small holder dairy farms in and around Kombolcha town located in Amhara Regional State of South Wollo zone, North East of Addis Ababa during the period between December

2013 and July 2014. Kombolcha town is located at 375 Km away from Addis Ababa at an altitude of 1500-1847 meter above sea level. It is geographically located between 11°5'N latitude and 39°44'E longitude. The annual rain fall of the area ranges from 581-1200 mm. The mean annual minimum and maximum temperature are 17.7 ^{0}C and 23.9 ^{0}C, respectively. The mean annual relative humidity is about 79 % and the area experiences a bi-modal rain fall patterns with a short rainy season which occurs from February to March and long rainy season which starts at the end of June and ends at early November. The remaining months are dry periods. Crop-livestock farming is the main farming system of the area and cattle are the second dominant species kept next to sheep.

Study population

The smallholder dairy cows in and around Kombolcha area were included in represent the study population. Lactating cows selected for this study were apparently healthy, and free of clinical mastitis and any other palpable udder lesion. The samples were randomly collected from cows reared under conventional and confined housing. The dairy cattle managed intensively were kept in exclusive stalls and provided with supplementary diets in addition to natural pasture and agricultural byproduct. The study area (Kombolcha) was selected as this is potential milk production area based on market availability, comparative advantage, biophysical potential and other socioeconomic parameters. The total cattle population of the South Wollo Zone is estimated to be 1 645 306; while that of Kombolcha district is 234 567, out of which 11 728 (5%) heads of cattle are cross breeds of Holstein Friesian and indigenous breeds (CSA, 2015).

Sample size determination

A cross-sectional study design with simple random sampling technique was carried out between December 2013 and July 2014. Due to lack of previous research on subclinical mastitis in the study area an expected prevalence of 50% was taken to estimate the sample size using the formula as described by Thrusfield (2005). Taking 95% confidence level, 5% precision and 50% expected prevalence, 384 lactating cows from different dairy farms were needed for this study. However, due to financial constraints, only 110 lactating cow milk were included in this study. All lactating cows included in this study had no visible signs of mastitis and had no history of treatment with antimicrobials in the preceding 30 days.

Milk samples collection

Milk samples were collected from all the quarters of the sampled cows by standard milk sampling techniques (Quinn *et al.,*1994; Sears *et al.,*1993). 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 pledged saturated in ethyl alcohol (70%). Recontamination of teats during scrubbing was avoided by scrubbing the teats on the far side of the udder first, then those on the near side. Separate pledged cotton was used for each teat. Teats towards sample collection were sampled first and then the far ones. 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 10 ml of milk was collected in sterile caped tubes and numbered.

Screening for subclinical mastitis

The selected smallholder dairy farms were visited once or twice in few cases. Cows with visible clinical mastitis were not included in this study. Clinical mastitis was diagnosed on the basis of manifestation of visible signs of inflammation. A quarter which is warm and swollen and shows pain upon palpation was considered to have acute clinical mastitis while misshaped, atrophied, hard and fibrotic quarter was considered to have chronic mastitis (IDF, 1987).

The quarter milk samples were screened for subclinical mastitis with CMT. For that a squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in CMT paddle. And then an equal amount of the CMT reagent was added to each cup. A gentle circular motion was applied to the

mixtures in a horizontal plane for 15 seconds. The test results were interpreted based on the thickness of the gel formed by CMT reagent and milk mixture. The results were interpreted as negative (0), weak positive (1), distinctive positive (2) and strong positive (3). A cow or a quarter was considered to have subclinical mastitis if CMT score is 1, 2 or 3 and the California *Mastitis* Test (CMT) was carried out according to the method described by Quinn *et al.,* (1999). Cows were considered as positive for CMT when at least one quarter turned out to be positive for CMT. Milk samples with positive results (1, 2, 3) on CMT were kept in icebox until transported to Kombolcha Regional Veterinary Laboratory and stored at +4OC for a maximum of 24 hours until they were inoculated into bacteriological media.

Bacterial isolation

Bacteriological culture was performed according to NMC standards (Hogan *et al.,*1999) at Kombolcha Regional Veterinary Laboratory. Briefly, from each sample, 0.01 mL of milk was plated on 7% blood agar and incubated aerobically for 24, 48 and up to 72 hrs at 37°C to rule out slow growing microorganisms. A mammary quarter was considered culture-positive when the growth of at least one colony is detected on the streaks. For primary bacterial identification, Gram reaction, colony size, shape, color and hemolytic characteristics were used. Additionally, For Gram-positive cocci ,catalase production with hydrogen peroxide (3%) was used to differentiate between catalase-positive *Staphylococci* and catalase-negative cocci. Coagulase tests were carried out using sterile rabbit plasma to distinguish *S. aureus* (coagulase-positive) from non-aureus Staphylococci, referred to as coagulase-negative Staphylococci; and Streptococci were subdivided into esculin-positive cocci and esculin-negative cocci (*Sterptococcus agalactiae* and *streptococcus dysgalactiae*). CAMP tests were used to differentiate *S. agalactiae* from *S. dysgalactiae*.

Antibiotic sensitivity test

In vitro antibiotic sensitivity profiles of bacterial isolates to 10 antibiotic impregnated discs [*gentamicin* (GEN10µg), *erythromycin*(E 15 µg), *chloramphenicol*(C 30µg), *pencillin* G(PG 10 units), *tetracycline*(TE 30 µg), Streptomycin(TE 30 µg), *doxycycline* (DO 30 µg), *ciproflaxin* (CIP 30 µg), *cefixime* (CF 15 µg), *ceftaxime* (CTX 30 µg)] were determined by Agar disc diffusion (Kirby - Bauer method) as described in Quinn *et al.,* (1999). The selection of the types of antimicrobial agents were made based on clinical considerations including frequent use of the drug especially for clinical mastitis in the study area and availability. Well-isolated colonies of the same morphology and characteristics were inoculated into 5 mL of nutrient broth and incubated at 37°C for 5 hrs until a visible turbidity appeared. The turbidity was compared to the 0.5 McFarland standard; and broth cultures with similar turbidly with 0.5 McFarland standard were uniformly spread on Muller Hinton agar plate surface with the help of sterile swabs. About 15 min after the plates were inoculated, antibiotic impregnated cultured discs were placed on the agar surface in such a way that the distance between the centers of two discs was not less than 24 mm. The plates so obtained were incubated at 35°C-37°C for 24 hours. For *Streptococcus* species, blood was added to Mueller – Hinton agar. Resistance was evaluated according to CLSI (2008) as susceptible (S), intermediate resistant (I) or resistant (R) after measuring the zone of inhibition by using measuring caliber. Zone of inhibition was taken to the nearest whole millimeter.

Data analysis

Data on distribution of bacterial isolates and antimicrobial susceptibility were determined as frequency and percentage . The proportion of bacteria susceptible to each antibiotic was calculated using the SPSS 20.0 statistical soft ware version.

In the CMT, 110 lactating cows from different far

Table 1: Bacterial isolates from milk samples of the cows with subclinical mastitis in Kombolcha, South Wollo.

Isolated bacterial species)	Number of isolates (%)
S.epidermidius	23(26.1%)
S. aureus	16(18.2%)
E.coli	14(15.9%)
S. dysagalactiae	10(11.4%)
S. fecalis	8(9.1%)
S.agalactiae	7(8%)
B.cereus	5(5.7%)
Entrobacter	5(5.7%)

Result

In total, 62 (56%) cows were diagnosed with subclinical mastitis. Out of 62 positive milk samples, a total of 88 different bacterial isolates were isolated and the biochemical tests identified 8 species as shown in Table 1.

The antimicrobial susceptibility and resistance profiles of bacterial isolates from subclinical cases of mastitis to antibiotics are shown in Table 2. In this study, *S. epidermidius* were found to be highly susceptible to *gentamicin* (100%), *erythromycin* (52%) and *Streptomycin* (65%). However, these isolates were resistant to *chloramphenicol* (52%), *penicillin G* (65%), *tetracycline* (52.2%), *doxacycline*(100%), cefixime (87%) and ceftaxime (69.6%). S. aureus isolates were found to be highly susceptible to *genetamicin* (100%) and *ciprofloxacin* (56.25 %). However, these isolates were highly resistant to *erythromycin* (62.5%), *cholarmphenicol* (56.2%), penicillin G (62.5%), *tetracycline* (68.8%), doxacycline (68%), cefixime (100%) and ceftaxime (62.5%). Similarly, *E.coli* was susceptible to gentamicin (50%), ciprofloxacin (78.6%), and cefixime (64.3%), while *S. dysgalactiae* was susceptible to gentamicin (100%), erythromycin (80%), *chloromphenicol* (100%), *tetracycline* (50%), *ciprofloxacin* (100%), *ceftaxime* (90%).Similarly; *B. cereus* isolates were susceptible to *gentamicin* (100%) and *ciprofloxacin* (100%). While *S. faecalis* was susceptible to *gentamicin* (62.5%), *tetracycline* (50%), *streptomycin* (100%), *doxacycline* (100%), *ciprofloxacin* (10%). Entrobactor and *S. agalactiae* were susceptible to *gentamicin*, *erythromycin*, *streptomycin*, *ciprofloxacin*. Overall most of the isolates were susceptible to *gentamicin*, *ciprofloxacin*, and *streptomycin*.

Discussion

Despite of many years of research mastitis remains the most economically damaging disease for the dairy industry worldwide. The present epidemiological study was applied through combination of the CMT with bacteriological cultures. Thus, subclinical mastitis was defined as a state when mammary glands without clinical abnormalities give apparently normal milk but was bacteriologically positive and with positive CMT (Mungube *et al.,* 2005). Karimuribo *et al.,* (2006) concluded the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable technique of diagnosis.

In the present study, out of 110 cows examined, 62 had subclinical mastitis, therefore making an overall prevalence of 56%. The overall prevalence of subclinical mastitis in the present study (56%) was much higher than previous studies in Ethiopia like Almaw et al. (2009), Almaw *et al.,* (2008), Bishi (1998) , Haftu et al. (2012) and Workineh *et al.,* (2002) who reported 25.22%, 34.4%, 34.3%, 33% and 38.2%, respectively. However, a study carried out in Tanzania in a similar herd structure as high as 90.3% (Kivaria *et al.,* 2004) subclinical mastitis prevalence was reported. Because mastitis is a complex disease involving interactions of several factors, mainly of management, environment, and factors relating to animal and causative organisms, its prevalence is expected to vary from place to place.

In Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Hussein *et al.,* 1997). According to Mungube *et al.,* (2005) losses associated with subclinical mastitis (SCM) in crossbred dairy cows in the central highlands of Ethiopia was

Table 2: Antimicrobial susceptibility of bacterial isolates from cows with subclinical mastitits to antibiotics-percentage of sensitive (S) , Intermediate (I) ,Resistant(R) isolates

Bacterial isolate		GEN	E	C	PG	TE	STR	DO	CIP	CFM	CTX
S.epidermidius (n=23)	S	100%	52.2%	39.1%	30.4%	34.8%	65.2%	-	34.7%	13%	21.7%
	R	-	34.8%	52%	65.2%	52.2%	26.1%	100%	30.4%	87%	69.6%
	I	-	13%	8.7	4.3%	13%	8.7	-	30.4%	-	8.7
S.aureus (n=16)	S	100%	31.2%	25%	31.2%	18.8%	25%	31.2%	56.25%	-	31.25%
	R	-	62.5%	56.2%	62.5%	68.8%	-	68.%	25%	100%	62.5%
	I	-	6.2%	18.8%	6.2%	12.5%	75%	-	18.8%	-	6.2%
E.coli (n=14)	S	50%	14.3%	21.4%	14.3	42.8%	-	35.7%	78.6%	64.3%	-
	R	28.6%	85.7%	7.1%	85.7%	57.1%	100%	64%	21.4%	35.7%	100%
	I	21.4%	14.3%	71%	-	-	-	-	-	-	-
S.dysgalactiae (n=10)	S	100%	80%	100%	-	50%	-	20%	100%	10%	90%
	R	-	20%	-	100%	30%	100%	60%	-	30%	10%
	I	-	-	-	-	20%	-	20%	-	60%	-
B.cerus (n=5)	S	100%	-	20%	20%	-	-	20%	100%	-	-
	R	-	100%	80%	60%	100%	100%	80%	-	100%	100%
	I	-	-	-	20%	-	-	-	-	-	-
S.fecalis (n=8)	S	62.5%	25%	-	25%	50%	100%	100%	100%	25%	-
	R	37.5%	62.5%	100%	75%	25%	-	-	-	50%	-
	I	-	-	-	-	25%	-	-	-	25%	100%
Entrobactor (n=5)	S	100%	40%	60%	-	-	100%	-	100%	-	-
	R	-	60%	20%	100%	40%	-	40%	-	100%	-
	I	-	-	20%	-	60%	-	60%	-	-	100%
S.agalactiae (n=7)	S	85.7%	14.3%	100%	42.8%	-	71%	28.6%	100%	28.6%	14.3%
	R	14.3%	-	-	57.1%	100%	28.6%	-	-	71%	28.6%
	I	-	85.7%	-	-	-	-	71%	-	-	57%
Total (88)	S	93.2%	36.4%	42%	22.7%	29.5%	42%	26.1%	73.9%	19.3%	22.7%
	R	9.1%	52.3%	39.8%	73.9%	55.7%	42%	62.5%	15.9%	69.3%	54.5%
	I	3.4%	13.6%	18.2%	3.4%	13.6%	15.9%	8%	11.4%	9.1%	22.7%

GEN= Gentamicin, E= Erythromycin, C=Chloromphenicol, PG= Pencilin G, TE=Tetracycline, STR= Streptomycin, DO= Doxacycline, CIP= Ciprofloxacin

found to be US$38 for each cow per lactation. Ethiopian farmers specially smallholders were unaware of the invisible loss from subclinical mastitis (Hussein, 1999) and were also true in Ugandan and Tanzanian farmers (Byarugaba *et al.,* 2008; Kivaria *et al.,* 2004). In this study, all of the farmers practice hand milking and their knowledge about mastitis was only on clinical mastitis; none of them new about subclinical mastitis. They were surprised during our field work when they saw CMT positive milk reaction while it appeared to them normal milk before the test was conducted.

The present study also revealed a close positive relationship between isolation of bacteria from mastitic milk samples and California mastitis test. As almost all milk samples were positive to CMT, specific bacteria were isolated. This means that CMT was a good diagnostic tool in the detection of sub-clinical mastitis; hence it could

be most the reliable test to be conducted to investigate sub-clinical mastitis in the dairy farms. On the other hand, the culture method may be used to confirm and aid proper treatment (Barnouin et al. 2005; Bekele and Molla, 2001; Bitew *et al.,* 2010; Tefera 2001).

The most commonly isolated pathogens in our study were S.epidermidius with a frequency of 26.1%, followed by S. aureus: 18.2%, E.coli : 15.9, and S.dysgalactiae: 11.4%. Getahun *et al.,* (2008) and Ayano et al. (2013) also reported S.epidermidius and S. aureus as the major pathogen from milk of subclinical mastitis cases in Ethiopia. However, the results were in contrary with the findings of Mekebib *et al.,* (2009), Sharif *et al.,* (2009) and Sori *et al.,* (2005), This variation may be due to season, managemental conditions at the farms, area, difference in sample handling in the laboratory and use of antibiotics.

The present study showed that staphylococci and streptococci represented more than half (72.7%) of bacterial isolates involved in subclinical mastitis, followed by other bacteria. Similar results were also reported by Moroni et al. (2006). The high rate of isolation of staphylococci may be attributed to the fact that the principal reservoirs of these organisms are the udder, skin and milk of the infected gland. The high frequency of staphylococcal mastitis is considered to be due to the existence of inadequate hygiene in the dairy industry, poor animal health services, and lack of proper attention to the health of the mammary gland in general.

In the present study interestingly environmental bacteria like Escherichia coli was isolated in high proportion (15.9%). This is in congruent with the reports of Mekonnen and Tesfaye (2010), Matios et al. (2009) who found 7.5% of the total isolates. In contrast, this figure is higher than isolates reported by Sori et al. (2005) and Getahun et al. (2008) who reported 0.75% and 0.5% in different parts of Ethiopia, respectively. The presence of environmental bacteria might be an implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area.

In this study, Anti-biogram test for 88 bacterial isolates was performed through a panel of ten antimicrobial drugs. The investigation result revealed that S.epidermidius isolates were susceptible to gentamicin, Streptomycin and erythromycin with efficacy rate of 100%, 65%, 52% in their decreasing order, respectively. Staphylococcus aureus isolates were also susceptible to gentamicin (100%) and ciprofloxacin (56.25%). Similarly, E.coli was susceptible to gentamicin (50%), ciprofloxacin (78.6%), and cefexime (64.3%), while S. dysgalactia was susceptible to gentamicin (100%), erythromycin (80%), chloramphenicol (100%), tetracycline (50%) , ciprofloxacin (100%), ceftaxime (90%). Similarly, B. cercius isolates were susceptible to gentamicin (100%) and ciprofloxacin (100%), While S. faecalis was susceptible to gentamicin (62.5%), tetracycline (50%), Streptomycin (100%), doxacycline (100%) and ciprofloxacin (100%). Entrobactor and S. agalactiae were susceptible to gentamicin, erythromycin, streptomycin, ciprofloxacin. Among anti-biotics tested in vitro, gentamicin was the most potent drug followed by ciprofloxacin, with the efficacy rate of 93.2% and 73.9% respectively. On the other hand, cefixime was found to be the least potent drug in the overall tested bacteria (19.3%). Anti- biogram test result in this study is in line with the report of Mustafa *et al.,* (2013) who found most of the pathogens from mastitic milk samples highly sensitive to gentamicin and norfloxacin. These findings are in agreement with findings of Mustafa *et al.,* (2007). Sumathi *et al.,*(2008) also found genatmicin effective while Ebrahimi *et al.,* (2002), Erskine *et al.,*(1986) , Guerin *et al.,*(2002) and Gianneechini *et al.,* (2002), found gentamicin resistant. The difference in susceptibility patters of bacteria to different anti-biotic might be attributed to difference in utilization of anti-microbial agents for treatment regimen and development of resistance due to repeated use of similar antibiotics in different farms for longer period.

Antibacterial sensitivity of bacteria isolated from different dairy farms in and around Kombolcha revealed 73.9%, 55.7%, 42% of the

total isolates were resistance against penicillin, tetracycline and streptomycin, respectively. These antibiotics are being routinely used for the treatment of bovine mastitis. At least 75.4% of the isolates from the farms were resistant to at least four antibacterial drugs. This multi-drug resistance is a real threat to effective control of the disease since the options are limited. Such high prevalence of resistance by udder pathogens was previously reported (Byarugaba *et al.,* 2008). The highest level of resistance was registered from samples obtained from the study area, is suggestive of high drug pressure and irrational use of antibiotics. Indeed, most of the farmers use penicillin, streptomycin and tetracycline for treatment of mammary and systemic bacterial diseases. The sale of antibiotics over-the-counter without prescription has offered unprecedented access to the drugs by farmers. No wonder, only 30% of the farmers relied on veterinarians for treatment but the majority treated their animals with the aid of herdsmen. Such treatments were carried without strict adherence to dosage requirements and treatment period due to lack of technical knowledge among farmers and herdsmen considering their low level of education. Such abuse of drugs is responsible for the emergence of multi-drug resistant which is a threat to both animal and public health.

Conclusion

In conclusion, this study provides the proof about a very high prevalence of subclinical mastitis on dairy farms in South Wollo. The high burden of subclinical mastitis and emergence of multidrug resistant pathogens could be one of the important constraints to dairy production in South Wollo. Most of the pathogens isolated from milk of affected cows were resistant to routinely used antibiotics, however susceptible to gentamicin and ciprofloxacin. Therefore, sensitization of dairy farmers in and around Kombolcha town on proper hygiene, appropriate milking techniques and dry cow therapy based on the results of antibiotic sensitivity tests are highly recommended. Further research is also needed to identify the most appropriate extension progamme on mastitis that can have a significant impact on effective control of the disease. Moreover, identification of mastitis pathogens and antimicrobial susceptibility testing on routine basis is essential to control the disease, achieve effective therapy and consequently improve the farms profitability.

Acknowledgement

The authors would like to thank research and community service office of Wollo University for providing fund for this project work. We are also very grateful to Kombolcha veterinary laboratory staffs for their collaboration to share their experiences during laboratory works

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BOVINE CYSTICERCOSIS AND HUMAN TENIASIS WITH PUBLIC HEALTH IMPLICATION AT ASELLA TOWN, ARSI ZONE OF OROMIA REGIONAL STATE, ETHIOPIA

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Abstract

The aim of study was to determine statues of bovine cystiercosis and human taeniasis in Asella, Arsi Zone of Oromia Regional State, Ethiopia. Simple randomly sampling followed by detailed meat inspection for cyst distribution with cyst count and variability test was done at Asella Municipal Abattoir. Questionnaire administration surveys was conducted on voluntarily in habitants of Asella town. Out of 450 examined animal, 22 (4.9%) were infected with *C. bovis* showing significantly higher in younger 8.2% (OR=3.4, 95%CI: 1.4-8.7) and in cross breed 14.2% (OR=4.3, 95%CI: 1.3-14.2) then their counters ($p<0.05$) but neither between sex nor among animals body condition ($p>0.05$). A total of 63 cyst consisting of 30 (47.6%) viable and 33 (52.4%) non-viable were recovered. Cysts count was 28.6%, 23.8%, 20.6%, 17.5%, 6.4% and 3.2% in the tongue, triceps muscle, heart, masseter muscle, liver and diaphragm respectively. In high viable cysts (61.1%) were found in the tongue. Out of 125 respondents, 89 (71.2%) had contracted taeniasis infection at least once in their life time. Men (OR=8.3, 95%CI: 1.9-36.4), occupationally high-risk groups (OR=2.1, 95%CI: 0.5-8.2), married persons (OR=7.1, 95%CI: 1.7-29.3), raw meat consumers (OR=35.2, 95%CI: 6.3-196.7) and low level of taeniasis/cysticercosis complex knowledge (OR=4.261, 95%CI: 1.2-15.6) were contracted taeniasis at higher risk than their counter groups ($p<0.05$). Age and educational background were no effect on the difference ($p>0.05$). The data indicated Taenia saginata/cysticercosis is prevalent in the study area which needs due attention for public awareness and strict routine meat inspection to safe guard public health and promote beef industry.

Key words: *Asella*, *Bovine*, *Cysticercosis*, *C. bovis*, *T. saginata*, *Taeniasis*.

LA CYSTICERCOSE BOVINE ET LA TENIASE HUMAINE ET LEURS IMPLICATIONS POUR LA SANTE PUBLIQUE DANS LA VILLE D'ASELLA, ZONE ARSI DE L'ÉTAT REGIONAL D'OROMIA EN ÉTHIOPIE

Résumé

Le but de l'étude était de déterminer l'état de la cysticercose bovine et de la téniase humaine à Asella, dans la zone Arsi de l'État régional d'Oromia en Éthiopie. Un échantillonnage aléatoire simple suivi d'une inspection détaillée de la viande pour déterminer la répartition des kystes (avec leur numération et un test de variabilité) a été effectué à l'Abattoir municipal d'Asella. Des enquêtes basées sur l'administration de questionnaires ont été menées sur une base volontaire chez les habitants de la ville d'Asella. Des 450 animaux examinés, 22 (4,9%) étaient infectés par C. bovis, avec un taux significativement élevé chez les juvéniles 8,2% (OR=3,4, IC 95% : 1,4-8,7) et les croisés 14,2% (OR=4,3, CI 95% : 1,3-14,2) par rapport aux autres groupes ($p <0,05$), le sexe et l'état corporel des animaux ($p> 0,05$) n'étant associé à aucun effet. Au total, 63 kystes dont 30 (47,6%) viables et 33 (52,4%) non viables ont été identifiés. Les nombres de kystes répartis dans différentes parties étaient 28,6%, 23,8%, 20,6%, 17,5%, 6,4% et 3,2%, respectivement pour la langue, le muscle triceps, le muscle cardiaque, le muscle masséter, le foie et le diaphragme. Des kystes hautement viables (61,1%) ont été trouvés dans la langue. Des 125 répondants, 89 (71,2%) avaient contracté une téniase au moins une fois dans leur vie. Les hommes (OR=8,3, IC 95% : 1,9-36,4), les groupes à haut risque professionnel (OR = 2,1, IC 95% : 0,5-8,2), les personnes mariées (OR = 7,1, IC 95% : 1,7-29,3), les

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consommateurs de viande crue (OR = 35,2, IC 95% : 6,3-196,7), et les groupes ayant peu de connaissances sur le complexe téniase / cysticercose (OR = 4,261, IC 95% : 1,2-15,6) étaient plus susceptibles de contracter la téniase par rapport aux autres groupes (<0,05). L'âge et le parcours éducatif n'ont montré aucun effet sur la différence (p> 0,05). Il ressort de ces données que Taenia saginata / cysticercose est répandue dans la zone d'étude et qu'elle mérite une attention particulière ; et elle devrait faire l'objet d'une sensibilisation auprès du public. De plus, une inspection de routine rigoureuse de la viande doit être faite pour préserver la santé publique et promouvoir l'industrie de la viande bovine.

Mots-clés : *Asella*, *bovine*, *cysticercose*, *C. bovis*, *T. saginata*, *téniase*.

Introduction

Bovine cysticercosis refers to the infection of cattle with metacestodes (Cysticercus bovis) of the human tapeworm (Taenia saginata) (Taylor *et al.,* 2007; Oladele *et al.,* 2004). Most incidents in cattle arise as a result of direct exposure to proglottids shed from infected individual contaminating environment (Gracey *et al.,* 1999; Hancock *et al.,* 1989) where by the ingested eggs localized in organs like heart, skeletal muscle and diaphragm, and developed into cysticerci (Gracey *et al.,* 1999). Human beings are become infected through ingesting of such metacestode stage within infected beef. The adult worm, developed in human small intestine (Pawlowski and Murell, 2001, Caparet *et al.,* 2002) causing taeniasis (Hancock *et al.,* 1989).

Cysticercosis and teniasis are worldwide distribution low in developed countries, being less than 1% in carcasses inspected (Onyango-Abuje *et al.,* 1996), their prevalence is high particularly, in Sub-Saharan Africa (WHO, 1995) reaching a level of 30-36% in Kenya, 20% in Guinea, 18% in Sierra Leone and 20% in Cameroon (Gebreab, 1995) where the lifecycle and transmission are maintained and characterized by unhygienic conditions coupled with poor hygiene, poor sanitation, poor livestock husbandry practices and inadequate meat inspection and control (Carlos *et al.,* 2003), and also where inhabitants traditionally raw beef or insufficiently cooked meat consumption (Hailu, 2005; Abunna *et al.,* 2007, Caparet *et al.,* 2002, Minozzo *et al.,* 2002).

Cattle are sources of about 45% of Ethiopian domestic meat consumption which is hampered by various animal health problems, among which, C. bovis/T. saginat is one (EARO, 2000) where bovine cysticercosis abattoir investigation ranged from 3.11% to 27.6% and while T. saginata taeniasis questionnaire survey ranged from 2.5 % to 89.41 %, respectively (Dawit, 2004; Hailu, 2005; Abunna *et al.,* 2007). The cultural habit of raw beef cut, minced beef and under cooked beef consumption in Ethiopia, has favored the disease spread (Dawit, 2004; Abunna *et al.,* 2007) with relatively lower prevalence of 2.3%-3.8% in Zeway Municipal Abattoir (Bedu *et al.,* 2011), 3%-3.1% in central Ethiopia (Tembo, 2001), 4.9% in Gondar (Dawit, 2004) and 7.5% in Addis Ababa (Nigatu, 2014) while higher prevalence of 17.5% in East shoa (Hailu, 2005), 21% in Nekemt (Ahmed, 1990) and 26.25% in Hawassa (Abunna *et al.,* 2007) reports. With regards to human taeniasis, relatively lowwhile higher 56.7% (Bedu *et al.,* 2011), had contracted T. saginata infection at least once in their life time. On the other hand, countrywide study on prevalence of bovine cysticericosis and human teaniasis were still very limited. It was aimed to assess the prevalence and associated risk factors for the occurrence of bovine cysticercosis and T. saginata taeniosis in Asella town, Arsi Zone, Oromia Regional State, Ethiopia.

Materials and Methods

Ethical clearance

Study were also involved human cases. Accordingly, the topic was presented to and approved by the appointed Ethical committee. Thus, international guidelines were followed under all circumstance.

Study area

The study was conducted at Asella town in 6o 59' to 8° 49' N and 38° 41' to 40o 44' E Arsi Zone, Oromia Regional State, located 175 km southeast of the capital city Addis Ababa, Ethiopia. The area has an altitude ranged from 2500 to 3000 meter above sea level, receiving mean annual rainfall and temperature of 200-400 mm and 22.5°C respectively. Agricultural production system of the area is mixed (livestock and crop production) farming system.

Study design and sample size determination

A cross-sectional study with simple random sampling method which involves abattoir surveys, laboratory cyst viability test and questionnaire administration were applied. The sample size was using Thrusfield (2007) with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Accordingly; the total number of required sample were 384 cattle where a total of 450 cattle were sampled. Similarly, 125 voluntary personals were interviewed.

Abattoir survey bovine cysticercosis

Study animals: The study animals were cattle originated from the high land and low land sedentary farming system of Arsi, Bale and East Shoa districts Oromia Regional State, and presented to the Asella Municipal Abattoir (AMA) for slaughtering. At abattoir, selected animal were categorized into <5 year and > 5 years age groups; poor, medium and good body condition using body condition scoring index (Nicholson and Butterworth, 1996), male and female sex groups, local and cross breeds during ante mortem inspection.

Slaughter operation procedures of the abattoirs: Butchers (small shop meat sellers) purchases cattle from markets of Arsi, Bale and East Shoa districts towns. They present cattle to the Asella Municipal Abattoir. Male are frequently parent than females. Technical meat inspector being register the animal and admit to the abattoir. Slaughter operation was done after 24 hrs of ante-mortem inspection. Routine meat inspection and supply of the whole carcasses to the butchers as fit for human conception were practiced at abattoir. The small shop meat sellers supply pubic with raw meat in kilo's.

Detailed meat inspection: Detail meat inspection procedures were applied examination of cyst distribution and numbers in organ. Hence, apes to ventral incision on heart, single ventral incision on tongue, double and parallel incision of internal and external masseter, incision on diaphragm, longitudinal incision on esophagus, two parallel incision on triceps muscles, and incision on liver were applied according to OIE (2004). Visual inspection and palpation were applied for the presence of C. bovis cyst (Gracey *et al.,* 1999). Cysticerci were carefully dissected from the tissues, counted in number in each organ, collected and transported to Asella Regional Veterinary Parasitology Laboratory at +4°C using ice box for viability test.

Laboratory cyst viability test

The viability test was done in Asella Regional Veterinary Parasitology Laboratory using normal saline solution with 30% ox bile and incubated at 37°C for 1 to 2 hrs. A cyst was regarded as viable if the scolex evaginated according to Gracey (1999). At the same time, the evaginated scolex was checked for characteristic T. saginata cysticerci based on the size of cysticercus, absence of hooks on the rostulum, and presence of four suckers on the (Opara, 2006).

Questionnaire survey on human taeniasis

Questionnaire survey was administered to 125 randomly-selected volunteer respondents from whom pre-informed consents were obtained. The interviews were conducted personally for having an exposure to taeniasis. The questionnaire focused on potential risk factors of taeniasis such as age, gender, occupation, educational level, habit of raw meat consumption, marital status, and level of knowledge about the taeniasis/cysticercosis complex, exposure risk, types of treatment taken, frequency of medication, and prognoses were considered. Occupationally groups

including those in contact with meat and meat products such as abattoir workers, butchers, meat inspectors, cooks and farmers were included. Another groups, the low-risk groups, those who were not as highly in contact with meat and meat products such as students, teachers, other civil servants and private workers were also included in the study.

Data management and analysis

The data collected were entered in Microsoft office excel 2007 program and analyzed STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). Prevalence was calculated as percentage values. The association between the independent factors and the prevalence of cysticercosis and taeniasis were evaluated using Chi-square test (χ^2). Logistic regression was used to determine level of significance of the considered potential risk factors associated with the prevalence. A P value < 0.05 considered statistically significant.

Results

Prevalence of bovine cysticercosis

Out of 450 inspected carcasses, 4.9% were positive for C. bovis (Table 1). The prevalence was 7% in female 4.7% in male. It was 8.1%, 4.5% and 3.3% in carcasses of poor, medium and good body condition animas. Statistically significant were observed neither between sex nor among body condition score gropes ($p > 0.05$). But, significant differences were observed between the breeds and the ages of animals ($p < 0.05$).

Logistic regression reveled animals below five years of age exhibited a higher risk of infection with cysticercosis (OR=3.430, 95%CI: 1.357-8.672) than those with above

Table 1: Prevalence of bovine cysticercosis at Asella Municipal Abattoir based on studied risk factors.

Variables	Level (group)	No inspected	No infected (%)	χ^2(p-value)
Age (year)	< 5	184	15 (8.2)	7.129(0.008)
	≥ 5	266	7 (2.6)	
Sex	Male	407	19 (4.7)	0.446(0.504)
	Female	43	3 (7.0)	
Breed	Cross	27	4 (14.8)	6.086(0.014)
	Local	423	18 (4.2)	
Body condition	Poor	85	7 (8.1)	2.755(0.252)
	Medium	245	11 (4.5)	
	Good	120	4 (3.3)	
Total		**450**	**22 (4.9)**	

Table 2: Multivariable logistic regression analysis of factors associated with the occurrence of C. bovis

Variables	Level	No inspected	No infected (%)	COR (95%CI)	AOR (95% CI)	p-value
Age (year)	< 5	184	15 (8.15)	3.284 (1.312-8.223)	3.430 (1.357-8.672)	0.009
	≥ 5	266	7 (2.63)	1	1	
Breed	Cross	27	4 (14.81)	3.913 (1.224-12.508)	4.298 (1.305-14.151)	0.016
	Local	423	18 (4.24)	1	1	

Note: COR = Crude Odds Ratio; AOR = Adjusted Odds Ratio; CI = Confidence Interval; 1 is Reference.

Table 3: Anatomical distribution and viability of cysts among inspected organs.

Organs/tissue inspected	Infected organs in (N=450) animal no. (%)	Total recovered cysts		
		Total no. (%)	Viable no. (%)	Dead no. (%)
Tongue	15 (3.33)	18 (28.6)	11 (61.1)	7 (38.89)
Triceps muscle	13 (2.9)	15 (23.8)	9 (60.0)	6 (40.0)
Heart	12 (2.7)	13 (20.6)	7 (53.9)	6 (46.1)
Masseter muscle	9 (2.0)	11 (17.5)	5 (45.4)	6 (54.6)
Liver	4 (0.89)	4 (6.4)	1 (25.0)	3 (75.0)
Diaphragm	2 (0.44)	2 (3.2)	0 (0)	2 (100)
Total		**63 (100)**	**30 (47.6)**	**33 (52.4)**

or equal to five years of age. Furthermore, breeds of cattle significantly enhance the risk for cysticercosis infection. Hence, cross breed were more likely to be infected (OR=4.298, 95%CI: 1.305-14.151) with C. bovis than local (Table 2).

Variable Anatomical distribution and viability of cysts were observed and shown in Table 3. The highest number of cysts encountered in the tongue (3.3%) followed by triceps muscle (2.9%), heart (2.7%), masseter muscle (2.0%), liver (0.9%) and diaphragm (0.4%) which is least infected from total. A total of 63 metacestodes consisting 30 (47.6%) alive but 33 (52.4%) dead were recovered. The highest number were encountered in the tongue (28.6%) followed by triceps muscle (23.8%), heart (20.6%), masseter muscle (17.5%), liver (6.4) and diaphragm (3.2%) in decreasing orders. Tongue had the highest proportion of viable cysts (11/18, 61.1%) but none of the cyst detected in diaphragm was viable.

Prevalence of human teaniasis

Of the total 125 interviewed respondents, 71.2% had contracted T. saginata infection at least once in their life time (Table 4). The majority of the respondents had an experience of raw meat consumption as a result of traditional and cultural practice. The

Table 4: Potential risk factors of Taenia saginata/ taeniasis infections in human respondents

Variables	Group	No. of respondents	Exposure (%)	χ^2 (p-value)
Age (year)	< 20	54	36 (66.67)	
	≥ 20	71	53 (74.65)	0.953 (0.329)
Gender	Male	63	55 (87.3)	
	Female	62	34 (54.84)	16.059 (0.000)
Occupation	High risk	45	38 (84.44)	
	Low risk	80	51 (63.75)	6.015 (0.014)
Marital status	Married	70	58 (81.43)	
	Single	55	31 (56.36)	10.543 (0.001)
Education level	Illiterate	49	34 (69.39)	
	Literate	76	55 (72.37)	0.129 (0.719)
Raw meat consumption	Frequent	63	61 (96.83)	
	Infrequent	62	28 (45.16)	40.675 (0.000)
Taeniasis knowledge	No	64	56 (87.5)	
	Yes	61	33 (54.1)	16.993 (0.000)
Total		**125**	**89 (71.2%)**	

Table 5: Multiple logistic regression analysis to predict the risk factors associated with taeniasis

Variables	Group	Respondents No.	Exposure no. (%)	COR (95%CI)	AOR (95% CI)	p-value
Gender	Male	63	55 (87.3)	5.662 (2.314-13.850)	8.303 (1.893-36.419)	0.005
	Female	62	34 (54.84)	1	1	
Occupation	High risk	45	38 (84.44)	3.087 (1.223-7.793)	2.065 (0.522-8.171)	0.301
	Low risk	80	51 (63.75)	1	1	
Marital status	Married	70	58 (81.43)	3.742 (1.650-8.485)	7.124 (1.734-29.270)	0.006
	Single	55	31 (56.36)	1	1	
Raw meat consumption	Frequent	63	61 (96.83)	39.037 (8.745-174.250)	35.161 (6.285-196.691)	0.000
	Infrequent	62	28 (45.16)	1	1	
Taeniasis knowledge	No	64	56 (87.5)	5.939 (2.425-14.547)	4.261 (1.166-15.570)	0.028
	Yes	61	33 (54.1)	1	1	

Note: COR = Crude Odds Ratio; AOR = Adjusted Odds Ratio; CI = Confidence Interval; 1 is Reference.

chi-square analysis Of risk factors associated with occurrence of human taeniasis, significant difference ($p<0.05$) were observed for the gender, occupation, marital status, education level, habit of raw meat consumption and level of taeniasis knowledge of respondents. But no between age groups and the education levels ($p > 0.05$) Table 5.

The results of logistic regression analysis of the association of different risk factors with the prevalence of taeniasis exposure of respondents are depicted in Table 5. Analysis of the association of risk factors with the prevalence using multivariable logistic regression showed that male individuals (OR=8.303, 95%CI: 1.893-36.419), high risk groups (OR=2.065, 95%CI: 0.522-8.171), married individuals (OR=7.124, 95%CI: 1.734-29.270), frequent raw meat consumers (OR=35.161-95%CI: 6.285-196.691) and low level of taeniasis knowledge (OR=4.261, 95%CI: 1.166-15.570) were at higher risk of infection with taeniasis as compared to respondents of female, low risk occupation, single individuals, infrequent raw meat consumers and high level of taeniasis knowledge, respectively.

Discussion

Bovine cysticercosis

Overall prevalence. Abattoir survey result of the present study revealed an overall prevalence of 4.9% cysticercosis in slaughtered cattle in Asella abattoir, which is similar with by 4.9% at Gonder (Dawit, 2004), and 3.1% in Central Ethiopia (Tembo, 2001); 5.4% in Kombolcha (Alula, 2010), 3.65% in Jimma (Taresa *et al.,* 2011), 4.4% (Megersa *et al.,* 2011) in Jimma; reported 3.6% in Addis Ababa abattoir (Ibrahim and Zerihun, 2012), 5.6% from Bishoftu (Emiru *et al.,* 2015) findings reported in different agro-ecological zones of Ethiopia. The present findings is also in agreement with reports from other African countries which reported 3% from Rwanda (Nzeyimana *et al.,* 2015) and 4.8% from Nigeria. (Karshima, 2013). However, the current finding was lower than the 13.8% in Debre Zeit (Getwchew, 1990), 26.3% in Awassa (Abunna *et al.,* 2008), 17.5% in East Shoa (Hailu, 2005), 13.3% in Wolaita Soddo municipal abattoir (Regassa *et al.,* 2009) and 12% at Yirgalem's abattoir (Abunna, 2013) all reported from Ethiopia. Unlike the present finding and reports of others, lower

prevalence of 0.26% in Croatia (Zivkovic *et al.,* 1996), 0.48–1.08% in Germany (Abusier *et al.,* 2006) and 0.9% in Cuba (Sau'rez and Santizo, 2005) were reported. The majority of the findings were based on surveys carried out on carcasses subjected to routine meat inspection which has same limitations shared globally with meat inspection. However, such variation in findings indicates differences in geographic distribution of the disease in Ethiopia. Compared to developed country, poor sanitary infrastructure, low awareness and improper disposal of sewage are major factors for higher prevalence of cysticercosis in developing countries (Fertig and Dorn, 1985; Frolova, 1982).

Prevalence within studied variables. This study revealed light infection among the inspected animals and this could be due to the practical limitations to the number of incisions allowed and many infestations could be undetected (Wanzala *et al.,* 2003). As gross mutilation lowers the marketability of carcasses and introduces contamination, owners do not allow multi incisions for the detail investigation, in addition to this postmortem inspection is less sensitive when the infection is at initial stage. Other possible reasons for such lower prevalence of bovine cysticercosis in the study area could be attributed to change in culture of raw meat consumption, variations in personal and environmental hygiene, awareness in using latrine, religion and low contamination from where animals were bought which minimizes rate of infection. Higher prevalence rates were recorded in female animals than males, but with no statistically significant difference between sex and prevalence of cysticercosis. The present finding is in agreement with report of Gomol et al. (2011) and Jemal and Haileleul (2011). However, the current finding is in contrast with earlier observation of Hailu (2005) and Tembo (2001). Higher prevalence rates in female animal could be due to the fact that females are more prone to parasitism during pregnancy and per-parturient period due to stress and decreased immune status (Urquhart *et al.,* 2007). Prevalence of cysticercosis in age groups were in line with the higher findings in younger animal than in olders (Gomol *et al.,* 2-011; Kebede *et al.,* 2008; Jemal and Haileleul, 2011; Garedaghi *et al.,* 2011) which could be due to the fact that younger animals are more susceptible than adult counter parts. Adult animals may acquire immunity to the parasites through frequent challenge and expel the ingested parasite before they establish infection (Urquhart *et al.,* 2007). The high C. bovis prevalence in cross than in local breeds could be associated with difference in management system, whereby cross breeds cattle were kept under few pasture. Poor public health hygiene such as infrequent uses of toilet leads to grazing pasture contamination. Present finding showed similar C. bovis infection among animals of different body condition. The finding was similar with reports of Gomol et al. (2011) and Jemal and Haileleul (2011).

Organ distribution of metacestode. Present finding showed wide organs and tissue distributing of C. bovis in infected animal. From the total of animal, tongue is the most frequently (15/450, 3.33%) affected organ with the highest number of cysts followed by triceps muscle (2.89%), heart muscle (2.67%), masseter muscle (2%), liver (0.89%) and diaphragm (0.44%) in decreasing order. This finding is similar with work reported by Belino (1975), Bedu et al. (2011), Abunna (2013) and Emiru et al. (2015) whose showed that tongue, triceps muscle, heart muscle and masseter muscle as the most frequent locations for C. bovis. These are frequently reported important predilection sites for C. bovis; a reason advanced for the frequent involvement of these muscles, being the increased blood supply due to frequent or continuous/rhythmic movement of the body parts (Gracey *et al.,* 1999). Generally, the method of meat inspection, the ability of meat inspectors to identify cases, differences in management, the sample size and sampling method, the number of cuts, and other factors can contribute to prevalence variations in bovine cysticercosis.

Viability profiles of metacestode. Of total encountered metacestode cysts, 47.62% alive but 52.38% dead cysts indicating thigh risk of zoonoses up on consumption of raw or

undercooked such edible organ with the cysts. Within the cyst observed from respective organ, tongue is the leading with 61.11% viable cysts followed by triceps muscle (60.0%), heart (53.9%), masseter muscle (45.5%) and liver (0.3%). None of the cyst detected in diaphragm was viable. Such distribution of viable C. bovies in aforementioned eadabletissues were also reported in Ethiopia (Megersa *et al.,* 2011; Abunna, 2013; Emiru *et al.,* 2015). The variations in anatomical distribution depend on a number of factors, such as blood kinetics and animals' daily activities. Any geographical and environmental factors affecting blood kinetics in the animal affect the distribution of oncospheres as well and hence the predilection sites during meat inspection (Gracey *et al.,* 1999). More importantly, most of these organs are consumed raw or undercooked in Ethiopia which could be a potential public health hazard in contracting taeniasis.

Human teaniasis

A well-formulated questionnaire is an important tool for the detection of T. saginata in the carrier population, in individual cases as in mass investigations (Fralova, 1985). The respondents who were questioned in this study disclosed findings of proglottids in their feces, underwears indicated the presence of T. saginata. World Health Organization's guidelines (WHO, 1983) state that T. saginata is known by its more frequent anal expulsion of proglottids than T. solium. The present 71.2% prevalence of human teniasis indicated its importance and widespread health problems in the studied area. This finding was agreed with 64% in Bishoftu (Emiru *et al.,* 2015), 70% in Gonder (Abunna, 2013), 64.2% in Wolaita Soddo (Dawit *et al.,* 2012), 56.7% in Zeway (Bedu *et al.,* 2011), 62.5% in Awassa (Abunna *et al.,* 2007), 79.5% in East Shoa (Hailu, 2005) and 69.2% in Yirgalem (Dawit, 2004) all from different parts of Ethiopia indicating food safety aspect, public health significance and zoonotic importance of this diseases in the country.

The high prevalence of in men, occupationally high-risk individuals, married persons, persons who consumed frequently raw meat and individuals with low taeniasis knowledge due to the habit of eating raw or undercooked meat consumption with no knowledge of zoonoses the area including the country. Zoonoses risk of consumption of such meat has been reported from parasitic zoonoses (Suarez and Santizo, 2005).

Contribution of age for the prevalence of taeniasis was in line with Bedu et al. (2011) and Abunna (2013) but contrast with report of Regassa et al. (2009); Taresa et al. (2011) and Dawit et al. (2012).

The present higher prevalence in males than females observed in this study was similar with the reports made by Tembo (2001), Dawit (2004), Hailu (2005), Bedu et al. (2011) and Emiru et al. (2015) in other parts of Ethiopia, and Fan (1997) in Taiwan. In contrast to the present finding, Gracey et al (1999) reported that females were more frequently affected than males. Among the residents from different occupational backgrounds, the high-risk groups were found to be more prone to infection than the counter groups indicating the more likelihood contact infection upon frequent exposure. Similar suggestion were also given (WHO, 1983; Dawit, 2004; Hailu, 2005; Abunna *et al.,* 2007; Bedu *et al.,* 2011 and Emiru *et al.,* 2015). The present finding of taeniasis, among raw meat consumers and those with lowleveles of diseases knowledge, which was similar with reports of Bedu *et al.,* (2011), Abunna et al. (2013) and Emiru et al. (2015) in Ethiopia, and in other countries like Union Soviet Socialist Republic, India, Thailand and Central and East Africa (Anataraman, 1974; Fan, 1997), indicated the role and risk of raw meat as sources of taeniasis in developing countries. The difference in prevalence of taeniasis infection could be attributed to variations in degrees of exposure, personal and environmental hygiene; awareness in using latrine and habit of raw and under cooked meat. Ethiopia where meat is an important component of human diet and traditionally it is consumed raw on several occasions.

Conclusion

Taeniasis/cysticercosis occurs most commonly in the environments characterized by poor sanitation, primitive livestock husbandry practice and inadequate meat inspection. High prevalence of human cases in the studied area with higher incidence in male, high risk groups, married, those frequently consume raw meat, and in those with low knowledge of taeniasis indicates the need for practice application on public health awareness on the risk associated with consumption of raw and under cooked meat. Moreover, routine meat inspection with individual and environmental hygiene such as use toilets/latrines training shall implemented for the control and prevention of the diseases both in human and animal which also promote the beef industry.

Conflict of Interests

The authors have not declared any conflict of interests.

Acknowledgments

The author would like to acknowledge Madda Walabu University for logistic and financial support. Asella Municipality Abattoir workers, cattle owners and questioner respondents are highly appreciated for their point in time cooperation and support during data collection.

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PARASITOSES GASTRO-INTESTINALES DES PETITS RUMINANTS ET DU GUIB HARNACHÉ AUTOUR DE LA FORÊT CLASSÉE DE WARI-MARO AU NORD-EST DU BÉNIN

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Résumé

La présente étude vise à comparer l'intensité d'infestation par les helminthes gastro-intestinaux des petits ruminants (ovins, caprins) et du guib harnaché (ruminant sauvage) utilisant les mêmes aires de pâturage dans trois campements riverains à la forêt classée de Wari-Maro située au Nord-Est du Bénin. Une coproscopie quantitative des échantillons de crottes a été effectuée par la technique de Mc Master. Les prélèvements ont été effectués pendant les saisons sèche et pluvieuse avec un total de 60 échantillons pour les ovins, 60 pour les caprins et 20 pour le guib harnaché. Les types de parasites communs aux petits ruminants ont été les strongles, Strongyloides, les coccidies ; les cestodes (Moniezia expansa) n'ont été retrouvés que chez les caprins et au cours de la saison pluvieuse. Aucun œuf d'helminthes ou d'oocyste de coccidies n'a été retrouvé dans les échantillons de crottes du guib harnaché. Les caprins ont présenté les plus forts taux d'infestation (>50%) pour tous les types de parasites au cours de la saison sèche. Les ovins quant à eux ont présenté les plus forts taux d'infestation en coccidies (>70% dans deux campements) au cours de la saison pluvieuse. L'excrétion fécale des œufs a varié de 50 à 2500 OPG au cours de la saison sèche et de 150 à 8400 OPG au cours de la saison pluvieuse. Des résultats de l'étude il ressort qu'aucun type de parasite gastro-intestinal n'est commun aux petits ruminants et au guib harnaché et qu'aucune relation n'existe entre leur intensité d'infestation.

Mots clés : parasites gastro-intestinaux, Mc Master, ovins-caprins, guib harnaché, Wari-Maro, Bénin

GASTROINTESTINAL PARASITOSIS OF SMALL RUMINANTS AND BUSHBUCK AROUND THE FOREST RESERVE WARI-MARO IN THE NORTH EAST OF BENIN

Summary

This study aims to compare the intensity of infestation by gastro-intestinal helminths of small ruminants (sheep and goats) and bushbuck (wild ruminants) using the same grazing areas in three coastal settlements in the classified forest Wari-Maro in the north-eastern Benin. A quantitative coproscopy of the droppings samples was carried out using the McMaster technique. The samples were collected during the dry and rainy seasons with a total of 60 samples for sheep, 60 goats and 20 bushbuck. The types of common parasites of small ruminants were strongyles, Strongyloides, coccidia; tapeworms (Moniezia expansa) have been found in goats and that during the rainy season. No helminth eggs or coccidia oocyst was found in samples of droppings bushbuck. Goats presented the highest infection rates (> 50%) for all types of parasites during the dry season. Sheep in turn presented the highest infection rates in coccidia (> 70% in both camps) during the rainy season. Fecal excretion of eggs ranged from 50 to 2500 OPG during the dry season and 150-8400 OPG during the rainy season. The results of the study it appears that no type of gastrointestinal parasite is common to small ruminants and bushbuck and no relationship exists between their infestation intensity.

Keys words: Gastrointestinal parasites, Mc Master, EPG, sheep-goat, bushbuck, Wari-Maro, Benin

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Introduction

En zone tropicale l'un des problèmes entravant le développement de l'élevage des ruminants est l'infestation par les helminthes (Fabiyi, 1987). Ces infestations induisent des pertes économiques importantes par morbidité et mortalité auxquelles s'ajoutent le coût de la lutte chimique (Baker, 1997). Au Bénin l'élevage des petits ruminants (ovins, caprins) arrive en deuxième position après celui des bovins et se rencontre dans presque toutes les exploitations (Djènontin, 2004). Cette activité est très importante dans la partie septentrionale et constitue pour les familles une épargne rapidement mobilisable pour résoudre des problèmes d'ordre financier (Ehouinsou *et al.,* 2006). L'alimentation des animaux dans cette partie du pays et plus précisément dans les départements de l'Alibori et du Borgou sur les pâturages naturels et les résidus de récolte (Djènontin, 2004). L'exploitation des pâturages comporte en corollaire une exposition accrue des animaux au parasitisme par des helminthes, dont la transmission est généralement associée à la consommation d'herbe (Hoste *et al.,* 2003). De plus des ruminants sauvages peuvent se retrouver sur les pâturages naturels et exploiter ces derniers. De nombreux parasites ont été identifiés pouvant infester aussi bien les animaux domestiques que sauvages (Grootenhuis, 1999 ; Martin *et al.,* 2011). Les nématodes gastro-intestinaux surtout les Trichostrongyloidea, sont communs chez tous les ruminants, les vers trouvés dans les ongulés sauvages (cervidés) sont généralement des espèces apparentées à celles des ruminants domestiques (Miller *et al.,* 1998). Certains nématodes tels que *Ostertagia lyrata*, *Marshallagia marshalli*, *Teladorsagia circumcincta*, *Haemonchus contortus* sont communs aux cervidés, aux petits ruminants et aux bovins (Pineau, 1994). Les œufs de Trématodes (*Fasciola hepatica* et *Dicrocoelium lanceolatum*) peuvent être maintenus dans l'environnement des ruminants domestiques par les cervidés. La cohabitation des ruminants domestiques et sauvages peut donc conduire à des échanges de parasites (Paploray, 2002). Ces possibilités d'échanges de parasites sont très peu étudiées (Grootenhuis, 1999). Ainsi l'objectif de la présente recherche est de comparer l'intensité d'infestation parasitaire gastro-intestinale des petits ruminants domestiques et du guib harnaché (ruminant sauvage) utilisant les mêmes aires de pâturage en vue d'élucider la possibilité d'échange de parasites entre ces différents groupes d'animaux.

Matériel et Méthodes

Milieu d'étude

La forêt classée de Wari-Maro se trouve entre 8°50' et 9°10' de latitude Nord et 1°55' et 2°25' de longitude Est, elle est à cheval sur les communes de Bassila et de Tchaourou, respectivement dans les départements de la Donga et du Borgou, situés dans la partie septentrionale du Bénin. Les campements choisis pour l'étude sont : Oubérou: (X= 414049,380 m, Y= 1017190,511 m) ; Samba: (X= 411672,401 m, Y= 1014962,094 m) ; Sinahou: (X=416723,481 m,Y=1017487,634 m). Le climat qui règne dans la forêt de Wari-Maro est de type soudano-guinéen avec une hauteur moyenne annuelle comprise entre 1.100 mm et 1.200 mm de pluies. Les températures varient entre 21 et 33 °C (Figure 1).

Matériel

Les prélèvements de crottes ont été effectués sur les ovins, les caprins et les guibs harnachés.

Le guib harnaché (Figure 2), un ruminant sauvage est une élégante antilope de 70 cm en moyenne au garrot avec une robe brun rougeâtre assez foncée chez le mâle, rouge vif chez la femelle et des raies blanches verticales réparties sur le thorax, les flancs et les cuisses. Il est largement répandu en Afrique subsaharienne dans les aires suffisamment couvertes pouvant lui permettre de se dissimuler. Il est un consommateur sélectif qui peut se nourrir d'espèces arbustives, graminéennes et d'herbage. Il peut également se nourrir des produits de plantations, des fruits gousses, de tubercules et de racines (Okiria, 1980 ; Corson, 2004).

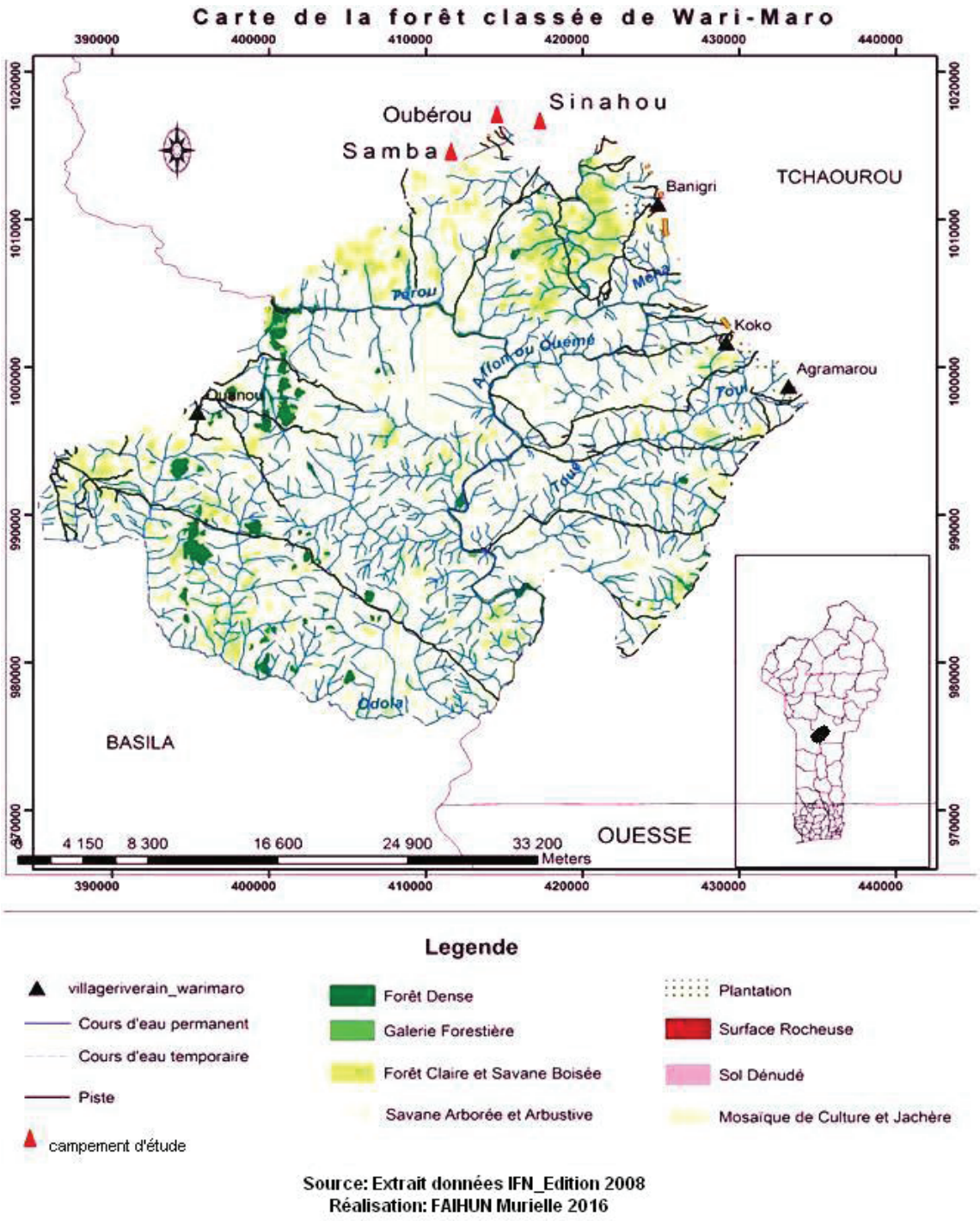


Figure 1 : Carte de la forêt classée de Wari-Maro présentant les trois campements d'étude

Figure 2 : Photo du guib harnaché (Anonyme) *http://www.dechevre.be/burkina/nouvelles/356-parc-de-la-pendjari-le-guib-harnache*

Les ovins et caprins prélevés au cours de cette étude sont des adultes de race Djallonké âgés de deux ans en moyenne élevés suivant un système extensif sur des parcours naturels à exploitation communautaire.

Méthodologie

Des échantillons de fèces ont été prélevés directement dans le rectum des ovins et caprins pendant les deux saisons pour un contrôle parasitaire. Dix animaux de chaque espèce ont été prélevés dans chaque campement. Chaque prélèvement est bien identifié (date, nom du campement, numéro du prélèvement), conservé dans un bocal en plastique. Les matières fécales sont acheminées au laboratoire vétérinaire de Parakou dans moins de six heures après le prélèvement sur les animaux. Elles sont immédiatement analysées ou le cas échéant sont conservées au réfrigérateur à 4 °C pendant au plus trois jours pour des analyses différées (Hansen *et al.,* 1995).

Pour le guib harnaché seules les fèces fraîches et non dégradées sont collectées dans les zones de pénétration des animaux domestiques. Les crottes ainsi collectées sont conditionnées dans des boites en plastique. Chaque boite est identifiée (la date, la zone de prélèvement) et rapidement acheminée au laboratoire pour être conservée dans les mêmes conditions que celles des animaux domestiques.

Un total de 30 échantillons de fèces pour les ovins, 30 pour les caprins, 10 pour le guib harnaché ont été analysés au cours de chacune des saisons (saison sèche et saison pluvieuse). Au cours de la saison sèche les prélèvements ont été effectués entre novembre et mars 2012 et au cours de la saison pluvieuse ils ont été effectués entre juin et septembre 2013.

La méthode de coproscopie utilisée est celle de Mc Master (Hansen et Perry, 1995) pour quantifier le nombre d'œufs de parasite présents par gramme de fèces.

Analyses statistiques

Le taux d'infestation a été calculé à partir de la formule suivante

$$\text{Taux d'infestation (parasite donné)} = \frac{\text{Nombre d'échantillons positifs}}{\text{Nombre total d'échantillons}} \text{x100}$$

L'analyse de la variance à un critère a été utilisée pour comparer les moyennes des OPG des différentes catégories d'animaux d'un campement à l'autre, d'une saison à l'autre et d'une espèce à l'autre ; le logiciel R 2.15 a été utilisé pour réaliser les différents tests statistiques.

Résultats

La coproscopie a révélé un polyparasitisme chez les petits ruminants domestiques par contre aucun œuf d'helminthes ou oocyste de coccidies n'a été identifié chez le guib harnaché (ruminant sauvage). Les ovins et les caprins ont présenté les mêmes types de parasites au cours de la saison sèche ce sont les strongles, les Strongyloides et les coccidies. Au cours de la saison pluvieuse les ovins ont toujours présenté les mêmes types de parasites, les caprins quant à eux ont présenté en plus de ces types de parasites les cestodes (Moniezia expansa) qui n'ont été rencontrés que chez les caprins du campement de Sinahou (Tableau 1). Au cours de la saison sèche les taux d'infestation pour tous les types de parasite ont été plus chez les caprins (>50%) quoique les ovins du campement de Sinahou

ont présenté les plus forts taux d'infestation en coccidies (100%) (Tableau 1).

Au cours de la saison pluvieuse les ovins ont présenté les plus forts taux d'infestation en coccidies variant de 20% à 100%. Seuls les caprins ont présenté des cestodes avec un taux d'infestation de 30%. Les animaux toutes espèces confondues ont présenté des taux d'infestation faibles en Strongyloides, ces taux varient de 10% à 60% (Tableau 1).

Les caprins ont présenté les plus fortes excrétions fécales pour les strongles au cours de la saison sèche et de la saison pluvieuse elles varient respectivement de 135±91,44 à 421,43±251,42 et de 657,14±214,92 à 937,50±698,85. Ils ont également présenté les plus forts OPG en Strongyloides au cours de la saison pluvieuse variant de 200±100 à 1200±1905,26. Les ovins quant à eux ont montré les plus fortes excrétions fécales en coccidies au cours des deux saisons, elles varient de 75±41,83 à 2500 au cours de la saison sèche et de 685,71±815,33 à 8400±565,69 au cours de la saison pluvieuse (Tableau 2).

La moyenne des OPG obtenus pour tous les types de parasites chez les ovins et les caprins n'ont pas présenté de différence significative d'un campement à l'autre au cours de la saison sèche. Au cours de la saison pluvieuse les ovins ont présenté des excrétions fécales en Strongyloides significativement différentes d'un campement à l'autre ; chez les caprins les moyennes des OPG en cestodes (*Moniezia expansa*) et en coccidies sont significativement différentes d'un campement à l'autre. Il faut aussi signaler que les petits ruminants du campement de Sinahou ont présenté les plus fortes excrétions fécales pour tous les types de parasites au cours de la saison pluvieuse (Tableau 2).

La saison a influencé l'intensité parasitaire des ovins et des caprins en les différences sont significatives d'une saison à l'autre. Chez les caprins il a eu également un effet de la saison sur les excrétions fécales des animaux en cestodes. Les plus fortes excrétions fécales pour les différents de types de parasites retrouvés ont été enregistrées au cours de la saison pluvieuse (Figure 3).

Au cours des deux saisons les OPG moyens en strongles enregistrés chez les caprins sont supérieurs à ceux obtenus chez les ovins et les différences sont statistiquement significatives au cours de la saison sèche. Les ovins quant à eux ont présenté les plus fortes excrétions fécales en coccidies que ce soit en saison sèche ou en saison pluvieuse et les différences sont statistiquement significatives au cours de la saison pluvieuse (Figure 3).

Tableau I : Taux d'infestation par type de parasites chez les catégories d'animaux étudiées au cours des saisons

Campement		**Espèces**	**Pourcentage d'échantillons positifs (%)**			
			Strongles	**Strongyloides**	**Cestodes (Moniezia expansa)**	**Coccidies**
Saison sèche	Oubérou n=10	Ovins	70	30	0	60
		Caprins	100	90	0	70
	Samba n=10	Ovins	30	30	0	10
		Caprins	70	90	0	50
	Sinahou n=10	Ovins	100	60	0	100
		Caprins	100	80	0	80
	n=10	Guib harnaché	0	0	0	0

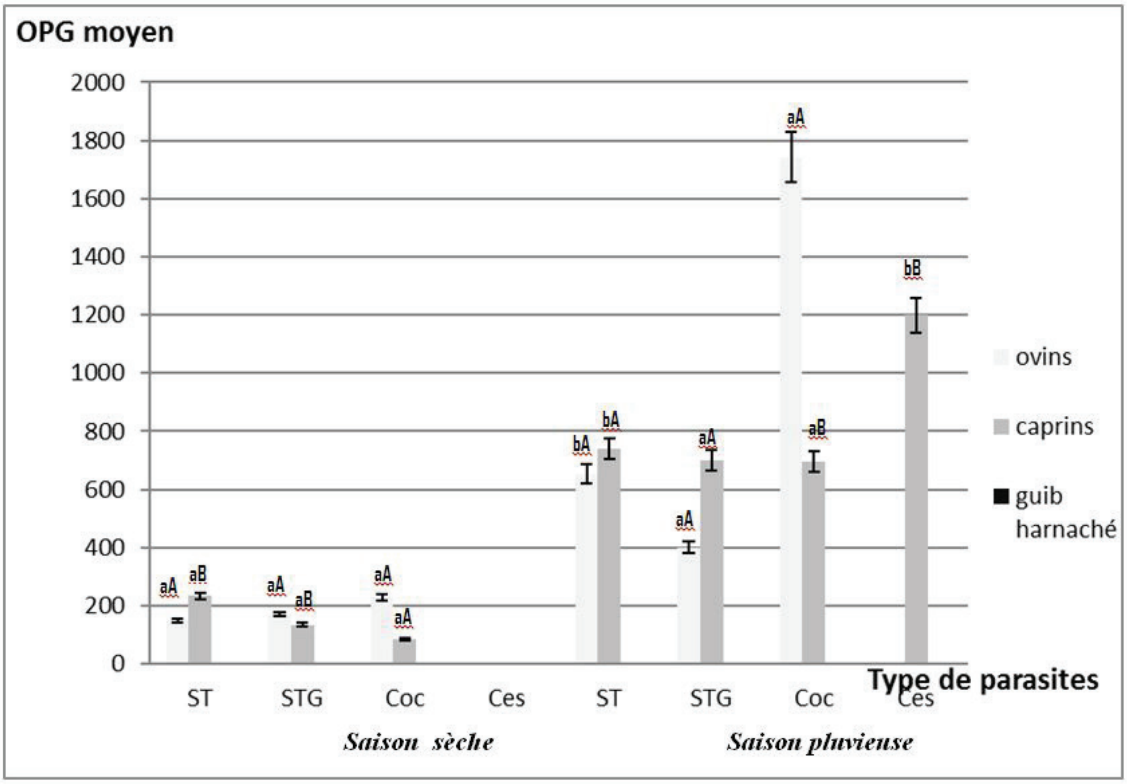
Campement		Espèces	Pourcentage d'échantillons positifs (%)			
			Strongles	Strongyloides	Cestodes (Moniezia expansa)	Coccidies
Saison pluvieuse	Oubérou n=10	Ovins	50	20	0	70
	Samba n=10	Caprins	70	60	0	60
		Ovins	80	10	0	100
	Sinahou n=10	Caprins	100	30	0	40
		Ovins	90	50	0	20
	n=10	Caprins	80	30	30	10
		Guib harnaché	0	0	0	0

Tableau 2 : OPG moyen par type de parasites dans les campements chez les ovins, les caprins et le guib harnaché au cours des saisons

	Espèce	Campement	OPG moyen ± écartype			
			Strongles	Strongyloides	Cestodes	Coccidies
Saison sèche	Ovins	Oubérou	150±115,47[a]	66,67±28,87[a]	0[a]	75±41,83[a]
		Samba	316,67±175,59[a]	516,67±510,72[a]	0[a]	2500[a]
		Sinahou	100±66,67[a]	50±0[a]	0[a]	95±59,86[a]
	Caprins	Oubérou	135±91,44[a]	183,33±108,97[a]	0[a]	92,86±53,45[a]
		Samba	421,43±251,42[a]	122,22±93,91[a]	0[a]	70±27,39[a]
		Sinahou	200±113,04[a]	93,75±56,30[a]	0[a]	112,50±69,44[a]
	Guib harnaché		-	-	-	-
Saison pluvieuse	Ovins	Oubérou	300±158,11[a]	250±70,71[a]	0[a]	685,71±815,33[a]
		Samba	887,50±683,35[a]	300[a]	0[a]	1150±992,47[a]
		Sinahou	677,78±582,62[a]	480±238,75[b]	0[a]	8400±565,69[a]
	Caprins	Oubérou	657,14±214,92[a]	700±352,14[a]	0[a]	933,33±280,48[a]
		Samba	640±464,76[a]	200±100[a]	0[a]	150±179,70[a]
		Sinahou	937,50±698,85[a]	1200±1905,26[a]	1200±556,78[b]	1100[a]
	Guib harnaché		-	-	-	-

(-) pas d'échantillons positifs pour le type de parasite

(ab) Les OPG moyens pour chaque type de parasite correspondant à chacun des campements sont statistiquement différents ou non suivant qu'ils ont respectivement une lettre différente ou non (par l'analyse de la variance) au seuil de 5 %



(ab) Les OPG moyens pour chaque type de parasite sont statistiquement différents ou non d'une saison à l'autre suivant qu'ils ont respectivement une lettre différente ou non au seuil de 5 %
(AB) Les OPG moyens pour chaque type de parasite sont statistiquement différents ou non d'une espèce à l'autre suivant qu'ils ont respectivement une lettre différente ou non au seuil de 5 %effet espèce
ST : strongles ; STG : strongyloides ; Coc : coccidies ; Ces : cestodes

Figure 3 : Comparaison des OPG moyen par type de parasites chez les ovins, les caprins et les guibs harnachés au cours des saisons

Discussion

Dans les zones tropicales et subtropicales les ovins et les caprins présentent les mêmes types de parasites (Ouattara et Dorchies, 2001). En condition d'infestation naturelle, les animaux hébergent très fréquemment plus d'une espèce de parasite (Gruner, 1991). Généralement les trois grands ordres sont rencontrés (Nématodes, Cestodes et Trématodes) avec un grand nombre de genres pour chacun de ces ordres (Hansen et Perry 1995). Les genres Strongyloides sp et *Moniezia expansa* ont été recensés lors de la présente étude de même que les œufs des strongles. Les échantillons de crottes analysés du guib harnaché n'ont révélé la présence d'aucun ookyste ou œuf d'helminthes. Van Wyk et Boomker (2011) qui ont examiné un guib harnaché en Afrique du Sud ont également rapporté l'absence d'œuf d'helminthe chez cet herbivore sauvage. Faihun *et al* (2017) ont par contre rencontré chez le guib harnaché deux types de parasites à savoir les strongles et Strongyloides. Les techniques de coproscopie qualitative (flottation simple et sédimentation) ont été utilisées par ces auteurs ; ces dernières permettent de mieux détecter la présence des œufs des différents parasites que la méthode de Mc Master dont le seuil de signification est 50 OPG. Boomker *et al* (1986) ont également recensé les genres *Trichostrongylus sp*, *Haemonchus sp*, *Cooperia sp*. chez le guib harnaché. L'hypothèse selon laquelle des

échanges de parasites pourraient se faire entre les ruminants domestiques et sauvages pâturant dans les mêmes environnements ne peut être infirmée ni confirmée. Les investigations doivent donc se poursuivre sur un effectif plus grand de guib harnaché pour étayer cette question. Deux explications peuvent cependant être données par rapport à l'absence d'infestation du guib harnaché rapportée par la présente étude. Le mode d'alimentation de ce dernier d'une part, il est un ongulé brouteur consommateur sélectif qui se nourrit d'herbe mais surtout de feuilles, de jeunes pousses, de gousses, de tubercules et de racines (Corson, 2004) ce qui l'expose moins aux infestations par les parasites gastro-intestinaux qui se trouvent au sol dans les herbages. D'autre part les guibs harnachés sont infestés mais les helminthes étaient en hypobiose et n'ont pas excrété des œufs ou ookystes détectables à la coproscopie (Camuset, 2010). Pour avoir des conclusions plus complètes il sera alors nécessaire de procéder à des bilans parasitaires chez quelques guibs harnachés pour avoir la certitude ou non de leur infestation.

Les niveaux d'infestation en strongles que ce soit chez les ovins et les caprins ont été faibles (de 100±66,67 à 316,67± pour les ovins et de135±91,44 à 421,43± 251,42 pour les caprins) au cours de la saison sèche et moyens (de 300±158,11 à 887,50±683,35 pour les ovins et de 650±464,76 à 937,50± 698,85 pour les caprins) au cours de la saison pluvieuse. Les ovins ont été plus infestés par les coccidies et en ont présenté les OPG les plus élevés quoi que les intensités des infestations ont été faibles (2500 OPG) au cours de la saison sèche et moyennes (8400± 565,69) au cours de la saison pluvieuse. Les coccidioses sont fréquentes en élevage ovin et causent une diarrhée nauséabonde verdâtre ou noirâtre (Poncelet, 2008). Les caprins quant à eux ont présenté les plus fortes excrétions fécales en strongles et la comparaison avec celles des ovins a montré une différence significative. En effet contrairement aux ovins l'infestation répétée des caprins par les larves de strongles ne conduit qu'à une réponse immunitaire faible ce qui augmente leur sensibilité à ces types d'infestation (Rozette, 2010). Le caractère rustique de la race Djallonké (Baker, 1997) des ovins et caprins concernés par notre étude ainsi que le mode d'élevage extensif adopté par les éleveurs expliquent l'infestation faible ou modérée de ces derniers par les parasites gastro-intestinaux. Que ce soit chez les ovins ou chez les caprins les plus fortes excrétions fécales ont été enregistrées au cours de la saison pluvieuse. Il est reconnu que la saison pluvieuse, favorable au développement des larves infestantes sur les pâturages, constitue la période d'explosion parasitaire particulièrement chez les ruminants (Fritsche *et al.,* 1993). L'humidité et l'oxygénation constituent des facteurs déterminants pour le développement des larves; la sécheresse limite la survie de toutes les espèces de strongles (Rozette, 2010). Les contaminations croisées entre les chèvres et les ovins sont importantes car de nombreuses espèces de strongles leur sont communes : *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Cooperia punctata*, *Cooperia pectinata*, *Trichuris ovis* (Ouatara et Dorchies, 2001). Il serait donc mieux d'éviter de mettre les ovins et les caprins sur le même pâturage (Rozette, 2010). Une attention plus soutenue doit être accordée aux petits ruminants du campement de Sinahou plus particulièrement les caprins qui ont présenté une intensité d'infestation importante en cestodes au cours de la saison pluvieuse. Les trématodes et les cestodes sont deux ordres de parasites posant des problèmes importants en zone tropicale (Baker, 1997). Les infestations aux cestodes pouvant causer parfois des symptômes nerveux sont surtout pathogènes aux jeunes sujets (Abassa, 1975).

Conclusion

Les espèces de ruminants domestiques (ovins, caprins) et de ruminants sauvages (guib harnaché) concernées par notre recherche n'ont pas montré de relation entre leur niveau d'infestation parasitaire. Les résultats de coproscopie ont révélé la présence de plusieurs types de parasites chez les petits ruminants

alors qu'aucun œuf d'helminthe ou oocyste de coccidies n'a été retrouvé chez le guib harnaché. Les types de parasites rencontrés chez les petits ruminants sont les strongles, Strongyloides, les cestodes et les coccidies. Les intensités d'infestation des petits ruminants par ces types de parasites ont été faibles au cours de la saison sèche et moyennes au cours de la saison pluvieuse. Les caprins semblent être plus sensibles aux infestations par les strongles et les cestodes alors que les ovins le sont plus par les coccidies. Les intensités parasitaires pour tous les types de parasites ne sont pas statistiquement différentes d'un campement à l'autre dans la majorité des cas cependant une attention plus particulière doit être accordée aux petits ruminants du campement de Sinahou. L'absence des œufs de parasites dans les échantillons de crottes du guib harnaché montrée par les résultats de notre étude doit être complétée par des autopsies helminthologiques pour une confirmation ou infirmation de celle-ci. Aussi un nombre plus important d'échantillons de crottes doit être analysé pour des résultats plus exhaustifs.

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SURVEY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN TRADE CATTLE SLAUGHTERED AT ABATTOIRS IN NORTH-CENTRAL NIGERIA

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Abstract

Contagious bovine pleuropneumonia (CBPP) is one of the most important infectious and contagious disease of cattle in sub-Saharan Africa and whose control is urgently needed. A cross-sectional study was conducted to investigate prevalence of CBPP and associated animal factors that predisposed to the disease in trade cattle slaughtered in Niger State, North-central Nigeria between January and May 2014. A total of 525 trade cattle were selected by systematic sampling approach from five purposively selected central abattoirs in the State. Serum samples were analyzed using c-ELISA and lung lesions determined at post-mortem inspections. The OpenEpi 2.3.1 software was used for statistical analyses. Descriptive and analytical statistics were used to present the results. Associations between animal characteristics (predictor variables) and sero-positivity as well as presence of CBPP lung lesions (outcome variables) were tested using Chi-square tests and likelihood backward logistic regressions. A sero-prevalence of 31.8% (95% CI: 27.93, 35.89) in live cattle before slaughtered was observed. Also, 29.5% (95% CI: 25.74, 33.53) of the slaughtered trade cattle had CBPP lung lesions at post-mortem inspections. Cattle in age group 4–5 years were more likely (OR 2.00; 95% CI: 1.17, 3.39) to be significantly exposed to Mycoplasma mycoides subsp. mycoides (Mmm) infection. Cows were more likely to be significantly predisposed to Mmm infections. Furthermore, bunaji breeds were more likely (OR 2.26, 95% CI: 1.42, 3.59) to be significantly predisposed to Mmm infections. This study has shown the need to combine more reliable serological tests with post-mortem examinations to improve active surveillance of CBPP in trade cattle. These dual approaches to investigation of CBPP and identification of intrinsic factors predisposing to infection should be institutionalized as elements of epidemio-surveillance and control strategies of the disease in sub-Saharan Africa.

Keyword: Abattoir, CBPP, c-ELISA, lung-lesions, trade cattle, Nigeria.

ÉTUDE DE LA PLEUROPNEUMONIE CONTAGIEUSE BOVINE CHEZ LES BOVINS COMMERCIALISES ABATTUS DANS LES ABATTOIRS DU CENTRE-NORD DU NIGÉRIA

Résumé

La pleuropneumonie contagieuse bovine (PPCB) est l'une des maladies infectieuses et contagieuses les plus importantes des bovins en Afrique subsaharienne dont le contrôle nécessite une intervention urgente. Une étude transversale a été menée, entre janvier et mai 2014, dans le but d'étudier la prévalence de la PPCB et les facteurs animaux associés prédisposant à la maladie, parmi les bovins commercialisés abattus dans l'État du Niger au centre-nord du Nigeria. Au total, 525 bovins commercialisés ont été choisis par échantillonnage systématique dans cinq abattoirs centraux sélectionnés à dessein dans cet État. Les échantillons de sérum ont été analysés en utilisant l'ELISA de compétition (c-ELISA), et les lésions pulmonaires ont été déterminées en procédant aux inspections post-mortem. Le logiciel OpenEpi 2.3.1 a été utilisé pour les analyses. Des statistiques descriptives et analytiques ont été utilisées pour la présentation des résultats. Les associations entre les caractéristiques (variables de prévision) et la séropositivité des animaux ainsi que la présence de lésions pulmonaires de la PPCB (variables de résultats)

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ont été évaluées en utilisant des tests de Chi-carré et les régressions logistiques de vraisemblance. Avant l'abattage, une séroprévalence de 31,8% (IC 95% : 27,93 ; 35,89) avait été observée chez les bovins. De plus, les inspections post-mortem ont révélé que 29,5% (IC 95% : 25,74 ; 33,53) des bovins abattus avaient des lésions pulmonaires dues à la PPCB. Les bovins âgés de 4 à 5 ans étaient plus susceptibles (OR 2.00; IC 95%: 1,17 ; 3,39) d'être significativement exposés à Mycoplasma mycoides subsp. Mycoïdes (Mmm). De plus, les vaches étaient plus susceptibles d'être significativement prédisposées aux infections à Mmm. En outre, les races bunaji étaient plus susceptibles (OR 2,26, IC 95%: 1,42, 3,59) d'être significativement prédisposées aux infections Mmm. Cette étude a fait ressortir la nécessité de combiner des tests sérologiques plus fiables avec des examens post-mortem, afin d'améliorer la surveillance active de la PPCB chez les bovins commercialisés. Cette double approche d'enquêtes sur la PPCB et l'identification de facteurs intrinsèques prédisposant à l'infection devraient être institutionnalisées en tant qu'éléments de stratégies de surveillance épidémiologique et de contrôle de la maladie en Afrique subsaharienne.

Mots-clés : abattoir, PPCB, c-ELISA, lésions pulmonaires, bovins commercialisés, Nigeria

Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious and contagious respiratory disease of cattle with high economic impacts in sub-Saharan Africa (Tambi *et al.,* 2006). It is caused by Mycoplasma mycoides subsp. mycoides (Mmm), previously further specified as small colony (SC) type (Manso-Silván *et al.,* 2009), and characterized by sero-fibrinous interlobular edema and hepatization giving a marbled appearance to the lung in acute and sub-acute cases, and capsulated lesions (sequestra) in the lungs of chronically infected cattle (Schnee *et al.,* 2011; Tardy *et al.,* 2011). Affected cattle developed dyspnea due to damage to the lungs, and a proportion of them died from respiratory insufficiency (Radostits *et al.,* 2007). The disease is transmitted by direct contact between infected and susceptible cattle (Vilei and Frey, 2010). While CBPP was eradicated from USA in the mid 20th century, it was not eradicated from Europe until the end of the 20th century and still persists in many African countries, especially in the West, Central, East, and parts of Southern Africa (Nicholas *et al.,* 2008), though has not been reported in North Africa (Amanfu, 2009).

CBPP is a major constraint to cattle production in sub-Saharan Africa and is regarded as the most serious infectious animal disease affecting cattle in the continent (Amanfu, 2008; Marobela-Raborokgwe, 2011). The economic impact of this disease is due to huge losses in cattle (Foluso, 2004; Tambi *et al.,* 2006; OIE, 2010). The decline in its outbreaks and burden reports in Nigeria and other affected African countries was due to absence of science-based evidence for disease prevalence, which does not augur well for the implementation of internationally coordinated control programmes as it underpins control and preventive actions (Amanfu, 2009).

Abattoir surveillance has been used in many countries as an important strategy for detection of disease cases and provides essential information that can be utilized for research and disease control purposes (Nunes–Pestica *et al.,* 1990; Nicholas *et al.,* 1996). The use of meat inspection to detect CBPP cases in slaughter facilities is particularly useful in Africa where laboratory capacity for routine disease diagnosis is limited (Phiri, 2006; Cadmus and Adesokan, 2009). Moreover, diagnosis and evaluation of diseases like cysticercosis, *fasciolosis* and CBPP can be made much more reliable at abattoir than other conventional diagnostic methods (Swai *et al.,* 2013). For accurate diagnosis of CBPP, gross pathological changes in the affected organs and tissues (Nunes–Pestica *et al.,* 1990) backed with sensitive and specific serological reactions (Egwu *et al.,* 1996) are required.

Although cattle production is a major economic activity in Nigeria, there is paucity of epidemiological based information on burden and associated predisposing biological factors for CBPP in trade cattle slaughtered at abattoirs in the country, while prevalence of the disease varies from one geographical area

to another. Many cattle traders are unaware of the existence of CBPP in apparently healthy animals at slaughter. Generation of such data would be a convenient and inexpensive source of information that would assist in the development of surveillance and control strategies for CBPP in endemic African countries. This study, therefore, was aimed at investigating abattoir based prevalence of contagious bovine pleuropneumonia in slaughter trade cattle using post-mortem and serological examinations in Niger State, North Central Nigeria. Our Null hypothesis was, that potential animal factors cannot influence occurrence of CBPP in cattle slaughtered at abattoirs. The examined cattle were under zero vaccination status because the last CBPP vaccination campaign in the state was carried out in November 2011. Moreover, the T1/44 vaccine used in Nigeria has limited efficacy as the immunity conferred is of short duration and vaccination must be repeated annually (Thiaucourt *et al.,* 2000). For vaccination to be effective, it must be repeated initially at short intervals of six months and thereafter annually over 3-5 years (FAO, 2002; FAO, 2004).

Materials and methods

Study area

The survey was conducted at five central abattoirs in Niger State, North-central Nigeria. These slaughterhouses are located in Minna (coordinate N09.64655°; E006.54436°), Suleja (N09.13716°; E007.20159°), Bida (N09.08570°; E006.02027°), Kontagora (N1o.41057°; E005.46677°) and New-Bussa (N09.86805°; E004.48343°). Niger State is located in the Southern Guinea Savannah ecological zone of Nigeria, between latitude 8o 20' N and 11o 30' N, and longitude 3° 30' E and 7° 20' E. It is the largest State in Nigeria in terms of land mass, covering an area of 86,000 km^2, representing about 9.3% of the total land area of the country. The State has large grazing areas, many stock routes and an international border with the Republic of Benin, which is porous. It experiences two distinct seasons, rainy season that spans between April and October, and dry season between November and March. The mean annual rainfall is 1600 mm with duration of about 180 days. It has humidity of 104% and average lowest and highest temperatures of about 27°C and 39°C, respectfully. The State has an estimated cattle population of about 2.4 million cattle in 2012 (MLFD, 2013).

Study design and population

A cross-sectional survey was conducted on cattle slaughtered at five slaughterhouses between January and May 2014. It involved blood sample collection from cattle prior to slaughter and post-mortem inspections of the slaughtered cattle from which blood samples were taken. The study population was apparently healthy cattle of ages two years and above, both sexes (bulls and cows), and available breeds (bunaji and bokologi) brought to the abattoirs by livestock traders and livestock keepers for slaughter during the period of the survey.

Selection study abattoirs and trade cattle

A total of five central abattoirs were purposefully selected for the study from the five major towns in the State. Accordingly, 525 cattle were selected from the target populations using systematic sampling procedure with, every 5th cattle examined for slaughter selected. We assumed a comparable number of cattle slaughtered in each of the five abattoirs and took an equal number of 105 cattle in each slaughterhouse. Studied animals, presented asymptomatic, were bled before slaughtered and blood samples taken for sera. Post-mortem inspections involved visual observations, palpations and incisions of the suspected lungs (Phiri 2006).

Post mortem and serum samples collection

Gross pathological lesions on the suspected lungs were recorded after inspection. Serum samples detailed for serological analysis was also collected before slaughtered. Age, sex, breed and CBPP gross lesions of sequestration, hepatisation, marbling, nodulation of lungs, hypertrophy of pulmonary lymph nodes and excessive plural fluid of amber colour were

recorded at post-mortem inspections (OIE, 2001).

For serum, 10 ml of whole blood was taken from jugular vein of each selected cattle, using a sterile 10-ml syringe and 18 × 1½" gauge needle per animal. This was immediately placed into an ice bath slanted and transported to the laboratory within seven hours. The clot was allowed to form in syringe at the lairage before transportation. All daily collected sera were later transferred into plastic tubes and centrifuged at 3000 rpm for 20 min and then decanted into cryovials, which were identified before storage at -20 °C until analyzed.

Serological analysis

A competitive enzyme linked immunosorbent assay (c-ELISA), developed by the OIE Collaborating Centre for the diagnosis and control of animal diseases in tropical countries (Le Goff and Thiaucourt, 1998) was used. It detected antibodies in infected herds even if they persist for a longer period of time (FAO, 2003; Niang *et al.,* 2006). The test performance on CBPP has sensitivity of 99.9 % and greater specificity of more than 63.8 % (OIE, 2014). The c-ELISA was conducted using commercial *M. mycoides subsp. mycoides* (*Mmm*) Antibody Test Kit (CIRAD/IDEXX Institut Pourquier Laboratories, Montpellier, France) according to manufacturers' instructions. Optical densities (ODs) were measured at 450 nm using the Photometer Spectra Fluor (Tecan, Crailsheim, Germany). Only positive serum samples with percentage inhibition (INH%) cutoff value of 50 % and above were considered as positive.

Data management and analyses

Collected data were summarized and entered into Microsoft Excel 7 spreadsheet (Microsoft Corporation, Redmond, WA, USA) and stored. The Open Source Epidemiologic Statistics for Public Health (OpenEpi) version 2.3.1 software (Dean *et al.,* 2009) was used for the statistical analysis. Descriptive and analytical statistics were used to describe the obtained data. Descriptive statistics was used to determine the prevalence of the disease. The associations between individual cattle characteristics (independent variable) with negative or positive serological outcomes and absence or presence of CBPP lesions in lungs (dependent variables) were first investigated using univariable analysis by Chi-square (X^2) tests. All factors found to be biologically plausible and significant were finally subjected to multivariate logistic regressions using likelihood stepwise backward logistic regression models to control for confounding and test for effect modification. Furthermore, $p<0.05$ was considered statistically significant at all analyses.

Ethical clearance

The study protocol was reviewed and approved by the Ethical Clearance Committee of the Niger State Ministry of Livestock and Fisheries Development, Minna, Nigeria. Permissions were obtained from the Area Animal Health Offices in the State to carry out the study at the respective abattoirs.

Results

CBPP sero-prevalence and associated animal factors in trade cattle at ante-mortem examinations

Sero-prevalence of CBPP in trade cattle examined at ante-mortem was 31.8% (167/525; 95% CI: 27.93, 35.89). The sero-prevalence of CBPP in live animals at the five slaughterhouses is presented in Table 1.

All animal factors of age, sex and breed were significantly ($p<0,05$) associated with CBPP sero-positivity during univariate analysis. On subsequent logistic regressions, only cattle in age group 4–5 years were more likely (OR 2.00; 95% CI: 1.17, 3.39) to be significantly exposed to *Mmm* infection. Also, cows were more likely (OR 2.00; 95% CI: 1.34, 2.93) to be significantly predisposed to *Mmm* infection than bulls. However, bunaji breeds were two times more likely (OR 1.72, 95% CI: 1.12, 2.65) to be significantly predisposed to *Mmm* infection than bokoloji breeds (Table 2).

Table 1: Sero-prevalence of CBPP in trade cattle sampled at ante-mortem examination in abattoirs of Niger State, North-central Nigeria: 2014

Abattoir	Number of live cattle sampled	Number of negative	Number of positive	Proportion (%)	95% Confidence interval
Suleja	105	86	19	18.10	11.60, 26.33
Minna	105	73	32	30.48	22.25, 39.77
Bida	105	68	37	35.24	26.56, 44.73
Kontagora	105	61	44	41.91	32.75, 51.50
New-Bussa	105	70	35	33.33	24.82, 42.75
Total	**525**	**358**	**167**	**31.81**	**27.93, 35.89**

Table 2: Animal factors associated with CBPP sero-prevalence in trade cattle examined at ant-mortem in abattoirs of Niger State, North-central Nigeria: 2014

Factors	Number negative Row (%)	Number positive Row (%)	Odds ratio	95% Confidence interval	P-value
Age					
2-3 years	73 (76.0)	23 (24.0)	1.00		
4-5 years	161 (61.5)	101 (38.5)	2.00	1.17, 3.39	0.009
≥6 years	124 (74.3)	43 (25.7)	1.01	0.61, 1.97	0.754
Sex					
Bulls	164 (76.6)	50 (23.4)	1.00		
Cows	194 (62.4)	117 (37.6)	2.00	1.34, 2.93	<0.001
Breed					
Bokoloji	115 (76.2)	36 (23.8)	1.00		
Bunaji	243 (65.0)	131 (35.0)	1.72	1.12, 2.65	0.012

Statistically significant at p<0.05

Table 3: Proportions of slaughtered trade cattle with CBPP lesions in their lungs at abattoirs in Niger State, North-central Nigeria: 2014

Abattoir	Number of slaughtered cattle sampled	Number of cattle without of CBPP lesions	Number of cattle with CBPP lesions	Proportion (%)	95% Confidence interval
Suleja	105	78	18	17.14	10.82, 25.26
Minna	105	87	27	25.71	18.05, 34.71
Bida	105	70	35	33.33	24.82, 42.75
Kontagora	105	64	41	39.05	30.07, 48.62
New-Bussa	105	71	34	32.38	23.96, 41.76
Total	**525**	**370**	**155**	**29.52**	**25.74, 33.53**

Table 4: Animal factors associated with CBPP lesions in lungs of trade cattle slaughtered at abattoirs in Niger State, North-central Nigeria: 2014

Factors	Number negative Row (%)	Number positive Row (%)	Odds ratio	95% Confidence interval	P-value
Age					
2-3 years	75 (78.1)	21 (21.9)	Ref.		
4-5 years	166 (63.4)	96 (36.6)	2.10	1.20, 3.56	0.007
≥6 years	129 (77.2)	38 (22.8)	1.05	0.58, 1.93	0.877
Sex					
Bulls	175 (81.8)	39 (18.2)	Ref.		
Cows	195 (62.7)	116 (37.3)	2.67	1.76, 4.05	<0.001
Breed					
Bokoloji	123 (81.5)	28 (18.5)	Ref.		
Bunaji	247 (66.0)	127 (34.0)	2.26	1.42, 3.59	<0.001

Statistically significant at p<0.05

Proportions of CBPP lung lesions at post mortem inspection in trade cattle and associated factors

Of the cattle subjected to post-mortem inspections at the slaughterhouses, 29.5% (155/525; CI: 25.74, 33.53) of them had CBPP lesions in their lungs. The proportions of CBPP lesions in the lungs of slaughtered cattle at the respective abattoirs are presented in Table 3.

At the post-mortem inspections, all animal factors of age, sex and breed were significantly (p<0,05) associated with CBPP lesions in the lungs of slaughtered cattle at univariate analysis. At final logistic regressions, only animals in age group 4–5 years were two times more likely (OR 2.10; 95% CI: 1.20, 3.56) to be significantly exposed to Mmm infection. However, cows were more likely (OR 2.67; 95% CI: 1.76, 4.05) to be significantly predisposed to *Mmm* infection than bulls. Also, bunaji breeds were more likely (OR 2.26, 95% CI: 1.42, 3.59) to be significantly predisposed to *Mmm* infection than bokoloji breeds (Table 4).

In the infected lungs, there was pleurisy, loss of lung parenchyma architecture, marbling, consolidation and thickening of interlobular septa, and focal necrotic areas with massive sero-sanquinous fluid. However, in the uninfected lungs, we observed that normal architecture was maintained with no obvious pneumonic lesions. A piece of lung tissue floated when suspended in water and both pleura were clear and distinct.

Discussion

Clinical diagnosis of CBPP is unreliable as initial signs may be slight or non-existent and may be indistinguishable from any severe pneumonia. Therefore, CBPP should be investigated by pathological, microbiological, molecular or serological diagnostic methods. As the pathological lesions of CBPP are distinctive and pathognomonic, abattoir surveillance for CBPP involving lung examination at post-mortem is a practical method for disease monitoring (OIE, 2014). The inherent obstacles against the successful control of CBPP particularly in the sub-Saharan Africa could be linked to inadequate abattoir surveillance and reliable diagnostic tests (Egwu *et al.,* 1996).

The present study had shown that CBPP is enzootic in Nigeria, with abattoir-based sero-prevalence of 31.8% in Niger State using c-ELISA. The observed sero-prevalence was similar to the 32.0% sero-prevalence reported from a CBPP surveillance, using similar test, in Nigeria (Aliyu *et al.,* 2003), but higher than 7.8% sero-prevalence reported from a similar

investigation in some abattoirs around Adama province in Ethiopia (Atnafie *et al.,* 2015). The sero-prevalence observed indicates that there was a considerable levels of cattle challenged with *Mmm* infections without manifesting clinical signs. The detection of positive sera in asymptomatic cattle in this study further confirms the salient nature of CBPP, with likely implication that some of the positive animals were in chronic stage and were carriers, as previously reported (Provost *et al.,* 1987; Alhaji and Babalobi, 2016a).

Using CBPP lesions in lungs, this survey observed post-mortem CBPP prevalence of 29.5% at the abattoirs. This was higher than previously reported 18.8% in a similar CBPP surveillance in Nigeria (Aliyu *et al.,* 2003), 18.4% at the Maiduguri municipal abattoir in Nigeria (Egwu *et al.,* 2012) and 8.6% in slaughter facilities in 10 regions of Tanzania (Noah *et al.,* 2015). However, our observed gross lesions prevalence was similar to the 29.7% reported at Garoua central abattoir in Cameroun (Wade *et al.,* 2015). Varying CBPP lesions manifested in the lungs of infected cattle may be due to differences in breed susceptibility as reported (Egwu *et al.,* 2012).

Movements of cattle across the State as well as the extensive nature of the cattle production system in Nigeria may have contributed to the current observed sero-prevalence and CBPP lesions in lungs. In addition, the position of the State as transit routes for nomadic pastoralists on seasonal migrations between northern and southern parts of the country may have also contributed to the high prevalence. These observations are in agreement with the reports that prevalence of CBPP tend to be higher in cattle herds on extensive management system (Nawathe, 1992; Nwanta and Umoh, 1992).

In this survey, sex, age and breed predispositions were significant ($p<0.05$) to the development of CBPP antibodies and lesions. However, cattle aged 4–5 years were more prone to CBPP lesions and sero-conversions, perhaps because antibodies tend to persist for longer periods during the chronic stage of infection in this age group. This observation is similar to previous reports that older cattle tend to be associated with chronic stage *Mmm* infection (Windsor and Masiga, 1977). Also, because CBPP is more a chronic disease, many cattle tend harbour the causative agent with time due to multiple exposures and other factors, without clinical manifestations. This may justifies why CBPP lesions were significantly found mostly in cattle within 4-5 years age group. The gender distribution of cattle with CBPP lesions significantly ($p<0.05$) shows more cows with the infection than the bulls. Our findings at abattoirs in this study confirmed the enzootic nature of CBPP in Nigeria, as previously reported (Alhaji and Babalobi, 2016a; Alhaji and Babalobi, 2016b).

The control of CBPP is not given the needed attention in trade cattle probably because of the insidious nature of the disease. Previous reports have indicated that majority of cases in apparently cattle remain sub-clinical and affected animals become carriers due to irregular vaccination and sequestration of lesions in the lungs (OIE, 2008; Ezanno and Lesnoff, 2009; Alhaji and Babalobi, 2016b). A very effective way for the control of CBPP is by test and slaughter policy (Tambi *et al.,* 2006), which is also not practicable at livestock markets in developing countries due to logistic reasons. However, this policy can be covered up by the use of post-mortem examinations in combination with serological diagnostic tests, as previously reported for the surveillance of CBPP in developing countries (Nicholas *et al.,* 1996; Danbirni *et al.,* 2010; Egwu *et al.,* 2012). The serological evidence provided in this study, in addition to post-mortem lesions, can provide the necessary platform needed for better monitoring and detection of this disease in Nigeria and other African countries.

Being a cross-sectional study, the survey does not show causal relationship but does demonstrated associations of animal characteristics with occurrence of CBPP at both ante-mortem and post-mortem levels in the State, which is the major limitation of this study.

Conclusions

This study has shown the need to combine more reliable serological tests with post-mortem examinations to improve active surveillance of CBPP in trade cattle. The high CBPP burden was partly due to absence of effective approach to surveillance of the disease in Nigeria. These dual approaches to investigations of CBPP and identification of intrinsic factors predisposing to infection should be institutionalized as elements of epidemio-surveillance and control strategies for the disease in sub-Saharan Africa. Aggressive veterinary extension services to educate cattle traders on the dangers of trading CBPP infected cattle should be pursued. It is also recommended that "trace back" of positive animals detected at abattoirs should be made to their original herds to obtain details of their vaccination history and previous outbreaks, so as to assist policy makers in instituting adequate control and immuno-prophylactic measures.

Acknowledgements

The authors wish to acknowledge efforts of the staff in charge of meat inspection at the five central abattoirs in Niger State, Nigeria during samples collection. We also appreciate the kind assistance of the technical staff of the Mycoplasmas Research Laboratory, National Veterinary Research Institute, Vom, Nigeria, during the serological analysis.

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A SURVEY OF ORGANS/OFFAL CONDEMNATIONS AND FOETAL LOSSES IN SLAUGHTERED TRADE CATTLE AT ABATTOIRS IN NORTH-CENTRAL NIGERIA: MAJOR CAUSES AND ASSOCIATED ECONOMIC IMPLICATIONS

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Abstract

Retrospective and prospective surveys of slaughtered trade cattle at five major abattoirs in North-central Nigeria were conducted to investigate organs/offal condemnations, foetal losses and associated economic implications. Of the 2,114,475 trade cattle slaughtered at the abattoirs between 2011 and 2015, 10.6% (n=223,869) of them had some of their organs/offal condemned due to disease conditions. The highest frequently condemned organ/offal was liver (33%), followed by intestine (31%), and the least was heart (1%). Of the condemned livers, *fasciolosis* (1.88%) was the leading cause, followed by hydatidosis (0.67%) and cysticercosis (0.56%). *Pneumonia* accounted for 0.89% of the condemned lungs, followed by cysticercosis (0.59%). Hydronephrosis (0.16%) and cysticercosis (0.12%) were the main causes of kidneys condemnations. Hydatidosis was the highest (0.03%) cause of heart condemnation. Pimply gut (1.06%) was the main cause of condemnation of intestines, followed by enteritis (0.59%). However, 4.6% (n=52,234) of the slaughtered trade cows were pregnant. There was significant ($p<0.001$) influence of seasons on organs/offal condemnations and foetal losses. Organs/offal were more likely to be condemned in the early rainy season (OR 1.08; 95% CI: 1.06-1.09) and early dry season (OR 2.22; 95% CI: 2.19-2.25). Foetal losses were more likely to occurred in the late rainy season (OR 1.19; 95% CI: 1.17-1.23) and early dry season (OR 1.18; 95% CI: 1.15-1.22). Total estimated economic loss due to condemned organs/offal was 5,467,126.40 USD and foetal loss was estimated at 8,789,630.10 USD. The present survey revealed relative burdens of organs/offal condemnations and foetal losses in Nigeria with seasonal influence. These represent significant loss of protein to human population and revenue to livestock industry, with overall consequences on national food security. Occurrence of zoonotic diseases is of great public health concern. Strict enforcement of meat inspection legislations, especially at ante-mortem examinations, with good standard operating procedure is recommended.

Keywords: Abattoir, economic loss, foetal loss, organs/offal condemnation, surveillance, Nigeria

UNE ENQUÊTE SUR LES CONDAMNATIONS D'ORGANES INTERNES/ ABATS ET LES PERTES FOETALES CHEZ LES BOVINS ABATTUS AUX ABATTOIRS DU CENTRE-NORD DU NIGÉRIA : PRINCIPALES CAUSES ET IMPLICATIONS ÉCONOMIQUES ASSOCIÉES

Résumé

Dans le cadre de la présente étude, des enquêtes rétrospectives et prospectives ont été menées dans cinq grands abattoirs du centre-nord du Nigéria dans le but d'évaluer les condamnations d'organes internes / abats, les pertes fœtales et les conséquences économiques associées. Des 2 114 475 bovins commercialisés abattus dans ces abattoirs entre 2011 et 2015, l'étude a constaté que les organes internes/ abats de 10,6% (n = 223 869) de ces animaux avaient été condamnés à cause des maladies. Les organes / abats les plus fréquemment condamnés était le foie (33%), l'intestin (31%), et le cœur (1%). En ce qui concerne les raisons de la condamnation des foies, la fasciolose (1,88%) venait en premier lieu, suivie de

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l'hydatidose (0,67%) et de la cysticercose (0,56%). La pneumonie était responsable de 0,89% des poumons condamnés, suivie de la cysticercose (0,59%). L'hydronéphrose (0,16%) et la cysticercose (0,12%) étaient les principales causes des condamnations des reins. L'hydatidose était la cause essentielle (0,03%) de la condamnation des cœurs. L'oesophagostomiase (1,06%) était la cause majeure de la condamnation des intestins, suivie de l'entérite (0,59%). Cependant, 4,6% (n = 52 234) des femelles abattues était en gestation. L'on a noté une influence significative (p <0,001) des saisons sur les condamnations d'organes / abats et les pertes fœtales. Les organes / abats étaient plus susceptibles d'être condamnés au début de la saison des pluies (OR 1,08 ; IC 95% : 1,06-1,09) et au début de la saison sèche (OR 2,22 ; IC 95% : 2,19-2,25). Les pertes fœtales étaient plus fréquentes à la fin de la saison des pluies (OR 1,19 ; IC 95% : 1,17-1,23) et au début de la saison sèche (OR 1,18 ; IC 95% : 1,15-1,22). L'ensemble des pertes économiques estimées attribuables aux condamnations d'organes / abats s'élevait à 5 467 126,40 USD, tandis que les pertes de fœtus étaient estimées à 8 789 630,10 USD. La présente étude a révélé des fardeaux relatifs constitués par les condamnations d'organes / abats et les pertes fœtales au Nigeria, avec une influence saisonnière. Tout ceci conduit à une importante perte de protéines pour la population humaine et de revenus pour l'industrie bovine, avec des conséquences globales sur la sécurité alimentaire nationale. La présence de zoonoses est un grand problème de santé publique. Il est recommandé d'appliquer avec rigueur les législations en matière d'inspection des viandes, en particulier lors des examens ante-mortem, et de se doter d'un bon mode opératoire normalisé.

Mots-clés : abattoir, perte économique, perte fœtale, condamnation d'organes internes /abats, surveillance, Nigeria

Introduction

The livestock sub-sector globally is highly dynamic, contributing 40% of the global value of agricultural output, and support the livelihoods and food security of almost a billion people (Thornton, 2010). Beyond their direct role in generating food and income, livestock are valuable assets, serving as stores of wealth, collateral for credit and an essential safety net during times of crisis (FAO, 2009). The mean protein intake per caput per day in Nigeria has been estimated at 14.85 g, with meat alone representing 6.8 g. The meat protein intake in Nigeria is much lower than the North American and European averages of 38.3 and 27.3 g per caput per day, respectively (FAO, 2006). Globally, Nigeria falls among countries with very low per caput production and consumption of animal protein (Nwakpu and Ugwu, 2004). However, the potential contribution of livestock cannot be fully exploited because of losses that occur as a result of livestock diseases. Diseases are considered as a major health problem and cause a significant economic loss in countries where livestock production is an important segment of the agricultural practice (FAO, 1994). The number of organs/offal condemned for various reasons implies serious nutritional deprivations to the growing human population and economic losses to the livestock industry (Mellau *et al.,* 2011; Amene *et al.,* 2012).

Abattoirs are good sources of valuable information on the prevalence and incidence of animal diseases and pathological conditions. They provide excellent opportunities for detecting diseases of both economic and public health importance, such as tuberculosis, cysticercosis, hydatidosis and *fasciolosis* (Cadmus and Adesokan, 2009; Asaaava *et al.,* 2009). Besides, abattoir provides epidemiological information that are needed for designing disease control programmes, especially the zoonoses, as well as for estimation of economic losses incurred through condemnations of affected organs and carcasses (Jibat *et al.,* 2008). Many diseases have been reported to be responsible for the condemnations of organs and carcasses in slaughtered cattle at abattoirs (Kambarage *et al.,* 1995). In addition to losses associated with organs/offal condemnations, others losses to the livestock industry through abattoirs are those from foetal wastages as a result of slaughtered pregnant cows. Previous studies have found cows slaughtered at Minna and Yola central abattoirs in Nigeria (Alhaji, 2011; Ardo *et al.,* 2013) and Arusha in Tanzania (Mellau *et al.,* 2011) to be pregnant.

Cattle play very important role in Nigerian economy. In view of the increasing human population, Nigeria requires a corresponding improvement in herd health management so as to increase its cattle population. With paucity of documented information on causes and rate of organs/offal condemnations, frequency of foetal losses and the associated economic implications in trade cattle slaughtered at abattoirs in Nigeria, it is important to carry out this study because prevalence may differs from one geographical region to another due to diseases, most especially the parasitic diseases, and quality of meat inspection activities. Further, such data could be a convenient and inexpensive source of information that can be used for the surveillance and control of livestock diseases, especially those that are of public health importance, and promotion of livestock development in African countries. This study, therefore, was aimed to investigate prevalence of organs/offal condemnations, foetal losses and associated economic implications at five municipal abattoirs in Northern Central Nigeria. Our Null hypothesis was that organs/offal condemnations and foetal losses cannot be associated with seasonal influence.

Materials and methods

Study area

The survey was conducted at five municipal abattoirs in Niger State, North-central Nigeria. These slaughterhouses are located in Minna (coordinate N09.64655°; E006.54436°), Suleja (N09.13716°;E007.20159°), Bida (N09.08570°; E006.02027°), Kontagora (N10.41057°; E005.46677o) and New-Bussa (N09.86805o; E004.48343o). Niger State is located in the Southern Guinea Savannah ecological zone of Nigeria, between latitude 8° 20' N and 11° 30' N, and longitude 3° 30' E and 7° 20' E. It is the largest State in Nigeria in terms of land mass, covering an area of 86,000 km^2, representing about 9.3% of the total land area of the country. The State experiences four distinct seasons, early dry season (October – December), late dry season (January – March), early rainy season (April – June), and late dry season (July – September). The mean annual rainfall is 1600 mm with duration of about 180 days. It has humidity of 104% and average lowest and highest temperatures of about 27°C and 39°C respectfully. In 2006, the human population in Niger State was estimated at 4.1 million people (NPC, 2006), and an estimated cattle population of 2.4 million cattle in 2012 (MLFD, 2014).

Study design and data collection

Both retrospective and prospective surveys were conducted to collect secondary and primary data on trade cattle brought from livestock markets and slaughtered in the designated abattoirs. The retrospective study involved retrieval of slaughtered trade cattle records of a five-year period, from 2011 to 2015 in the five abattoirs. As a means of quality control of data, all records with no proper diagnosis of organ lesions and ambiguous information on the pathological conditions were excluded from the study. Routine meat inspections at the abattoirs were carried out by veterinarians and animal health technologists who have undertaken special training in meat inspection and identification of gross pathologies at postmortems. Postmortem inspections were conducted through visualizations, palpations and systematic incisions of visceral organs particularly the livers, lungs and kidneys for the presence of cysts, adult parasites and lesions (Getaw *et al.,* 2010). Lesions and parasites from partially and totally condemned organs that needed further investigations after abattoir examinations and identifications as means of external validation were taken to the Niger State Veterinary Hospital Minna diagnostic laboratory for confirmations.

During routine meat inspection, the disease conditions were grossly diagnosed based on pathological changes such as color, size, morphology, consistency and presence of lesions or parasites as described by Gracey (1986). Judgment on whether an organ was fit or unfit for consumption was based on guidelines described by Gracey (1986) and the Food and Agriculture Organization of the

United Nations (FAO, 1994). Furthermore, foetal losses were assessed by observations and palpations of uteri of all slaughtered cows.

Also, a prospective study was carried out by the researchers in the designated abattoirs through active meat inspections conducted between August and December 2015 using the same approaches as for the retrospective study. One month was spent in each abattoir for the investigations.

Data management and statistical analysis

Data obtained from the five months prospective investigations in 2015 were added to those routinely collected by the designated meat inspectors and entered into Microsoft Excel 7 spreadsheet (Microsoft Corporation, Redmond, WA, USA). The Open Source Epidemiologic Statistics for Public Health (OpenEpi) software version 2.3 (Dean *et al.,* 2009) was used to analyzed the data. Descriptive statistics was used to determine the proportions of organs/offal condemned, defined as the number of condemned organs/offal due to a specific disease condition against the total number of slaughtered trade cattle and inspected at the abattoirs during the five-year period.Also, rate of foetal losses was assessed by descriptive analysis, defined as the proportion of cows found to be pregnant at post-mortem inspections (since ante-mortem examination is rarely performed) to the total number of slaughtered trade cows and inspected during the period under review (Thrusfield, 2009). Associations of seasons with the occurrence of condemned organs/offal and loss foetuses were first subjected to univariate analysis using Chi-square tests (Dohoo *et al.,* 2009). Furthermore, likelihood stepwise backward multivariate logistic regression models were used to determined final associations on factors that were significant at $p<0.05$ during univariate analysis. Outcomes with $p<0.05$ were considered statistically significant in all analyses.

Economic analysis

The estimated economic loss that resulted from organs/offal condemnations at abattoirs was established based on combined secondary and primary data recorded during the survey period. Economic loss incurred due to organs/offal condemnations for the five-year period was computed by multiplying the average weight of the organs/offal by the average price per kg of organs and the total number of condemned organs/offal (Mwabonimana *et al.,* 2009). The annual economic loss was obtained by assuming that the parameters (organs/offal condemnation rates per annum and average price) remained relatively constant during the five-year period. Average local market price of each organ was obtained from the butchers and meat sellers at the abattoirs and meat markets in the five cities.

Economic loss from foetal losses was estimated by multiplying estimated average calf birth weight with cost per kg live weight (Alhaji, 2011). Average foetal birth weight was 25.5 kg and current cost per live birth weight of cattle was 1,300 Nigerian Naira (NGN).

Results

Causes of organs/offal condemnations in trade cattle and associated seasonal influence

A total of 2,114,475 trade cattle were slaughtered and inspected at the five abattoirs during the period 2011–2015. Suleja municipal abattoir had the highest slaughtered (n=1,122,863; 53.1%), while the lowest slaughtered (n=164,340; 7.8%) was from New-Bussa municipal abattoir (Table 1). A total of 223,869 slaughtered cattle had some of their organs condemned due to disease conditions, with overall prevalence of 10.6% (Tables 2). Organ-specific condemned organs were: livers (n=74,374; 33.2%), lungs (n=68,118; 30.4%), kidneys (n=11,458; 5.1%), hearts (n=1,136; 0.5%) and intestines (n=68,783; 30.7%). Highest frequently condemned organ/offal was observed in liver, while heart had the least (Fig. 1).

Of the condemned livers, *fasciolosis* (1.88%) was the leading cause of condemnations, followed by hydatidosis (0.67%) and cysticercosis (0.56%). Pneumonia accounted for 0.89% of the condemned lungs,

Table 1: Annual slaughtered trade cattle at five municipal abattoirs in North-central Nigeria: 2011 - 2015

Abattoir	2011	2012	2013	2014	2015	Total
Minna	38,228	89,782	129,999	44,174	39,208	341,391
Suleja	133,846	301,418	389,979	148,642	148,978	1,122,863
Bida	31,052	49,609	94,967	56,793	50,891	283,312
Kontagora	23,789	35,798	49,971	45,107	47,904	202,569
New Bussa	23,465	29,100	35,763	39,055	36,957	164,340
Total	**250,380**	**505,707**	**700,679**	**333,771**	**323,938**	**2,114,475**

Table 2: Annual prevalence of condemned organs/offal due to disease conditions in slaughtered trade cattle at five municipal abattoirs of North-central Nigeria: 2011–2015

Year	Number of cattle slaughtered	Number of cattle with organs/offal condemned	Proportion (%)	95% Confidence interval
2011	250,380	42,730	12.2	12.09, 12.35
2012	505,707	62,964	12.4	12.29, 12.47
2013	700,679	54,019	7.9	7.88, 8.01
2014	333,771	31,412	7.8	7.74, 7.92
2015	323,938	32,744	10.1	10.01, 10.21
Total	**2,114,475**	**223,869**	**10.6**	**10.55, 10.63**

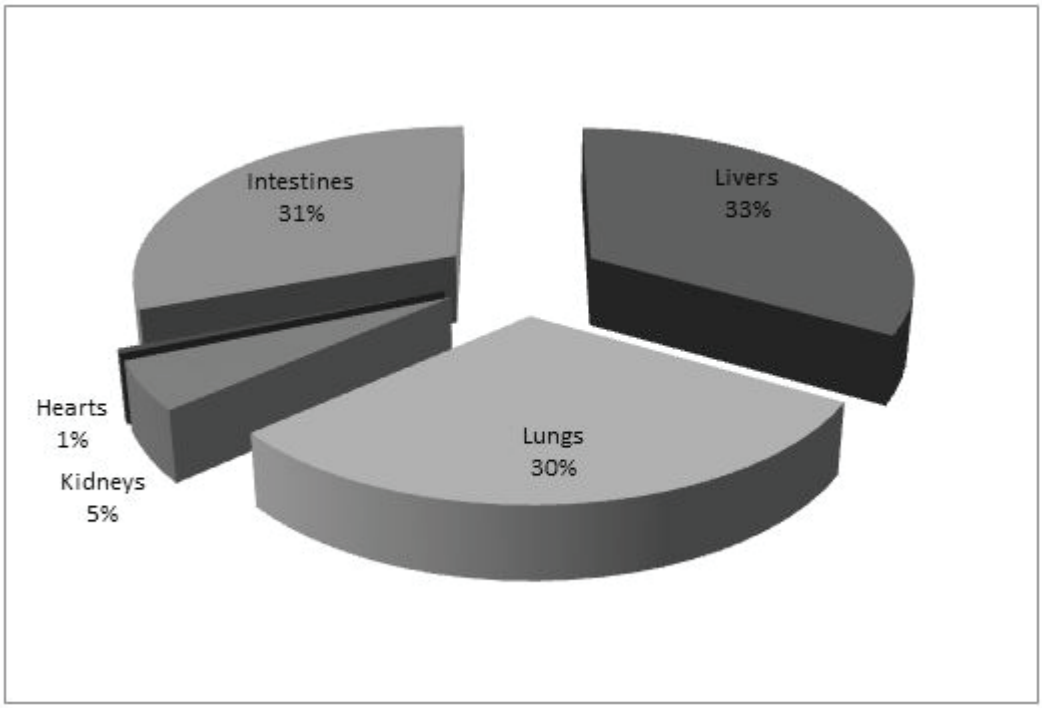

Figure 1: Proportions of condemned organs/offal in slaughtered trade cattle at five municipal abattoirs in North-central Nigeria: 2011 – 2015

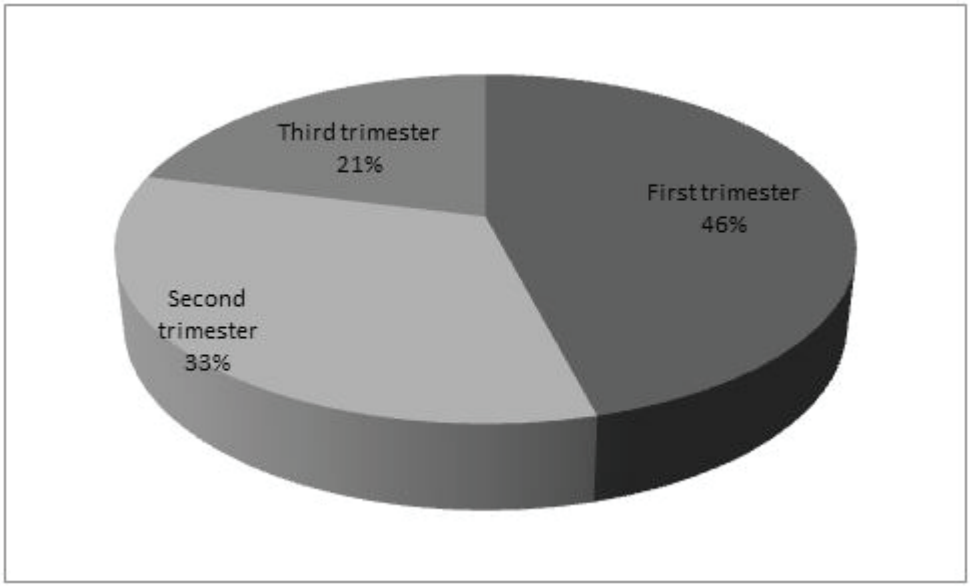

Figure 2: Proportions of foetal losses in relation to pregnancy stages of slaughtered trade pregnant cows at five municipal abattoirs in North-central Nigeria: 2011 – 2015

Table 3: Proportions of trade cattle with condemned organs/offal and associated causes of condemnations at five municipal abattoirs of North-central Nigeria: 2011–2015

Organs/ offal	Disease conditions	2011	2012	2013	2014	2015	Total	Proportion (%)
Livers	*Fasciolosis*	1,969	2,9133	2,959	2,731	2,998	39,790	1.88
	Cirrhosis	1,409	1,315	1,226	1,214	1,389	6,553	0.31
	Cysticercosis	1,658	2,699	3,397	1,448	2,637	11,839	0.56
	Hydatidosis	1,856	3,138	3,075	3,129	2,982	14,180	0.67
	Hepatitis	336	419	655	271	331	2,012	0.10
Lungs	Pleurisy	1,308	2,337	3,421	1,354	2,325	10,745	0.51
	Pneumonia	2,589	4,390	4,681	3,136	4,044	18,840	0.89
	Cysticercosis	1,854	2,805	3,502	1,668	2,679	12,508	0.59
	Abscesses	1,115	1,211	2,101	1,141	2,118	7,686	0.36
	Emphysema	1,198	1,272	2,156	1,211	2,177	8,014	0.38
	Hydatidosis	1,449	2,334	2,219	1,407	2,387	9,796	0.46
	Tuberculosis	103	111	108	93	114	529	0.03
Kidneys	Hydronephrosis	511	713	919	616	713	3,472	0.16
	Nephritis	223	425	779	311	589	2,327	0.11
	Infarcts	105	344	562	114	103	1,228	0.06
	Hydatidosis	246	440	638	239	436	1,999	0.10
	Cysticercosis	319	527	721	349	516	2,432	0.12

Organs/ offal	Disease conditions	2011	2012	2013	2014	2015	Total	Proportion (%)
Hearts	Pericarditis	45	40	53	47	39	224	0.01
	Cysticercosis	88	90	71	53	84	386	0.02
	Hydatidosis	121	103	101	90	111	526	0.03
Intestines	Pimply gut	6,055	7,859	9,117	5,008	7,265	27,445	1.06
	Enteritis	1,717	2,610	3,774	1,652	2,701	12,454	0.59
	Abscesses	1,132	1,069	2,781	1,140	1,264	7,386	0.35
	Peritonitis	1,591	2,603	3,589	1,583	2,568	11,934	0.56
	Cysticercosis	1,960	2,836	1,414	1,407	1,947	9,564	0.45
Total		**30,957**	**62,964**	**54,019**	**31,412**	**44,517**	**223,869**	**10.59**

Table 4: Association of season with occurrence of organs/offal condemnation due to disease conditions in slaughtered trade cattle at five municipal abattoirs of North-central Nigeria: 2011–2015

Season	Number of cattle with condemned organs/offal (%)	Number of cattle without condemned organs/offal (%)	Odds ratio	95% confidence interval	P-value
Late dry season	47,722 (12.7)	329,177 (87.3)	1.00		
Early rainy season	50,928 (11.9)	378,840 (88.1)	1.08	1.06, 1.09	<0.001
Late rainy season	64,983 (14.4)	385,248 (85.6)	0.86	0.85, 0.98	<0.001
Early dry season	52,563 (6.1)	805,014 (93.9)	2.22	2.19, 2.25	<0.001

Significance at p<0.005

Table 5: Annual foetal losses in slaughtered trade cows at five municipal abattoirs of North-central Nigeria: 2011–2015

Year	Number of cattle slaughtered	Number of cows slaughtered	Number of pregnant cows slaughtered (%)
2011	250,380	131,950	8,846 (6.7)
2012	505,707	268,023	12,034 (4.5)
2013	700,679	378,367	15,595 (4.1)
2014	333,771	175,564	7,577 (4.3)
2015	323,938	178,166	8,182 (4.6)
Total	**2,114,475**	**1,132,070**	**52,234 (4.6)**

followed by cysticercosis (0.59%). Of the kidneys that were condemned, hydronephrosis (0.16%) and cysticercosis (0.12%) were the main causes. Hydatidosis (0.03%) was identified as the leading cause of heart condemnation, followed by cysticercosis (0.02%). Further, lumps in the gut, that is, pimply gut (1.06%), was the main cause of condemnation of intestines and enteritis (0.59%) followed (Table 3).

There was significant ($p<0.05$) influence of seasons on the condemnations of organs/offal in slaughtered trade cattle during the study period. Organs/offal were more likely to be condemned in early rainy season (OR 1.08; 95% CI: 1.06, 1.09) and early dry season (OR 2.22; 95% CI: 2.19, 2.25) than in the late dry season. However, late rainy season was less likely (OR 0.86; 95% CI: 0.85, 0.98) not to have more condemned organs/offal as the late dry season (Table 4).

Table 6: Association of season with foetal losses in slaughtered trade cows at five municipal abattoirs of North-central Nigeria: 2011–2015

Season	Number pregnant cows slaughtered	Number of non pregnant cows slaughtered	Odds ratio	95% Confidence interval	P-value
Late dry season	9,621 (4.8)	190,573 (95.2)	1.00		
Early rainy season	14,929 (6.0)	234,697 (94.0)	0.79	0.77, 0.82	<0.001
Late rainy season	17,193 (4.0)	407,804 (96.0)	1.19	1.17, 1.23	<0.001
Early dry season	10,491 (4.0)	246,762 (96.0)	1.18	1.15, 1.22	<0.001

Significance at p<0.005

Table 7: Estimated economic losses associated with condemned organs/offal from slaughtered trade cattle at five municipal abattoirs in North-central Nigeria: 2011–2015

Organs	Number of organs condemned	Mean weight of organs/ offal (kg)	Unit cost (NGN)/kg	Annual economic loss (NGN 1,000)	Total five-year loss (NGN 1,000)
Livers	74,374	4.8	1,100.0	78,538.9	392,694.7
Lungs	68,118	2.9	900.0	35,557.6	177,788.0
Kidneys	11,458	0.5	1,100.0	1,26.88	6,301.9
Hearts	1,136	0.7	1,100.0	174.9	874.7
Intestines	68,783	6.6	1,100.0	99,872.9	499,364.6
Total	-	-	-	**214,144.3**	**1,077,023.9**

Table 8: Estimated economic losses associated with foetal losses from slaughtered trade cattle at five municipal abattoirs in North-central Nigeria: 2011–2015

Stage of pregnancy	Number of foetus wasted	Mean foetal birth weight (kg)	Unit cost (NGN)/kg	Annual economic loss (NGN 1,000)	Total five-year loss (NGN 1,000)
First trimester	24,007	25.5	1,300.0	159,166.5	795,832.1
Second trimester	17,236	25.5	1,300.0	114,276.7	571,373.4
Third trimester	10,991	25.5	1,300.0	72,870.4	364,351.7
Total	**52,234**	-	-	**346,313.6**	**1,731,557.2**

Foetal losses in trade cattle and associated seasonal influence

A total of 1,132,070 trade cows were slaughtered and their uteri inspected for pregnancy during the five-year period. Of all cows slaughtered, 52,234 (4.6%) were found to be pregnant (Table 5). Slaughtered pregnant cows at first trimester recorded the highest proportion (46%) of foetal losses (Fig. 2). Also, there was significant (p<0.001) influence of seasons on foetal losses in slaughtered trade cattle in the period under review. Foetal losses were more likely to occurred in the late rainy season (OR 1.19; 95% CI: 1.17, 1.23) and early dry season (OR 1.18; 95% CI: 1.15, 1.22) than in the late dry season. Also, early rainy season was less likely (OR 0.79; 95% CI: 0.77, 0.82) not to have more foetal losses as the late dry season (Table 6).

Estimated economic losses

The prices that were used in the estimation of economic losses were those that prevailed in the cities where the abattoirs

were domiciled. The average weight of organs/offal, total number of organs/offal condemned, average unit cost per kg of organs/offal and the total economic losses attributable to organs condemnations are presented in Table 7. The total estimated cost of all organs/offal condemned during one year was 215,753,630.00 NGN. However, the total economic loss due to organs/offal condemnation during the five-year period was estimated at 1,077,023,900.00 NGN (5,467,126.40 USD or 4,986,221.80 EURO).

The number of foetal losses per trimester, mean birth weight of foetuses, average unit cost per kg of meat and the total economic losses attributable to foetal losses are presented in Table 8. The total estimated economic loss from 52,234 wasted fetuses from 211 to 2015 in the abattoirs was 1,731,557,200.00 NGN (8,789,630.10 USD or 6,763,895.30 EURO).

Discussion

This study revealed that a number of disease conditions are responsible for the condemnations of organs/offal at abattoirs with consequent nutritional and economic implications. Also observed was high foetal losses due to frequent slaughtered of pregnant cows, which constitutes economic and animal welfare concerns. Organs/offal condemnations and foetal losses reduce availability of necessary protein, vitamins and minerals needed for the teaming human populations and deprive livestock industry of valuable income. The existence of hydatidosis, *fasciolosis*, cysticercosis, and tuberculosis in slaughtered trade animals is an indication of public health threats to meat handlers and consumers. Therefore, consequences associated with the public health and food security from these negative activities cannot be over emphasized.

Livers constituted 33% of the overall condemned organs. Condemnation due to *fasciolosis* occurred in 1.88% of all slaughtered cattle during the study period. This was less than 14.6% five-year prevalence reported in condemned livers at Makurdi abattoirs in Nigeria (Ejeh *et al.,* 2015). This observed prevalence was also less than 4.5% found in Dodoma, Tanzania (Tembo and Nonga, 2015). A very high livers condemnation (30%) due to *fasciolosis* has been reported in Sumbawanga, Tanzania (Kamwela *et al.,* 2013). Furthermore, previous studies have also revealed higher liver condemnations due to *fasciolosis* at slaughterhouses in Nigeria and Ethiopia (Njoku-Tony 2011; Mulugeta *et al.,* 2011; Mohammed *et al.,* 2012). All the compared studies were conducted in single abattoirs, unlike the present survey. The identification of pathological conditions associated with *fasciolosis* at post-mortems found this study is an indication that it is a serious problem in cattle in sub-Saharan Africa. We also found that hydatidosis contributed 0.67% to causes of liver condemnation in the five abattoirs. High rate (14.8%) of hydatid cysts have been reported to responsible for liver condemnation in slaughtered cattle at Shire municipal abattoir in northern Ethiopia (Asfaw and Afera, 2014).

This is study found that 30% of the total condemned organs were lungs from the slaughtered cattle. Pneumonia was observed to be the major cause of lungs condemnation in this study. However, emphysema and tuberculosis were the least. Cattle have well-developed interlobular septa and lack of collateral ventilation, especially during transportation or stocking at cattle markets, can predisposed them to be more susceptible to interstitial emphysema (Mellau *et al.,* 2010a). Pulmonary emphysema may be caused by obstruction of airflow or by extensive gasping respiration during the slaughter process (FAO, 1994). Exposure of animals to stress factors like dust, overcrowding and exhaustion from long trekking in search of pasture and water can also exacerbate respiratory disease conditions (Kusiluka and Kambarage, 1996).

Kidneys accounted for 5% of all organs/offal condemned in the study period. The disease conditions that caused kidneys condemnations were hydronephrosis, hydatidosis and cysticercosis. Also, nephritis and infarcts were other causes of kidneys condemnations observed. Asfaw and Afera (2014) reported up to 0.9% hydatidosis in the kidneys of slaughtered

cattle at Shire municipal abattoir in northern Ethiopia. This study found heart to be the least condemned organ, constituting about 1% of the total condemned organs/offal. Hydatidosis, cysticercosis and pericarditis were the causes of heart condemnation observed. However, previous studies reported that pericarditis and hydatid cyst were the most common causes of heart condemnation (Genet *et al.,* 2012).

The present study found intestines to constitute about 31% of the whole condemned organs/offal. Also, we observed pimply gut (oesophagostomosis) to be the leading cause of cattle intestinal condemnations at slaughtered with 1.06% rate. This rate was less than those earlier reported in Nigeria and in Tanzania (Cadmus and Adesokan, 2009; Mellau *et al.,* 2011; Tembo and Nonga, 2015). In addition, intestinal condemnation from pimply gut was observed to be more during the rainy than the dry seasons. This may be due to favourable conditions that increased helminthes activities in the season. It is, therefore, imperative that control of helminthes be made routine in cattle during the rainy season in order to reduce wastages of valuable organs/offal in slaughter cattle.

The results of this study have shown the presence of hydatidosis, cysticercosis, *fasciolosis* and tuberculosis in slaughtered cattle, which are not only of economic significance but also of public health importance, as previously reported (Berhe, 2009; Ernest *et al.,* 2009; Swai and Schoonman, 2012; Alembrhan and Haylegebriel, 2013; Endalew and Nuradddis, 2013). Because of these diseases zoonotic importance, public health education needs to be strengthened to reduce their impacts. Variations in prevalence of the condemned organs/offal may be due to differences in the anatomical and physiological characteristics of the affected organs as well as extent of infections.

We found high number of slaughtered cows to be pregnant, with rate of 4.6% in the five surveyed abattoirs. However, previous studies in single abattoirs found higher fetal loss rates of 5% (Cadmus & Adesokan, 2010), 4.4% (Alhaji, 2011) and 14.4% (Ardo et al,. 2013) at some abattoirs in Nigeria, and 8.6% reported at Arusha (Mellau et al, 2011). The effects of these high foetal losses threaten growth of cattle population and beef supply in Nigeria where meat production is current far less than consumption.

Seasons were found to have significant influence on organs/offal condemnations and foetal losses in this study. The highest numbers were recorded in the late and early rainy seasons, and least during the late dry season. The high rates of losses in the rainy seasons could be due to climatic factors that favour farming seasons thereby providing very little land areas for livestock grazing; high incidence of diseases, which also predisposed to abortions. These findings are in consensus with the reports of Oyekunle et al. (1992) that observed high foetal losses during the rainy seasons, which they attributed to availability of feeds and cows' gravidity that make them robust to command higher prices. However, our findings were in contrast to the reports of Nwakpu and Osakwe (2007) and Cadmus and Adesokan (2010) that recorded high foetal losses in the dry season due to increased liquidation of pregnant cows for fear of dry season stress.

The total economic loss due to organs/offal condemnation during the five-year period was estimated at 5,467,126.40 USD or 4,986,221.80 EURO. Liver condemnations alone accounted for annual economic loss of 398,674.82 USD, which is comparably about three times more than the result previously obtained in Nigeria that indicated an annual financial loss of 110,968.00 USD (Cadmus and Adesokan, 2009) and the reported annual financial loss of 118,701.80 USD in Tanzania while (Tembo and Nonga, 2015). The estimated economic loss as a result of 52,234 foetal losses in the five abattoirs from 2011 to 2015 was 8,789,630.50 USD or 6,763,895.30 EURO with annual losses of 1,757,926.10 USD or 1,352,779.10 EURO. These losses in revenue to the livestock industry have serious economic implications and a negative impact on the socio-economic wellbeing of those involved in the livestock value chain. Therefore, disease control

strategies and prevention of slaughtering of pregnant cows should be strictly instituted.

This survey has demonstrated the use of slaughterhouse records as an important tool in assessing food animal diseases status, as previously reported (Mellau *et al.,* 2010b). Although, abattoir survey generally provides useful information regarding the quality of meat and does not require many resources, it is faced with some limitations because diagnosis of disease conditions based on gross pathology has sensitivity of 63-71% (Khaitsa *et al.,* 1994) and some conditions are likely to be missed at routine meat inspections. Also, disease prevalence may have been underestimated because of likely poor record keeping. However, the available retrospective evidences were discussed in the light of the conjoined prospective investigations.

Conclusion

The present survey revealed relative burdens of organs/offal condemnations and foetal losses in Nigeria with seasonal influence. These represent significant loss of protein to human population and revenue to livestock industry, with overall consequences on national food security. Occurrence of hydatidosis, cysticercosis, *fasciolosis* and tuberculosis is of great public health concern and, therefore, their control requires collaborations between the public health and veterinary authorities. With high burdens of livestock diseases at slaughter, there will be need for appropriate surveillance and institution of effective control measures at livestock markets before slaughter. Screening of cows for pregnancy before slaughter will promote animals' welfare. Strict enforcement of meat inspection legislations, especially at ante-mortem examinations, with good standard operating procedure (SOP) is recommended.

Acknowledgements

The authors extend their gratitude to all staff of the Niger State Ministry of Livestock and Fisheries Development, especially those attached to Minna, Suleja, Bida, Kontagora and New-Bussa abattoirs for their assistance during data collection for the survey.

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BLOOD BIOCHEMICAL OF NILE CROCODILE (CROCODYLUS NILOTICUS) IN KANO ZOOLOGICAL GARDEN, NIGERIA

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Abstract

The potential application of blood reference range for crocodile is a basis that can provide important clinical information about health and physiological condition of animal. This study investigates serum biochemistry of Nile crocodile from Kano Zoological Garden, Kano, Nigeria. Six (6) adult Nile crocodile (Crocodylus niloticus) were captured from crocodile pond. Blood was collected from post occipital sinus of the physically restrain crocodile and used for serum biochemical parameters. The results revealed the Total Serum Protein (TSP) concentration of 9.2g/L, albumin concentration which is a common plasma protein is 43g/L while globulin concentration is 54g/L. Cholesterol concentration measure is registered at 5.2mmol/L with High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) of 1mmol/L and 1.35mmol/L respectively. Creatinine: a breakdown product of creatinine which is an important part of muscle tissue is 44umol/L. Uric acid which is a primary catabolic end product of protein is 0.18mmol/L while glucose and triglyceride are 4.94mmol/L and 2.24mmol/L respectively while enzymes which include Alanine aminotransferase (ALT) concentration is 6U/L, Aspartate aminotransferase concentration is 5U/L while Alkaline Phosphatase is 20U/L. Clearly, nutritional status, age, gender, season, physiology and environment should be considered if clinical pathology is to be employed as a diagnostic tool.

Keywords: Biochemical, clinical information, plasma, enzymes, physiological

BIOCHIMIE SANGUINE DU CROCODILE DU NIL (*CROCODYLUS NILOTICUS*) DANS LE JARDIN ZOOLOGIQUE DE KANO AU NIGERIA

Résumé

L'application potentielle de la gamme de valeurs de référence pour le crocodile est une base susceptible de fournir des informations cliniques importantes sur la santé et l'état physiologique de cet animal. La présente étude analyse la biochimie du sérum du crocodile du Nil du jardin zoologique de Kano au Nigeria. Six (6) crocodiles du Nil (Crocodylus niloticus) adultes ont été capturés dans un étang de crocodiles situé dans le zoo. Du sang a été prélevé du sinus post-occipital du crocodile immobilisé, et utilisé pour déterminer les paramètres biochimiques du sérum. Les résultats ont révélé un taux de protéine sérique total (TSP) de 9,2 g / l, un taux d'albumine (une protéine plasmatique commune) de 43 g / l, et un taux de globuline de 54 g / l. Le taux de cholestérol enregistré est de 5,2 mmol / l, avec des taux de lipoprotéine de haute densité (HDL) et de lipoprotéine de basse densité (LDL) respectivement de 1 mmol / l et 1,35 mmol / l. La créatinine, un sous-produit résultant du métabolisme du phosphate de créatinine, une partie importante du tissu musculaire, est de 44umol / l. L'acide urique, un produit final du catabolisme primaire des protéines, est de 0,18 mmol / l. Les taux de glucose et de triglycérides sont respectivement de 4,94 mmol / l et 2,24 mmol / l, tandis que les concentrations d'enzymes sont établies comme suit : l'alanine-aminotransférase (ALT) 6 U/5U/l, l'aspartate-aminotransférase 5U/l, la phosphatase alcaline 20U /l. Les valeurs biochimiques enregistrées ont été comparées avec les données disponibles sur le crocodile du Nil de ferme. De toute évidence, l'état nutritionnel, l'âge, le sexe, la saison, la physiologie et l'environnement devraient être pris en considération si l'on veut utiliser la pathologie clinique comme outil de diagnostic.

Mots-clés : biochimie, information clinique, enzymes plasmatiques, physiologique

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Introduction

Blood profile studies in wildlife or captive croco¬diles are being carried out for scientific, educational or commercial reasons; they are being applied to conser¬vation or reproduction projects, such as to skin and meat exploitation (Oliveira, 2004). Blood analysis is a relatively noninvasive method that can provide important clinical information about health and physiological condition of animals (Stein, 1996).

Success in animal disease control and prevention depend greatly on a precise and rapid diagnosis. This is a key to improve the crocodile production industry. Among several parameters, blood biochemical is definitely necessary in assisting clinicians to proximate the status of the animal body (Kanchanapangka *et al.,* 1999) and because of the difficulty in obtaining meaningful reference interval for each species of reptile (Campbell, 2006), decision level are often use where assessing reptilians.

Conditions detected through evaluation of blood biochemistry and haematology of reptile includes anaemia, inflammatory disease, parasistemias, hematopoietic disorders and haemostatic alterations (Campbell, 2006). According to Campbell (2006), normal hematologic values for reptiles (including crocodilians), as determined by different laboratories, vary significantly due to differences in blood sampling, handling, analytic techniques, difference in environmental condition of the reptiles habitat, physiologic status of the reptile, its age gender and nutrition and the use of anaesthetics.

Development in diagnosis of the crocodile diseases has not gone far enough to a satisfy level. Additional information concerning the morphological and physi¬ological characteristics of crocodilian blood profile is needed to make a successful differential diagnosis and disease monitoring (Kanchanapangka *et al.,* 1999).

Knowledge of blood biochemical and their physiological variations are incomplete and controversial in captive Nile crocodile. Lack of uniformity on biochemical de¬nominations, such as a confusion caused by the different species affinity is frequently verified in publications.

Materials and Methods

Study area

Established in 1972, Kano Zoological Garden, Kano State covers a total area of 40.47 ha. It is located 3km away from the old city of Kano nationally renowned for its tourist attraction and lies between latitudes 11058'4"N and longitude 8031'32'E. The Zoo currently had 58 different species, consisting of 300 individual animals which includes; four Lions, eight Hyena (stripped and spotted), two adult Ostriches, Elands, Elephant, Giraffes, Hippopotamus, Horney badger, Duikers, Bushbuck, Baboons, Jackal, Civet cat, Buffalo, Chimpazee, Monkeys, porcupines, Gazelles Zebra, Warthogs, Horse, Pythons, Crocodiles, Crane crown, Marabus stock, Geese, Peacock etc.. It was designed to conserve animals for people to see, render recreational services, serve as a research centre and provide practical educational instructions. Kano Zoo was the second of its kind in West Africa after Cote d'Ivoire and was accredited by the Pan-African Association of Zoos and Aquaria (PAAZA) (Lawan, 2011).

Crocodilians House

The enclosure is approximately 100 x 40 (triangular) square meters designed with circular concrete pond of approximately 1.2m height. Fence has a combination of material chain link fencing of the enclosure is properly constructed to prevent escape. This is achieved with an extension concrete above and below ground, as crocodiles can be excellent triggers and climbers. Solid cement wall is used for the circular enclosure while chain is used to link extension below to provide bush viewing and improved safety.

Fence height is 0.5m with a turn back at the top to force a climbing animal to fall.

A Shallow concrete pool, with drain system and pump for changing water is within. The water area covers 20% of the total enclosure, leaving enough dry land area for all

specimens to basking.

Materials

Material used for the study include experimental animal (Nile crocodile), Rope or line, Hand tower (10 pieces), Weighing Balance, 21G needle, 5ml syringe, 14 ordinary sample bottle, thermometer, flexible measuring tape, writing material (field note book, pen, biro and marker).

Methodology

All crocodile was starved for two days before sample collection.

All crocodiles caught were physically restrained without the use of narcotics and were released within 15 minutes of being caught.

Once captured and restrained, blood samples ranging between 3 and 5 ml each were collected from the post-occipital venous sinus, dorsal midline and just caudal to the base of the head using a 21 gauge needle and a 5ml syringe as described by Guillette et al. (1996) and Millan et al. (1997). The volume of blood collected depended on the size of the animal. All blood collected was immediately transferred to blood tubes and kept cool with ice packs. Samples were centrifuged at the end of each evening and plasma samples placed in Cryovails and frozen in liquid nitrogen until analyzed in the Clinical Pathology Laboratory of General Hospital, Kano. Blood samples were only collected from living healthy animals and all animals found with wound or look unhealthy in the study area were intentionally disregarded and did not form any part of the study.

Blood samples were analyzed for Total Serum Protein (TSP), Albumin, Globulin, Glucose, Cholesterol, Creatinine, Uric Acid, Triglycerides, High density lipoprotein (HDL), Lower density lipoprotein (LDL), and palsma enzymes: Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST),

Blood biochemistry analysis

Biochemical analyses were done using a Next/Vetex Alfa Wassermann Analyzer (Alfa Wassermann B.V., Woerden, The Netherlands). Total protein was determined using a modified Weichselbaum biuret method (Weichselbaum, 1946). Albumin was measured using the bromocresol green method (Cheesbrough, 2005) globulin and the albumin-globulin ratio were calculated (Johnson *et al.,* 2002) while cholesterol was determined by enzymatic methods (Abell *et al.,* 1952; Bergmeyer and Grassl, 1983), creatinine was determined by the picrate method (Cheesbrough, 2005) and uric acid by the uricase method (Bauer, 1982). Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were determined by using standard IFCC methods (Bergmeyer *et al.,* 1977; Tietz *et al.,* 1983; Bergmeyer *et al.,* 1986). The glucose oxidase method (Marks, 1996) was used to determine glucose in the samples.

Statistical analysis

All values were presented descriptively.

A comparison of blood biochemistry range with report previously for Nile crocodiles in the literature was drawn.

Results

Blood biochemical profile of Nile crocodile in Kano Zoological Garden

The results of the biochemical analysis of blood sample collected from six (6) Nile crocodiles in Kano Zoological garden are presented in table 1.

Total Serum Protein (TSP) concentration is 97g/L, albumin concentration which is a common plasma protein is 43g/L while globulin concentration is 54g/L. Cholesterol concentration measure is registered at 5.2mmol/L with High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) of 1mmol/L and 1.35mmol/L respectively.

Creatinine: a breakdown product of creatine which is an important part of muscle tissue was 44.2umol/L. Uric acid which is a primary catabolic end product of protein is 0.18mmol/L while glucose and triglyceride are 4.94mmol/L and 2.24mmol/L respectively.

Blood plasma enzymes of Nile crocodile in Kano Zoological Garden.

Table 2 revealed the biochemical enzymes of six (6) Nile crocodiles from study area.

The biochemical enzymes are important tools in diagnosing the state of the animal external organs and bones.

Alanine aminotransferase (ALT) concentration is 6U/L, Aspartate aminotransferase concentration is 5U/L while Alkaline Phosphatase is 20U/L.

Comparison of biochemical parameters of captive Nile crocodile in the current study and previous studies.

Table 3 shows the comparison of blood biochemical with previously reported values for Nile crocodile in captivity.

Total Serum Protein (TSP), albumin and globulin concentrations of 97g/L, 43g/L and 54g/L respectively are much higher than the reported concentration for the Nile crocodile by Osagiobare (2013) in FCWM zoo of Nigeria and Foggin et al. (1987) in Zimbabwe farm.

Cholesterol concentration of 5.2mmol/L is also higher than 4.5mmol/L for Nile crocodile in FCWM zoo while creatinine concentration (44.2umol/L) recorded in the current study is lower to 66umol/L by Osagiobare (2013) in FCWM Zoo. Uric acid of 0.18mmol/L is also lower to 0.43mmol/L for FCWM zoo Nile crocodile and 0.24mmol/L from same animal in Zimbabwe Farm (Foggin, 1987).

Glucose concentration (4.9mmol/L) in the study is much closer to 5.0mmol/L recorded in FCWM (Osagiobare, 2013) and 4.57mmol/L at Zimbawe Farm (Foggin, 1987).

Triglyceride of 2.2mmol/L is substantially higher than 0.4mmol/L recorded for Nile crocodile in FCWM Zoo (Osagiobare, 2013).

Table 1: Blood biochemical profile of Nile crocodile in Kano Zoological Garden.

Parameters	Value (n= 6)
Total Serum Protein TSP (g/L)	97
Albumin (g/L)	43
Globulin (g/L)	54
Cholesterol (mmol/L)	5.2
Creatinine (umol/L)	44.2
Uric Acid (mmol/L)	0.18
Glucose (mmol/L)	4.94
Triglycerides (mmol/L)	2.24
High density lipoprotein (HDL) (mmol/L)	1
Lower density lipoprotein (LDL) (mmol/L)	1.35

Table 2. Blood plasma enzymes of Nile crocodile in Kano Zoological Garden.

Enzyme(s) (u/L)	Value (n=6)
Aspartate aminotransferase (AST)	5
Alanine aminotransferase (ALT)	6
Alkaline phosphatase (ALP)	20

Table 3. Comparison of biochemical parameters of captive Nile crocodile in the current study and previous studies.

Parameters	**Current study (n=6)**	**Federal College of Wildlife Mgt, (FCWM) New Bussa, Nigeria (Osagiobare, 2013)**	**Zimbabwe farm (Foggin *et al.*, 1987)**
Total Serum Protein TSP (g/L)	97	33	53
Albumin (g/L)	43	16	19
Globulin (g/L)	54	17	31
Cholesterol (mmol/L)	5.2	4.5	-
Creatinine (umol/L)	44.2	66	-
Uric Acid (mmol/L)	0.18	0.43	0.24
Glucose (mmol/L)	4.9	5.0	4.57
Triglycyrides (mmol/L)	2.2	0.45	-
High density lipoprotein (HDL) (mmol/L)	0.4	--	-
Lower density lipoprotein (LDL) (mmol/L)	0.6	--	-

Table 4. Comparison of plasma enzymes captive Nile crocodile in the current study and previous studies.

Enzyme(s) (u/L)	**Current study(n=6)**	**Federal College of Wildlife Mgt. (FCWM) , New Bussa, Nigeria (Osagiobare, 2013)**	**Zimbabwe farm (Foggin *et al.*, 1987)**
Aspartate aminotransferase (AST)	5	27	13.1
Alanine aminotransferase (ALT)	6	39	--
Alkaline phosphatase (ALP)	20	46	16.6

Comparison of plasma enzymes Nile crocodile in the current study and previous studies.

Table 4.4 shows that Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration of 5U/L and 6U/L respectively measured in the plasma of the Nile crocodiles in the study area were much lower than the value recorded in the FCWM Zoo (Osagiobare, 2013). Plasma Alkaline Phosphatase (ALP) concentration of 20U/L in the study is relatively lower to the value recorded (46U/L) in FCWM zoo but higher to that of Zimbabwe Farm (16.6U/L) (Foggin, 1987).

Discussion

Blood biochemical interpretation in captive crocodile is very challenging due to influence of variability species, nutritional status, age, gender, seasons, physiology (Thrall *et al.,* 2004; Lawrence, 1987) and environment. Though, there is paucity in publication in terms of establishing reference range for captive Nile crocodile however, biochemical parameters seems variable when compared with previous work even in the same species within a geographical location.

High Total Serum Protein and Albumin concentration in the current study could indicate possible inflammation or other

parasitism (Campbell, 1996). Elevated Globulin concentration is an indicative in reptiles of altered immune activity and presence of infections in the population (Campbell, 2006; Thrall *et al.,* 2004).

High Cholesterol concentration could be as a result of high nutritional plane and general lack of physical activity of the captive crocodile (Padilla *et al.,* 2011). Presence of both the High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) has shown that there is no deficiency of lipoprotein which could have resulted to organs dysfunctional.

Creatinine value in the study is within the range for Nile crocodile in the wild (Lovely *et al.,* 2007) but lower to the value recorded in captivity (Osagiobare, 2013). Low creatinine is a breakdown product of creatine which is an important part of muscle tissue. Low cratinine is indicative of low muscle mass. However, creatinine is not considered important for assessing renal diseases in reptiles (Campbell, 1996).

Uric acid concentration is lower than those reported by Osagiobare (2013) and Foggin et al. (1987) for captive Nile crocodile but higher the value reported for wild Nile crocodile (Lovely *et al.,* 2007). Uric acid is the main nitrogenous waste product in urine and faeces of reptile, with 80 - 90% of nitrogen excreted as uric acid. The values of uric acid in most reptiles range from 0 mg/dl to 10mg/dl and values above 15mg/dl are considered high (Campbell, 1996; Frye, 1991). High values of uric acid tend to occur a day after an individual has eaten (Campbell, 1996). For Glucose, the concentration were similar with the value documented in captive Nile crocodile (Osagiobare, 2013; Foggin, 1987) and fall within the range reported by Lovely et al (2007) in free ranging wild Nile crocodile. Glucose might be related to diet, feeding before capture and time between blood sample collections and processing (Campbell, 1996). Elevated blood glucose concentration in reptiles are often related to metabolic conditions, systemic diseases and stress associated hyperclycemia resulting from glucocortocoid and epinephrine release (Campbell, 2006; Thrall *et al.,* 2004) and this may result to pansteatis related die off of Nile crocodile (Botha, 2010). However, the observed in this study shows that the studied animal are neither suffering from hyperglycemia nor hypoglycemia (<200mg/dl) (Botha, 2010).

Triglyceride in this study is substantially higher than the previously reported values in Captive Nile by Osagiobare (2013) but within the range reported in free ranging wild crocodile (Botha, 2010). Low triglyceride concentrations is an indicative of malnutrition in reptiles (Campbell, 2006; Thrall *et al.,* 2004) , Hence the observed values is considered normal for Nile crocodile as the recorded value is above most of the values recorded for Captive crocodile at Olifant River where it was hypothesized that animal are suffering from malnutrition (Botha, 2010).

Plasma Enzymes

Aspartate aminotransaferase (AST) and Alanine aminotransferase (ALT) are serum transaminase activity. Elevation of serum levels of both enzymes can occur with states of altered hepatocellular membrane permeability. The activity of these enzymes is not related to functioning of specific organs in Nile crocodile (Campbell, 2006) though may be useful in evaluating muscle and liver damage or degeneration [23]. In this study, AST and ALT are comparatively lower to values documented for captive Nile crocodile (Osagiobare, 2013; Foggin, 1987) and free ranging Nile crocodile (Lovely *et al.,* 2007; Botha, 2010).

Alkaline Phosphatase (ALP) concentration in Nile crocodile from Kano Zoo were just below the concentration recorded in Nile crocodile from Federal College of Wildlife Management, new Bussa, Nigeria (Osagiobare, 2013) but above the reported concentration from Zimbabwe farm (Foggin, 1987). The observed values in this study fall within the range reported by Lovely et al. (2007) in free ranging Nile crocodile Okavango Delta, Botswana. Though, this enzymes are not necessarily associated with veterinary diseases rather an implication of environmental contamination (Botha, 2010), therefore it's hypothesized that crocodile pool at Kano Zoological Garden is probably free of pollutant.

Conclusion

Based on the results of this study, the conclusion can be drawn that, on average, the blood biochemical of the Nile crocodiles in Kano Zoological Garden fall within the range for healthy crocodiles. However there are pertinent exceptions to this statement as reported that the elevated plasma protein (Total serum protein, albumin and globulin) is an indication of possible inflammation possibly due to malnutrition associated with general weakness and immune problems.

Recommendation

This study provides information on biochemical profiles of the large body sized Nile crocodile, it is then important that further studies should be conducted between classes and sex of Nile crocodile in the study area if clinical pathology is to be used as predictive biomarkers of health status of Nile crocodile in the study area.

Other variables that needed to be considered for future investigations are; the role of geographical distribution, dietary requirement, seasonal variability and age determination. Moreover the data from this study can also be used as reference range for future studies

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EVALUATION OF BLOOD PARAMETERS OF BROILER CHICKENS FED DIET SUPPLEMENTED WITH EMBACERYL AND CHAYA LEAF (Cnidoscolus aconitifolius)

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Abstract

Haematological and serum indices of broiler chickens fed diets supplemented with Embaceryl (EMB) and chaya (Cnidoscolus aconitifolius) leaf powder (CLP) at the finisher phase were evaluated in this study using one hundred and twenty (120) chickens. Twenty four (24) chickens were assigned each to five (5) experimental treatments at varying levels of chaya leaf and embaceryl viz: 0g/kg, 2.5g/kg embaceryl, 2.5g/kg CLP, 5.0g/kg CLP and 7.5g/kg CLP in a complete randomized design. The birds were replicated 3times and the experiment lasted for 56 days. Data generated from this study was subjected to one way analysis of variance and significant means were separated using Duncan multiple range test. The PCV (%) and Hb (g/dl) values varies significantly ($p < 0.05$) from 25.00% to 30.00 % and 08.50g/dl to 10.00g/dl respectively with birds fed basal diet with 5.0g/kg chaya leaf powder recording the highest values. The RBC and WBC were significantly influenced by the treatments, the RBC increased with increased level of phytobiotics, while WBC reduced as the levels of phytobiotics increased. Broilers fed diet supplemented with 5.0g/kg chaya leaf recorded the higher values for total protein (66.50g/dl-55.25g/dl) and albumin (39.95g/dl-34.95g/dl) respectively. However, the cholesterol level decreased with increased level of chaya leaf powder (206.50mg/dl-152.85g/dl). The results of this study suggest that broiler chickens can be fed with diet supplemented with chaya leaf powder without any detrimental effects on haematological and serum biochemical indices.

Keywords: Chaya leaf powder, embaceryl, blood, broilers

ÉVALUATION DES PARAMÈTRES SANGUINS DES POULETS DE CHAIR SOUMIS A UNE ALIMENTATION ADDITIONNÉE D'EMBACERYL ET DE POUDRE DE FEUILLES DE CHAYA (*Cnidoscolus aconitifolius*)

Résumé

La présente étude, qui a porté sur cent vingt (120) poulets en phase de finition, a évalué les indices hématologiques et sériques des poulets soumis à une alimentation supplémentée en embaceryl (EMB) et en poudre de feuilles de chaya Cnidoscolus aconitifolius (CLP : chaya leaf powder). Vingt-quatre (24) poulets ont été répartis en cinq (5) traitements expérimentaux à différents niveaux d'inclusion de poudre de feuilles chaya et d'embaceryl, soit : 0 g / kg ; 2,5 g / kg d'embaceryl ; 2,5 g / kg de CLP ; 5,0 g / kg de CLP : et 7,5 g / Kg CLP, dans un schéma complètement aléatoire. Les traitements ont été répétés 3 fois, et l'expérience a duré 56 jours. Les données générées par cette étude ont été soumises à une analyse de variance à un facteur, et les moyennes significatives ont été séparées en utilisant la méthode de Duncan. Les valeurs PCV (%) et Hb (g / dl) étaient significativement ($p <0,05$) variables, respectivement 25,00% à 30,00% et 08,50g / dl à 10,00g / dl, les oiseaux soumis au régime de base comportant 5,0g / kg de poudre de feuilles de chaya enregistrant les valeurs les plus élevées. Les numérations érythrocytaires (RBC) et leucocytaires (WBC) ont été influencés de façon significative par les traitements : les RBC ont augmenté parallèlement à l'élévation du niveau de phytobiotiques, tandis que les WBC diminuaient au fur et à mesure que les taux de phytobiotiques augmentaient. Les poulets soumis au régime alimentaire additionné de 5,0 g / kg de poudre

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de feuilles de chaya ont enregistré les valeurs les plus élevées pour la protéine totale (66,50 g / dl-55,25 g / dl) et l'albumine (39,95 g / dl-34,95 g / dl). Cependant, le taux de cholestérol a diminué avec l'augmentation du niveau d'inclusion de poudre de feuilles de chaya (206.50mg / dl-152.85g / dl). Les résultats de cette étude portent à croire que les poulets de chair peuvent être nourris avec un régime supplémenté en poudre de feuilles de chaya sans aucun effet préjudiciable sur leurs indices hématologiques et biochimiques sériques.

Mots-clés : poudre de feuilles de Chaya, embaceryl, sang, poulets de chair

Introduction

In Nigeria, the State of nutrition of the populace is predominantly marked by inadequate protein intake both in quantity and quality (Adegbola, 1985). The protein intake per average Nigerian is grossly inadequate and the shortage has given rise to high prices of animal protein. Efforts should therefore, be directed towards exploring all reasonable options to meet the recommended level at reduced cost. One of the efforts include the practice of feeding livestock with sub therapeutic levels of antibiotics for improving performance, reducing some pathogenic microorganisms in the intestinal tract has been in use for over fifty years (Gibson and Fuller, 2000). Also, The fighting of bacterial, germs and other blood related diseases in poultry animals has geared farmers towards the use of various growth promoters 'antibiotics' drugs and phytobiotics from plant sources to fight these numerous blood diseases. However, antibiotics used as growth promoters in animal feed have been banned recently due to possible development of drug resistance, cross resistance and multiple resistances (Mehala and Moorthy, 2008). With the removal of antibiotics growth promoters from livestock diets in different parts of the world, numerous additives are now being used or proposed as means to reduce or eliminate pathogens or to improve growth (Joerger, 2002). They include probiotics (Awad, *et al.,* 2006), Organic acids (Gunal, 2006), Enzymes and phytogenics – plant materials and their extracts. Herbs and their extracts have been reported to have beneficial effects on broiler performance and carcass quality (Schleicher, 1998) and some of these are indigenous to Africa, they include Ginger (Zingiber officinale), Garlic (Allium sativum), Bitter leaf (Vernonia amygdalina) and Chaya leaf (Cnidoscolus aconitifolius). Cnidoscolus aconitifolius, commonly known as Chaya leaf is a large, fast growing leafy perennial shrub that is believed to have originated in Mexico. It has succulent stems which exude a milky sap when cut.

Tree Spinach or "Iyana Ipaja" as it is called in some parts of Nigeria is easy to grow, a tender perennial in the United State and suffers little insect damage. It is tolerance of heavy rainfall and has some drought tolerance. Some varieties have stingy hairs and require gloves for harvesting. Cooking destroys stingy hairs. Chaya is one of the most productive vegetables, chaya is a good source of protein, vitamins, calcium and iron and it is a rich source of antioxidants (Kuti, 2004). Hence, examination of blood provides a valuable opportunity to clinically evaluate the quality of feeds. This study is thus to assess the haematological and serum biochemical values of broiler finishers fed diets supplemented with Cnidoscolus aconitifolius.

Materials and Methods

Experimental site

The research was carried out at the Poultry Unit, Agricultural Science Department, Federal College of Education, Abeokuta in Odeda Local Government Area. It falls within the rainforest vegetation zone of South-Western Nigeria at latitude 7o13` 49.46"N, longitude 3o26` 11.98"E and altitude of 76 meters above sea level. The climate is humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity is 43.7oC and 82% respectively.

Experimental birds and management

120 day old broiler chicks were used for this study, after brooding which lasted for

two weeks the birds were randomly assigned to 5 treatments with 24 birds per treatment with each replicate containing 8 birds each. Routine poultry management practices which include daily inspection of birds for symptoms of diseases, mortality, cleaning of feeding and drinking troughs, provision of fresh feed and water were maintained. The recommended vaccine regime was strictly adhered to.

Experimental diet

A concentrate diet was formulated (Table 1). Chaya leaves (Cnidoscolus aconitifolius) were obtained from a nearby village around Osiele, Abeokuta. It was chopped and dried under room temperature. It was then milled into powder form before inclusion into the feed.

Table 1: Composition of experimental diets fed broiler chickens at g/kg

Ingredients	**Control 0**	**EMB 2.5**	**CLP 2.5**	**CLP 5.0**	**CLP 7.5**
Maize	470.00	470.00	470.00	470.00	470.00
Soya bean	260.00	260.00	260.00	260.00	260.00
Vegetable oil	45.00	45.00	45.00	45.00	45.00
Fish meal	15.00	15.00	15.00	15.00	15.00
Wheat offal	150.00	150.00	150.00	150.00	150.00
Embaceryl	-	+	-	-	-
Chaya leaves	-	-	+	++	+++
Bone meal	20.00	20.00	20.00	20.00	20.00
Oyster shell	30.00	30.00	30.00	30.00	30.00
Salt	2.50	2.50	2.50	2.50	2.50
*Premix	2.50	2.50	2.50	2.50	2.50
Methionine	2.50	2.50	2.50	2.50	2.50
Lysine	2.50	2.50	2.50	2.50	2.50
Total	1000	1000	1000	1000	1000
Calculated analysis (%)					
ME(MJKg-1)	12.74	12.74	12.74	12.74	12.74
Crude Fibre	3.88	3.88	3.88	3.88	3.88
Crude Protein	20.00	20.00	20.00	20.00	20.00
Ether extract	3.51	3.51	3.51	3.51	3.51
Calcium	1.47	1.47	1.47	1.47	1.47
Av. Phosphorus	0.45	0.45	0.45	0.45	0.45
Lysine	1.25	1.25	1.25	1.25	1.25
Methionine	0.6	0.6	0.6	0.6	0.6

**Vitamin and mineral premix based on 2.5Kg per ton; Vit A: 4,000,000Iu, Vit D:80000, VitB12:25mg, Niacin:6000mg, Vit E40000, Vit k3 800mg, VitB3 1000mg, Vit B26000mg, Vit B6:5000mg panthotenic Acid:20000, Folics Acid:200mg, Biotine 8mg, Maganese:30000, Iron8000mg, Zinc:2000mg, Copper: nill, Cobalt:80mg, Iodine:400mg, Selenium:40mg, Choline:80000mg*

Experimental design

The design used for this experiment was a complete randomized design techniques, one hundred birds and twenty birds were randomly allocated to five (5) dietary treatment group of twenty (24) birds each and was further divided into three replicates with eight (8) birds per replicate.

Data collection

At 8th week of the experiment, 2.5ml of blood was collected from one bird per replicate with syringe through the wing veins of the birds into tube containing Ethylene Diamine Tetra Acetic Acid (EDTA). This was used to determine the haematological components which include Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (HB) and White Blood Cell (WBC). This was put in a cooler containing iced pack so as to prevent the blood from clotting before it gets to the laboratory for test. Blood samples for serum biochemical indices were collected into another sample bottle containing no anticoagulant. All the haematology and serum biochemical parameters were analysed as outlined by the procedure of Ochei et al. 2000.

Statistical analysis

All data generated from this research were subjected to Analysis of Variance (ANOVA), while significant ($p<0.05$) differences were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The haemetological and serum components obtained for broiler chickens in this study are presented in tables 2 and 3 respectively. The PCV (%) and Hb (g/dl) values vary significantly ($p < 0.05$) from 25.00% to 30.00 % and 08.50g/dl to 10.00g/dl respectively. It was observed that values obtained for both PCV and Hb in this study reduced with inclusion of antibiotics (embaceryl), and increased with more addition of Cnidoscolus aconitifolius inclusion in the diet. The PCV values obtained in the present study for birds fed diet supplemented with 2.5 g/kg CLP falls within the normal range but was higher than values reported for chicken in Nigeria (Oyewole and Ajibade, 1990). Daramola *et al.,* 2005 reported concentration of haemoglobin (Hb) in the

Table 2: Haematological parameters of broiler chickens fed diets supplemented with Emberceryl and (Cnidoscolus aconitifolius) chaya leaf (g/kg)

Parameters	Control 0	EMB 2.5	CLP 2.5	CLP 5.0	CLP 7.5	S.E.M
PCV (%)	26.50^{cd}	22.00^{d}	30.00^{a}	29.00^{a}	28.50^{ab}	**0.58**
Hb (g/dl)	8.90^{b}	8.50^{bs}	10.00^{a}	9.85^{a}	9.30^{a}	**0.58**
RBC (X 1012)	2.44^{ab}	2.00^{b}	2.615^{a}	2.689^{a}	2.65^{a}	**0.48**
WBC (Cumm3)	27.45^{a}	20.40^{c}	23.00^{b}	20.25^{c}	21.65^{bc}	**0.58**
Neutrophyl (%)	18.50	18.50	18.50	17.50	17.50	**0.54**

^{ab}*Mean on the same row having different superscripts are significantly different (P<0.05)*

Table 3: Serum parameters of broiler chickens fed diets supplemented with Emberceryl and (Cnidoscolus aconitifolius) chaya leaf (g/kg)

Parameters	Control 0	EMB 2.5	CLP 2.5	CLP 5.0	CLP 7.5	S.E.M
Glucose(mg/dl)	167.00	166.25	165.55	168.55	167.80	0.58
Protein(g/dl)	58.00^{bc}	55.25^{c}	63.20^{b}	61.65^{b}	66.50^{a}	0.49
Albumin(g/l)	36.60^{b}	34.95^{b}	35.90^{b}	35.25^{b}	39.95^{a}	0.56
Globulin(g/dl)	31.40	30.30	27.45	32.40	33.55	0.58
Cholesterol(mg/dl)	206.5^{a}	191.35^{b}	193.90^{b}	152.85^{e}	175.95^{d}	0.51

^{abcde}*Mean on the same row having different superscripts are significantly different (P<0.05)*

cytoplasm of the red blood cells and this gives an indication of an oxygen carrying capacity of the blood of an animal. Pellet and Young (1980) confirmed that haemoglobin levels are positively correlated with protein quality and level in the diets. Udo (1987) also reported that nutrition is the most important factor affecting haemoglobin levels of the blood. The values of RBC values obtained in this study ranged from 8.50 g/dl to 10.00 g /dl. Low values of RBC and Hb could be attributed to anemia (Muhammad *et al.,* 2003), and also may indicate congenital heart disease, dehydration and lung disease (Agbabiaka *et al.,* 1999). Experiment has shown that the concentration of CLP in the diet had no effect on feed consumption and mortality rates of birds. Higher levels of WBC have been produced in farm animals in a bid to fight against the foreign bodies introduced. The increasing WBC values recorded with no CLP are an indication of health hazard, this could be due to infectious inflammatory conditions, stress condition or conditions induced by steroids or tannin residues (Brij *et al.,* 1977). WBC played protective roles and also associated with the production of antibodies and recognition of foreign substances such as bacteria and viruses.

The serum traits of broiler chickens fed CLP supplemented diet is presented in table 3. Serum protein, albumin and cholesterol were significantly ($p<0.05$) affected by the dietary treatment. Highest values (66.50g/dl and 39.95mg/dl) of total protein and albumin recorded for broiler chickens fed 7.5g/kg CLP is an indication of improved health status (Olsen, 1975). The increased total serum protein, albumin and glucose obtained for broiler chickens fed CLP based diets were due to improvement in protein synthesis, carbohydrate and lipid metabolism (Rosa *et al.,* 2001). The reduction in serum cholesterol of broiler chickens fed CLP could be attributed to reduced absorption or synthesis of cholesterol in the gastro intestinal tract by probiotic, prebiotic and phytobiotics supplementation (Mohan et al. 1996; Ghiyas et al. 2008).

Conclusion

The incorporation of chaya leaf powder at 5.0g/kg promotes both the haematological and serum qualities in broiler chickens. Increasing knowledge of micro organisms' resistance to antibiotics used by poultry has contributed to the use of alternatives like phytobiotics.

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EFFECTS OF GENOTYPES AND SEX ON GROWTH PERFORMANCE OF YORUBA ECOTYPE AND CROSSBRED GROWER CHICKENS

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Abstract

Information on adaptability and crossbreeding potentials of exotic birds are important to improve animal protein supply in the tropics. The aim of the present study was to evaluate the performance of exotic grower chicks and their crossbred under a humid tropical environment. A total of 547 chicks which belong to 10 genotypes (Yoruba ecotype, YEC; Sussex, SS; Goliath, GO; Marshall, ML and six crossbred genotypes) were evaluated for growth performance from 8 to 20 weeks of age. Marshall grower chicks were significantly higher ($p<0.05$) in weekly body weights than, Yoruba ecotype, Sussex and Goliath, and the six crossbred genotypes throughout the 12 weeks study. Crossbred chicks that were produced from Marshall cocks and Yoruba ecotype hens were significantly heavier ($p<0.05$) in weekly body weights than SS x SS, Go x GO and other crossbred chicks. Male chicks were significantly higher ($p<0.05$) in weekly body weights than female chicks from 9 to 20 weeks of age. There were significant ($p<0.05$) interaction between genotype and sex on body weights of crossbred chicks. Reciprocal effect was significant ($p<0.05$) on body weight of chicks in all the crossbred groups. Reciprocal effect favoured chicks that were born to purebred cocks and Yoruba ecotype hens. The study therefore recommends the use of Marshall cocks and Yoruba ecotype hens to improve poultry meat production in Nigeria.

Key words: Body weight, Crossbred chicks, Genotype, Interaction, Reciprocal effect, Tropical climate

EFFETS DES GENOTYPES ET DU SEXE SUR LA PERFORMANCE DE CROISSANCE DES POULETS DE L'ÉCOTYPE YORUBA ET DES POULETS DE RACE CROISEE

Résumé

La disponibilité de l'information sur l'adaptabilité et le potentiel de croisement des oiseaux exotiques est importante pour l'amélioration de l'approvisionnement en protéines animales dans les régions intertropicales. La présente étude avait pour objectif d'évaluer la performance des poussins exotiques et de leurs croisements dans un environnement tropical humide. Au total, 547 poussins appartenant à 10 génotypes (écotype Yoruba, YEC, Sussex, SS, Goliath, GO, Marshall, ML et six génotypes croisés) ont été évalués en vue de déterminer l'état de leur croissance à l'âge de 8 à 20 semaines. Les poids corporels hebdomadaires des poussins Marshall étaient significativement plus élevés ($p <0,05$) que ceux de l'écotype Yoruba, Sussex, Goliath, et des six génotypes croisés, tout au long de l'étude de 12 semaines. Les poussins croisés issus de coqs Marshall et de poules de l'écotype Yoruba avaient des poids corporels hebdomadaires significativement ($p <0,05$) plus lourds que les SS x SS, Go x GO et les autres poussins croisés. Les poussins mâles avaient un poids corporel hebdomadaire significativement ($p <0,05$) plus élevé que les femelles à l'âge de 9 à 20 semaines. On a relevé une relation significative ($p <0,05$) entre le génotype et le sexe et le poids corporel des poussins croisés. L'effet réciproque était significatif ($p <0,05$) sur le poids corporel des poussins dans tous les groupes croisés. L'effet réciproque a favorisé les poussins issus de coqs de race pure et de poules de l'écotype Yoruba. L'étude recommande donc l'utilisation de coqs Marshall et de poules de l'écotype Yoruba pour améliorer la production de viande de volaille au Nigeria.

Mots-clés : poids corporel, poussins croisés, génotype, interaction, effet réciproque, climat tropical

Introduction

Poultry production is one area of animal enterprise with significant contribution to human food production. Demand for poultry meat is higher because of the perceived superiority of white meat over red meat produced by ruminant animals. Chicken meat also has comparably low contents of fat and cholesterol. Furthermore, the meat is relatively low priced, with typically convenient portions and lack of religious restriction (Jaturashita, 2004).

Indigenous chickens in Africa represent valuable resources for livestock development because of their extensive genetic diversity and adaptation to varied environmental conditions (Ajayi, 2010). However, a commonly reported problem is their low productivity compared with exotic chickens (Tadelle *et al.,* 2000; Sola-Ojo *et al.,* 2012). However, Akinokun (1990) noted that exotic chicken are generally inferior in the tropical environment than in their place of origin in terms of survivability, adaptability and prolificacy. Furthermore, Permin (2008) and Islam and Nishibori (2009) opined that previous attempts at wholesale replacement of indigenous chickens with exotic strains have failed because of the inability of exotic birds to adapt to adverse environmental conditions, such as high temperature, disease and shortage of feed which are common decimals in small holders' farm.

Crossbreeding is the mating of two or more genetically unrelated or different breeds; strains or inbreed lines. Crossbreeding is a fast tool for breeders to improve many traits in farm animals (Oseni *et al.,* 1997). It has been successfully employed by the present commercial meat and egg type poultry industries with focus on such traits as auto-colour sexing potentials, growth rate and meat yield. According to Guéye (1998), the genetic potential of indigenous chicken could be improved by crossing them with selected but still robust exotic breeds. The goal of crossbreeding program in hot climates should be a new breed or hybrid that is resistant to harsh tropical conditions and at the same time produces reasonable amount of egg and meat (Mekki *et al.,* 2005). The difference in the mean phenotypic values from the crosses of two breeds in which their role as the male parents is interchanged is called reciprocal effect. Its economic exploitation has been of interest of some researchers in developing tropical countries where the importation of exotic animals is fast gaining ground (Fayeye, 2001). However, much is still needed to be learnt on reciprocal effects on body weights of crossbred chickens in Africa.

Sussex and Goliath are dual purpose British chickens. Sussex chickens are popularly known as backyard chicken in many countries because they are good foragers (Citrus County fair, 2010-2011). There is the need for information on the adaptability and crossbreeding potentials of both Sussex and Goliath with indigenous chicken like Yoruba ecotype chicken. An un-publish work by the same authors showed that genotypes and sex influenced the early (0-8 weeks) growth performance of Yoruba ecotype; Marshall; Sussex and Goliath and their crossbred chicks in Nigeria. The present work is aimed at evaluating the growth performance of Yoruba ecotype, Marshall, Sussex, Goliath and their crossbred grower (8-20weeks old) chickens in Nigeria.

Materials and Methods

A total of 547 eight-week old chicks which belong to four purebred (Yoruba ecotype, Sussex, Goliath and Marshall) and six crossbred genotypes (Table 1) were used to evaluate weekly bodyweight performance of grower chicken. The birds were obtained from an earlier study on early growth characteristics and feed efficiency of 583 chicks produced by the authors. All the birds were raised in deep litter floored pens at a poultry facility located at Oyan (Osun state, South-West Nigeria). The coordinates of the experimental site is latitude 8.05 and longitude 4.77 and an elevation of 422 meters above sea level. Birds were supplied with feed and water ad libitum. All birds were given unrestricted access to feed and water. The composition of experimental diet is

shown in Table 2. Birds were vaccinated against Newcastle and Gumboro diseases. Data were collected on weekly body weights (measured in grammes) for a period of 12 weeks, until the birds were 20 weeks of age. Data on weekly bodyweight were subjected to Analysis of Variance (ANOVA) suitable for a 10x2 factorial design to evaluate the fixed effects of genotypes, sex and their interaction. Mean genotypic values were separated using Duncan multiple range test.

The mathematical model of ANOVA used is as follows:

$$Y_{ijk} = \mu + G_i + S_j + GS_{ij} + e_{ijk}$$

Where:Yijk is the phenotypic value obtained for kth animal which belong to the ith genotypes and jth sex. Gi, Sj, represent the fixed effects of genotypes and sex, respectively. GSij represents the interaction between genotypes and sex. μ and eijk represent overall mean and error residual, respectively.

Table 1: Genotypic groups involved in the study

Genotype	Number of chicks
Purebred groups	
YEC x YEC	139
GO x GO	58
SS x SS	75
ML x ML	92
Crossbred groups	
GO x YEC	38
YEC x GO	34
SS x YEC	28
YEC x SS	34
ML x YEC	19
YEC x ML	30
Total	**547**

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

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

Ingredients	% composition
Calcium %	1.00
Available phosphorus%	0.45
Na%	0.38
Cl % (min)	0.30
Methionine digest%(min)	0.46
Cystine digest% (min)	0.30
Lysine digest%(min)	0.90
Threoinine digest%(min)	0.70
Crude fibre%(max)	7.00
Crude fat%(min)	5.00
Crude Protein %	16.00
ME Kcal/kg	2450.00

Results

Mean body weight (+ Standard Error) of Yoruba ecotype, exotic and crossbred grower chickens are shown in Table 3. The three exotic chickens were significantly heavier ($p<0.05$) in weekly body weights than the local chicken from 9 to 20 weeks of age. Marshall grower chicks were significantly heavier ($p<0.05$) in weekly body weights than Sussex, Goliath and the six crossbred chicken genotypes throughout the study. All the crossbred chicks were significantly heavier ($p<0.05$) in weekly body weights than Yoruba ecotype chicken, except YEC x ML. Crossbred chicks that were produced from Marshall cocks and Yoruba ecotype hens were significantly heavier ($p<0.05$) in weekly body weights than SS x SS, Go x GO and other crossbred chicks. The male chicks were significantly higher ($p<0.05$) in weekly body weights than female chicks from 9 to 20 weeks of age (Table 3).

There were significant ($p<0.05$) interaction between genotype and sex on body weights of crossbred chicks. MLxYEC male chicks were significantly ($p<0.05$) heavier than other genotypes at week 1, whereas the heaviest female chicken at week 1 was the MLxML chicks. There was no significant ($p>0.05$) difference between YECxGO and YECxSS male chicks at weeks 7, 8 9, 10, 11 and 12, whereas the female YECxGO chicks were significantly ($p<0.05$) heavier within the same period. The GOxYEC male chicks were consistently superior ($p<0.05$) to SS x YEC male chicks at weeks 2, 3, 4, and 5, whereas there was no significant ($p>0.05$) difference between female chicks of the two genotypes during the same period. Reciprocal effect on body weight was significant in all the crossbred groups. Reciprocal effect favoured chicks that were born to purebred cocks and Yoruba ecotype hens.

Table 3: Effects of genotypes and sex on body weight of pure bred and cross bred grower chickens.

Genotype	Weekl	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	week20
YECxYEC	459±7^{h}	513±8^{h}	559±8^{h}	617±9^{g}	617±9^{g}	789±28^{e}	798±9^{h}	887±10^{g}	1026±12^{g}	1103±14^{g}	1183±14^{g}	1248±14^{g}
GOx GO	917±9^{c}	1038±11^{c}	1157±12^{c}	1272±12^{c}	1272±12^{c}	1583±40^{c}	1756±13^{c}	1852±14^{c}	1952±17^{c}	2024±19^{c}	2093±20^{c}	2161±20^{c}
SS x SS	776±8^{d}	865±10^{d}	977±11^{d}	1066±11	1066±11^{d}	1254±36^{d}	1353±12^{e}	1458±13^{e}	1556±15^{e}	1644±17^{e}	1744±18^{e}	1836±18^{e}
MLx ML	1151±9^{a}	1324±11^{a}	1595±12^{a}	1891±12^{a}	1891±12^{a}	2202±40^{a}	2458±13^{a}	2674±14^{a}	2925±17^{a}	3130±19^{a}	3381±20^{a}	3621±20^{a}
GOxYEC	706±12^{e}	790±14^{e}	920±16^{e}	1047±16^{d}	1047±16^{d}	1284±53^{d}	1407±17^{d}	1521±18^{d}	1642±22^{d}	1766±25^{d}	1903±26^{d}	2025±26^{d}
YECxGO	495±12^{g}	551±14^{g}	602±16^{g}	660±16^{f}	660±16^{f}	780±53^{e}	836±17^{g}	896±19^{g}	963±23^{h}	1039±25^{h}	1093±26^{h}	1145±26^{h}
SSxYEC	613±14^{f}	716±16^{f}	813±17^{f}	949±18^{e}	949±18^{e}	1145±58^{d}	1227±19^{f}	1306±20^{f}	1394±25^{f}	1476±28^{f}	1546±29^{f}	1602±29^{f}
YECxSS	490±13^{g}	539±15^{gh}	584±16^{gh}	635±17^{fg}	635±17^{fg}	741±55^{e}	797±17^{gh}	870±19^{g}	931±23^{h}	987±27^{h}	1036±27^{h}	1083±27^{h}
MLxYEC	1101±17^{b}	1277±20^{b}	1455±22^{b}	1628±22^{b}	1628±22^{b}	1979±73^{b}	2164±23^{b}	2324±26^{b}	2470±31^{b}	2626±35^{b}	2778±36^{b}	2932±36^{b}
YECxML	491±13^{g}	543±15^{gh}	590±17^{gh}	643±17^{fg}	643±17^{fg}	743±56^{e}	800±18^{gh}	855±20^{g}	915±24^{h}	987±27^{h}	1058±28^{h}	1126±28^{h}

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

Table4: Details of interaction between genotype and sex on weekly body weights in Yoruba ecotype, Goliath, Sussex, Marshal and their crossbred chicks

		GENOTYPE									
Week	**Sex**	**YECxYEC**	**GOxGO**	**SSxSS**	**MMxMM**	**GOxYEC**	**YECxGO**	**SSxYEC**	**YECxSS**	**MLxYEC**	**YECxML**
1	M	500±9^{jl}	1093±14^{c}	939±13^{e}	1246±16^{b}	847±20^{f}	565±19^{i}	729±21^{g}	533±21^{ij}	1322±22^{a}	571±21^{hi}
	F	417±9^{k}	741±12^{g}	612±11^{h}	1055±9^{d}	564±14^{i}	424±16^{k}	496±17^{j}	447±14^{k}	880±26^{f}	411±16^{k}
2	M	561±11^{i}	1255±16^{c}	1031±15^{d}	1425±18^{b}	944±23^{e}	625±22^{h}	838±24^{f}	591±24^{hi}	1529±25^{d}	631±24^{h}
	F	465±11^{j}	822±14^{f}	700±12^{g}	1222±11^{c}	636±16^{h}	477±18^{j}	595±20^{hi}	488±17^{j}	1026±30^{d}	456±18^{j}
3	M	612±12^{h}	1413±18^{b}	1184±17^{c}	1754±20^{a}	1125±26^{c}	674±25^{fg}	945±27^{d}	642±27^{gh}	1714±28^{a}	686±27^{fg}
	F	506±12^{i}	902±16^{d}	771±14^{e}	1435±12^{b}	714±18^{f}	529±20^{i}	680±22^{fg}	525±18^{i}	1196±33^{c}	494±20^{i}
4	M	671±12^{k}	1573±18^{d}	1287±17^{e}	2061±21^{a}	1283±26^{e}	737±25^{j}	1128±27^{f}	707±27^{jk}	1904±29^{b}	745±27^{j}
	F	562±12^{l}	971±16^{g}	844±14^{h}	1720±12^{c}	811±19^{hi}	583±20^{l}	770±23^{ij}	564±19^{l}	1353±34^{e}	505±21^{l}
5	M	731±12^{m}	1771±18^{d}	1407±17^{f}	2301±20^{a}	1434±25^{f}	811±24^{kl}	1269±27^{g}	769±27^{g}	2097±28^{b}	799±27^{kl}
	F	623±12^{n}	1091±16^{h}	908±13^{i}	1830±12^{c}	902±18^{ij}	632±20^{n}	846±22^{jk}	602±18^{n}	1523±33^{e}	586±20^{n}
6	M	792±40^{gh}	1956±59^{b}	1508±56^{cd}	2475±68^{a}	1571±86^{cd}	872±82^{fg}	1384±90de	837±90^{fg}	2280±94^{a}	856±90^{fg}
	F	786±40^{gh}	1209±53^{e}	1000±45^{f}	1929±40^{b}	997±61^{f}	688±67^{hi}	906±74^{fg}	644±62^{ij}	1679±112^{c}	631±68^{j}
7	M	860±13^{l}	2198±19^{c}	1589±18^{g}	2789±22^{a}	1713±27^{f}	928±26^{k}	1473±29^{h}	914±29^{kl}	2481±30^{b}	919±28^{kl}
	F	735±13^{m}	1314±17^{i}	1117±14^{j}	2126±13^{d}	1102±19^{j}	744±21^{m}	982±24^{k}	681±20^{n}	1847±36^{e}	680±22^{n}
8	M	972±14^{l}	2313±21^{d}	1698±20^{g}	2974±24^{a}	1840±30^{f}	995±29^{kl}	1557±32^{h}	1021±32^{kl}	2665±33^{b}	982±32^{kl}
	F	802±14^{m}	1390±18^{i}	1219±18^{j}	2374±14^{c}	1202±21^{j}	797±23^{m}	1055±26^{k}	719±22^{n}	1983±39^{e}	729±24^{n}
9	M	1189±17^{k}	2437±25^{d}	1805±24^{g}	3203±29^{a}	1976±37^{f}	1070±35^{lm}	1635±38^{h}	1107±38^{kl}	2822±40^{b}	1048±38^{m}
	F	862±17^{n}	1467±22^{j}	1308±19^{j}	2648±17^{c}	1309±26^{j}	857±28no	1153±32^{kl}	756±26^{p}	2117±48^{e}	782±29^{op}
10	M	1312±19^{k}	2538±28^{d}	1909±27^{g}	3401±33^{a}	2130±41^{f}	1156±39^{lm}	1705±43^{h}	1169±43^{lm}	2981±45^{b}	136±43^{m}
	F	894±19^{n}	1512±25^{i}	1379±22^{j}	2859±19^{c}	1402±29^{j}	922±32^{n}	1248±36^{kl}	795±30^{o}	2271±54^{e}	838±33no
11	M	1407±20^{i}	2633±29^{c}	2034±28^{e}	3612±34^{a}	2316±42^{d}	1215±41^{k}	1770±44^{f}	1236±44^{jk}	3148±46^{b}	1226±44^{jk}
	F	959±20^{l}	1553±26^{g}	1454±22^{hi}	3150±20^{b}	1490±30^{gh}	970±33^{l}	1322±37^{j}	836±31^{m}	2409±55^{d}	891±34^{lm}
12	M	1488±20^{j}	2723±29^{d}	2148±28^{f}	3811±34^{a}	2476±42^{e}	1269±41^{k}	1829±44^{g}	1288±44^{k}	3283±46^{c}	1300±44^{k}
	F	1008±20^{l}	1600±26^{h}	1524±22^{ij}	3431±20^{b}	1575±30^{h}	1022±33^{l}	1375±37^{k}	878±31^{m}	2581±56^{e}	952±34^{lm}

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

Discussion

The present results suggest differences in genetic potentials of the 10 genotypes of chickens for growth performance and matured body weights. Amusan et al. (2013) reported that the effect of chicken genotype was significant on the growth performance, feed intake and feed efficiency of the progenies resulting from pure, straight and reciprocal cross of Giriraja and Alpha chickens in South West Nigeria. An earlier study by Ajayi and Ejiofor (2009) showed differences in body weight and body size parameters between Ross and Anak broiler chickens that were raised in high rain-forest belt of Nigeria. These results are consistent with the submission of Deeb and Lamont (2002) that there exist differences in growth pattern of animals belonging to the same species.

The observed reciprocal difference agrees with the reports of Adebambo *et al.,* (2011) who opined that sire and dam genotypes had significant effects on growth rate of chicken. Previous reports in chicken have shown that reciprocal effects could be an important factor in crossbreeding programmes (Singh et al. 1983, Hanafi and Iraqi, 2001, Keambou et al, 2010). According to Keambou *et al.,* (2010), while heterosis was in general weak, the reciprocal effect was found to be significant for growth and conformation traits in the cross between local and Hubbard chickens in Western highlands of Cameroon. Reciprocal effect is a maternal effect and may also be due to possible difference in the combining aptitudes between males and females of the local and exotic breeds (Keambou et al, 2010).

The sexual dimorphism observed in the present study is in agreement with previous reports (Fayeye *et al.,* 2005, Dusan Tercic and Antonija Holeman, 2008, Bekele *et al.,* 2010, Kgwatalala *et al.,* 2012). According to Bekele *et al.,* (2010), 4-8 months male Red F1 crosses with indigenous breed as paternal lines and exotic breeds as maternal lines were significantly higher in body weight than female grower chicks. Similar observation was made by Kgwatalala *et al.,*(2012) in their work on the body weights of male and female naked and normal strains of indigenous Tswana chicken that were raised from 14 to 20 weeks of age. Singh et al. (982) attributed the male biased sexual dimorphism observed in many avian species to effective male growth hormones compared with female hormones. In another report, 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 chicks consumed more feed, utilized the feed more efficiently, and gained more body weight than females but had a higher mortality rate. Furthermore, the heavier body weight observed in male chicks compared with their female counterparts may possibly be due to better feed efficiency. Meizehof, (1988) observed that male broiler utilize feed more efficiently than female birds. Earlier, Gonzales and Sartori (2002) reported that the muscle development in meat type birds occurs through the interaction of several factors, among which sex and hormonal factors have significant influence. Male chicks have more androgen hormones responsible for muscle anabolism, which results in higher growth rates than female chicks.

The genotype x sex interaction on body weight of crossbred grower chicks suggests variation in the growth pattern of male and female chicks of different genotypes at the same chronological age. Del Castilho et al. (2013) observed no genotype x sex interaction in their evaluation of six genotypes of free ranged broiler chickens that were raised for 91 days

Public brief on the study for the benefit of policy makers

Previous attempts at wholesale replacement of indigenous chickens with exotic strains have failed because of the inability of exotic birds to adapt to adverse environmental conditions in tropical Africa. There is a strong advocacy for improving the genetic potential of indigenous chicken by crossing them with

selected but still robust exotic breeds. This approach could lead to new breeds or hybrids that are resistant to harsh tropical conditions and at the same time produces reasonable amount of egg and meat. The present work is aimed at evaluating the growth performance of Yoruba ecotype, Marshall, Sussex, Goliath and their crossbred grower (8-20weeks old) chickens in Nigeria. Reciprocal crossbreeding favoured the production of bigger chicks using purebred exotic cocks and Yoruba ecotype hens. The authors therefore recommend the use of Marshall cocks and Yoruba ecotype hens to improve poultry meat production in Nigeria.

Conclusion

It was concluded that exotic chickens were superior in weekly body weights than the local chicken. Crossbred chicks that were produced by Marshall cocks and Yoruba ecotype hens were heavier in weekly body weights than all pure and crossbred genotypes. All the 10 genotypes displayed male biased sexual dimorphism. Reciprocal and interaction effects on body weight of chicks were pronounced among crossbred groups. Reciprocal effect favoured chicks that were born to purebred cocks and Yoruba ecotype hens. The study therefore recommends the use of Marshall cocks and Yoruba ecotype hens to improve poultry meat production in Nigeria.

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EFFECTS OF TWO FEED FORMS ON THE GROWTH PERFORMANCE, CARCASS YIELD AND DUODENAL VILLUS MORPHOLOGY OF LOCALLY-ADAPTED TURKEYS

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Abstract

This study was conducted to determine the performance, carcass yield and duodenal villus morphology of locally-adapted turkeys on feed forms (mash and pellet) of the same nutrient contents. A total of 120 day-old turkey poults were used for the experiment. The poults were brooded for 3 weeks using kerosene stove as source of heat. They were thereafter divided into two treatment groups of 60 poults each with 3 replicates of 20 birds per replicate. They were managed intensively on litter-covered floor for 8 weeks. Data on performance characteristics were collected and at the end of the experiment two birds of average weight for each replicate were selected and starved overnight to clear the guts and thereafter sacrificed for evaluation of the carcass yield. The duodenal villus morphology of a part of the small intestine was also determined. Data were subjected to studentized t-test at 5% level of significance. Poults on pelleted feed form had significantly ($P<0.05$) better feed conversion ratio (3.45) than birds on mash (4.23). However, a significantly ($P<0.05$) higher cost of feed intake per poult per day (N13.96) was obtained in birds fed pelletized form of feed compared to N8.30 obtained in poults on mash feed form. The drumstick was also significantly ($P<0.05$) influenced by the forms of feed with poults on mash feed having a greater value. The results showed that poults on mash had significantly ($P<0.05$) higher villus height (1.00µm) than poults fed pellet (0.60µm). This implies that mash diet enhanced the duodenal villus morphological development in locally-adapted turkeys relative to mash diet. It was thereby concluded that for better nutrient utilization, pelletized feed should be adopted for locally-adapted turkey production but its use could be limited by higher cost compared to mash diet. However, it might in the long run yield sufficiently better gain than obtainable in the mash feed form.

Keywords: Mash, pellet, growth performance, carcass yield, villus, duodenum, cost benefit

EFFETS DE DEUX FORMES D'ALIMENTS SUR LA PERFORMANCE DE CROISSANCE, LE RENDEMENT DE CARCASSE ET LA MORPHOLOGIE DES VILLOSITES DUODÉNALES DE DINDES ADAPTÉES AUX CONDITIONS LOCALES

Résumé

La présente étude a été réalisée dans le but de déterminer la performance, le rendement en carcasse et la morphologie des villosités duodénales de dindes adaptées aux conditions locales, soumises à deux formes d'aliments (purée et boulettes) d'une même teneur en nutriments. Au total, 120 dindonneaux âgés d'un jour ont été utilisés pour l'expérience. Les dindonneaux ont été incubés pendant 3 semaines en utilisant un poêle à kérosène comme source de chaleur. Ils ont ensuite été divisés en deux groupes de traitement de 60 dindonneaux chacun, avec 3 répétitions de 20 oiseaux par répétition. Ces volailles ont été gérées de manière intensive sur des sols recouverts de litière, pendant 8 semaines. Les données sur les caractéristiques de performance ont été recueillies, et à la fin de l'expérience, deux oiseaux de poids

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moyen sélectionnés dans chaque répétition ont été privés de nourriture toute une nuit afin qu'ils puissent vider les intestins, et être sacrifiés pour évaluation du rendement en carcasse. La morphologie des villosités duodénales d'une partie de l'intestin grêle a également été déterminée. Les données ont été soumises au test-t de Student à un niveau de signification de 5%. La boulette a montré un indice de consommation (3,45) nettement (P <0,05) plus élevé que la purée (4,23). Cependant, un coût d'ingestion alimentaire significativement (P <0,05) plus élevé par dindonneau par jour (N 13,96) a été observé chez les oiseaux sur boulettes par rapport aux dindonneaux sur purée. De plus, le pilon a été significativement (P <0,05) influencé par les formes d'aliments, les dindonneaux nourris à la purée enregistrant une plus grande valeur. Les résultats ont montré que les dindonneaux sur purée présentaient une hauteur de villosité duodénale (P <0,05) plus élevée (1,00µm) par rapport aux oiseaux recevant des boulettes (0,60µm). Ceci implique que le régime de purée a amélioré le développement morphologique des villosités duodénales des dindonneaux adaptés aux conditions locales par rapport à ceux soumis au régime de purée. Ainsi, il a été conclu que pour une meilleure utilisation des nutriments, l'alimentation sous forme de boulettes devrait être adoptée pour la production de dindes adaptées aux conditions locales, mais son utilisation est susceptible d'être limitée par un coût plus élevé par rapport au régime de purée. Cependant, elle est susceptible de produire, à terme, un rendement suffisamment meilleur que celui qui pourrait être obtenu à la suite de l'alimentation sous forme de purée.

Mots-clés : purée, boulette, performance de croissance, rendement en carcasse, villosité, duodénum, rapport coût /avantage

Introduction

An increase in poultry production which is only realistic by reducing the cost of production and also improving the yield of poultry useful products will help to correct the situation attributable to the ever-increasing high feeding costs and thereby make protein readily available to the teeming population.

Poultry feeds can be subjected to different processing techniques such as mash, pellets and crumbles in order to improve on the productivity of the birds. Many feed ingredients, especially cereals are ground before they are incorporated into poultry diets. Reduction of feed particle size increases both the number of particles and the surface area per unit volume thereby allowing greater access to digestive enzymes (Goodband *et al.,* 2002).

The physical forms of feed have different effects on the overall performance and intestinal morphology of the birds (Aderibigbe *et al.,* 2013). Mash is a form of a complete feed that is finely ground and mixed so that birds cannot easily separate out ingredients; each mouthful provides a well-balanced diet. Mash diet gives greater unification of growth, less losses due to mortality and quite economical. However, ground feed is not so palatable and does not retain its nutritive value as much as un-ground feed (Jahan *et al.,* 2006). A modification of the mash form of feed 'pellet' is thereby expedient. Pelleting consists of mechanically pressing the mash into hard dry pellets or "artificial grain". Pellet is a form of complete feed that is compacted and is extruded to about 1/8 inch in diameter and ¼ inch in length (Jahan *et al.,* 2006). The major advantage of pellet feed is that it prevents wastage of feed and the disadvantage is that pellets are expensive. The beneficial effects of pelleted feed on digestibility of nutrients may arise from their influence on intestinal morphology, but published data on this aspect are so scanty. The available research (Amerah *et al.,* 2007; Aderibigbe *et al.,* 2013) related to this aspect, just focused on broiler chicken production.

Villi and crypts are the functional units of the small intestine assuming the role of digestion and absorption of nutrients. The gastrointestinal tract can adapt and react morphologically to external factors, such as changes in diet. An increase in the mucosal surface area for example, could result in an improved capacity to absorb available nutrients. While performance parameters may be the most common way of evaluating feeding trials, they can be evaluated through histological examination of the gastrointestinal tract, as it is well known that many substances

can affect the development of intestinal villi. The gastrointestinal tract can adapt and react morphologically to factors such as dietary changes, for example to the addition of probiotic and prebiotics in the diet and investigation to determine changes reveal useful information on intestinal function (Michela-Mohnl, 2011).

Turkey, guinea fowl and other avian species account for a lower percentage of the world total poultry production. A rise in turkey (Meleagris gallopavo) production particularly with the use of locally-adapted breed will also increase food production and availability to the teeming population. Turkey meat is an excellent protein source and has a good-price quality ratio (Roberson *et al.,* 2003). Therefore, ensuring optimal intestinal development and the functional capacity of poults should never be overlooked. The experiment thereby investigated the growth performance, carcass yield and duodenal villus morphology of locally-adapted turkeys fed mash and pelletized diets.

Materials and Methods

Experimental Site

The experiment was carried out at the poultry unit of the Directorate of University Farms and the Animal Products and Processing Laboratory of the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site falls within latitude 7o15'N and longitude 3°21'E and has a tropical humid climate with mean annual rainfall of 1037mm and 34.7oC temperature. Relative humidity averages 85% throughout the year.

Experimental Turkeys and Management

A total of 120 day-old locally-adapted poults were purchased from a reputable hatchery and used for the experiment. Brooding operation commenced and lasted for a period of three weeks. The birds were intensively managed (deep litter system) by use of the dietary feed forms of the same nutrient composition from the end of the 3rd week to the end of the 11th week of the experiment.

Table 1: Percentage composition of the experimental diets

Ingredient (%)	Starter diet	Grower diet
Maize	48.00	40.00
Soybean meal	35.00	20.00
Fish meal (72 % CP)	10.00	1.00
Wheat Offal	-	31.50
Palm kernel cake	-	2.50
Limestone	2.00	1.50
Bone meal	4.00	2.50
Lysine	0.20	0.25
Methionine	0.20	0.25
Common salt (NaCl)	0.30	0.25
*Vitamin/Min. Premix	0.30	0.25
Total	**100.00**	**100.00**
Calculated diet analysis		
Crude protein (%)	27.00	18.51
Ether extract (%)	3.91	4.18
Crude Fibre (%)	4.21	5.82
ME (MJ/kg)	12.63	11.82

**Turkey chick premix composition per kilogramme diet: vit.A 40,000IU; vit. D3 4000IU; vit. E 40.0 mg;vit. K3 8mg; vit. B11 0mg; vit. B2 8mg; vit. B6 5 mg; vit. B12 0.025 mg; niacin 60 mg; pantothenic acid 20 mg; folic acid 2000 mg; biotin 150 mg; iron 32 mg; Manganese 64 mg; Zinc 40 mg; Copper 8 mg; Cobalt 80 mg; Iodine 0.15 mg; Selenium 0.2 mg; Choline 300 mg*

Experimental procedures

After the third week of brooding, the poults were randomly allocated to the experimental diets (pellet and mash). Each treatment consisted of 60 poults which was subdivided into three replicates of 20 poults per replicate. The birds were allocated to pens (1.2m × 1.2m) at random on the basis of their weights balanced to reduce variation in the total weight of poults per replicate. The poults were managed intensively for eight weeks (3rd – 11thweek) on feed forms; mash and pellets (2mm) of the same nutritional content as shown in Table 1. Feeds and water were provided ad libitum on daily basis.

Experimental diet mix

The mash diet was produced by thorough milling and mixing of feed ingredients

while the pelletized diet was produced by mixing the milled ingredients with water as binding agent. Thereafter, it was pelletized by the aid of a pelletizing machine with die producing 2mm sized pelletized feed. The pellets were then air-dried.

Performance parameters

Feed intake

This was taken weekly and recorded for each replicate. Feed left over was subtracted from the amount of feed offered to the birds weekly to determine the feed intake. Average feed consumed by a bird was noted by calculating the average feed consumed by the relation

Feed intake = total feed offered – total feed left over

Average feed intake (g/bird) = feed intake/number of birds

Body weight gain

This was also recorded for each replicate. Average weight gain per bird was noted by deducing the difference between the final body weight and initial body weight and dividing this value by the number of birds per replicate.

Average body weight gain (g/bird) = {final weight (g) – initial weight (g)}/number of birds

Feed conversion ratio (FCR)

This was calculated by finding the ratio of the feed intake to the body weight gain.

FCR = total feed consumed (kg)/Weight gain (kg)

Mortality (%)

This was calculated on replicate basis as the ratio of the number of dead poults in each replicate to the total number of poults stocked in a replicate expressed as a percentage.

*Percentage mortality = (number of dead poults in a replicate/number of poults stocked)*100*

Carcass yield parameters and duodenal morphology

At the 56thday of the experiment, two birds of average weight for each replicate were selected and starved over night to clear the guts. Thereafter, they were slaughtered via neck slit and eviscerated for carcass evaluation. The dressed weight was determined and expressed as a percentage of the live weight. Cut-up parts (head, wings, drumstick, shank, thigh, neck, breast and back muscles) were also weighed. Internal organs such as liver, kidney, gizzard and heart were weighed. The weights of these parts were expressed as percentages of the live weight. The entire intestine was removed and placed immediately into a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1M cacodylate buffer (pH 7.4). The duodenum was cut and prepared as slide for light electron microscopy as enunciated by Shamoto and Yamauchi (2000).

Cost benefits analysis

The prevailing market costs at the time of the study (N338.95 = €1) were used to calculate the cost of feed intake per gram (N) and cost of feed consumed per bird in a day (N).

*The daily cost of feed intake per bird (N) = daily feed intake per bird*cost per gram of feed*

Statistical Analysis

Data collected were subjected to studentized t- test at 5% level of significance. Significantly different means were separated using Duncan's Multiple Range Test as contained in SAS (2000).

Results and Discussions

The effects of feed form on the growth performance of locally-adapted turkeys are shown in Table 2. Significant ($P < 0.05$) differences were obtained only in the feed conversion ratio, the cost of feed (N) and the cost of feed intake per bird (N). A better (3.45) feed conversion ratio was obtained in poults fed pellets compared to that obtained in poults fed mash diet (4.23). This could be

Table 2: Performance of local turkeys fed feeds of different forms (pellet and mash)

	Feed form	
Parameter	**Pellet**	**Mash**
Initial weight (g/bird)	448.41±11.03	455.55±14.70
Final weight (g/bird)	2025.40±42.38	1917.22±37.89
Weight gain (g/bird/day)	28.16±0.62	26.10±0.66
Feed intake (g/bird/day)	97.30±3.10	110.54±5.56
Feed Conversion Ratio	3.45±0.06[b]	4.23±0.20[a]
Mortality (%)	0.00±0.00	5.56±5.56
Cost of feed/kg (N)	126.35±0.00[a]	85.34±0.00[b]
Cost of feed intake/bird/day (N)	13.96±0.71[a]	8.30±0.27[b]

[a,b] : Means in the same row with different superscripts differ significantly (P<0.05)
€1 = N338.95

Table 3: Carcass characteristics of local turkeys fed feeds of different forms (pellet and mash)

	Feed form	
Parameter	**Pellet**	**Mash**
Live weight (g)	2006.67±120.19	1816.67±120.19
Dressing percentage	79.79±0.44	79.09±2.10
Cut-up parts[1]		
Head	2.88±0.12	3.05±0.09
Shanks	3.70±0.33	3.72±0.28
Thighs	7.43±1.64	9.34±0.28
Wings	7.97±1.38	10.07±0.31
Drumsticks	9.45±0.09[b]	10.04±0.15[a]
Neck	4.87±0.12	4.98±0.25
Breast	15.83±0.50	15.61±0.52
Back	8.40±1.56	9.59±0.22
Organs[2]		
Heart	0.39±0.03	0.40±0.05
Kidney	1.16±0.64	0.43±0.06
Liver	1.51±0.51	2.38±0.22
Gizzard	2.79±0.08	2.93±0.13
Crop	1.15±0.04	0.87±0.10
Spleen	0.10±0.03	0.11±0.02
Lungs	0.43±0.04	0.53±0.08
Proventriculus	0.42±0.05	0.39±0.02

[a,b] : Means in the same row with different superscripts differ significantly (P<0.05).
[1, 2]:Values expressed as percentage of the live weight.

due to greater reduction in nutrient separation and a reduced feed wastage in pelletized diet. This result is corroborated by the findings of Zakeri et al. (2013) who reported a better feed efficiency in birds fed pelletized diets.

The feed intake of the birds on mash and pellet diets showed no significant (P > 0.05) difference with mean values of 97.30g/bird/day and 110.54g/bird/day for pellet and mash diets respectively. This result disagrees with Jahan et al. (2006) who reported that pelleted diets gave greater feed intake than mash diet. However, birds fed mash diet had numerically increased feed intake compared with birds fed pellets. This agreed with Parsons et al. (2006) who opined that the increased feed intake was due to excessive feed wastage.

The similarity in the percentage mortality recorded in the study is in line with the earlier findings by Sogunle et al. (2013) that the physical forms of feed had little or no effect on percentage mortality of birds.

A higher value of *N*126.35 was obtained for pellet feed form while a lower value of *N*85.34 was obtained by feeding mash feed to the poults. The difference in the cost is due to the extra cost of processing pelleted diets. This is in line with the findings of Jean and Trevidy (2000) who reported that, pellet is about 10% more expensive than mash diet.

Table 4: Intestinal villus morphology of local turkeys fed feeds of different forms (pellet and mash)

	Feed form	
Parameter (µm)	**Pellet**	**Mash**
Villus height	0.60±0.06[b]	1.00±0.12[a]
Lamina propia depth	76.67±12.02	100.00±25.00
Apical width	0.90±0.21	0.93±0.07
Basal width	3.77±0.83	4.07±1.84

[a,b] *: Means in the same row with different superscripts differ significantly (P < 0.05)*

The effects of feed forms on the carcass yield of the locally-adpted turkeys are presented in Table 3. Statistical similarities (P > 0.05) were obtained in all the carcass characteristics considered, except the drumstick, where a significant (P < 0.05) higher drumstick was found in birds on mash feed form. The non-significant (P > 0.05) difference obtained in other parameters were in disagreement with the report of Shafiee et al. (2006) who found that weight of breast, thigh, abdominal fat, heart was significantly heavier in broiler chickens fed pellet diets than in those fed mash diet.

In Table 4, the results on duodenal villus morphology of locally-adapted turkeys fed the two forms (pellet and mash) are shown. The processing of feed as reported by Kemp and Kenny, (2003) can affect the ileum and ceacum microflora, growth and feed efficiency. Significant (P<0.05) difference was obtained only in the villus height of the birds fed the two feed forms. The villus height was higher (1.00µm) in birds fed mash diets than 0.60µm obtained in birds on pelletized diet which can be due to coarse nature of pellet feed which may not enhance the increase in length of the villus height of the birds. This result contradicted the works of Cera et al. (1988) who reported that pelletized diet caused extension of the villus, enlargement of total luminal villus absorptive area and subsequently resulted in adequate digestive enzymes action and higher transport of nutrients at the villus surface.

Conclusion

It could be concluded that:

- The physical form of diet enhanced the growth performance in terms of the feed conversion ratio with reduced feed wastage in locally-adapted turkeys on pelletized diets. However, a better drumstick was obtained in the carcass yield of turkeys fed on mash diet.

- Mash form of diet enhanced the development of duodenal villus morphology relative to pellet diet in locally-adapted turkeys.
- A better cost-benefit was obtained with the use of mash diet relative to pelletized feed

Impact

The study demonstrated that pelletized feed could be adopted for the production of locally-adapted turkey but its use could be limited by higher cost compared to mash diet. However, it may in the long run yield sufficiently better gains than obtainable in the mash feed form.

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EFFECTS OF ANTI-TICK VACCINES, RECOMBINANT SERINE PROTEASE INHIBITORS (RAS-1-2) AND RIM 36 ANTIGENS AGAINST *RHIPICEPHALUS* APPENDICULATUS TICKS' FEEDING ON ZEBU CATTLE IN UGANDA

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Abstract

A preliminary trial of a cocktail of recombinant RAS-1-2 and RIM 36 antigens was conducted in Uganda to assess the effects of ant-tick vaccines against *Rhipicephalus* appendiculatus tick feeding on Zebu cattle under both experimental and natural conditions. Under experimental conditions, over a period of 28 days, the vaccinated group (n = 5) registered a mean female tick engorgement weight of 147.7 ± 41.8, mean tick egg weight of 90.5 ± 37.9, mean egg hatchability rate of 87.0 ± 12.1 and mean days ticks took to drop off of 6.4 ± 0.4 as opposed to the unvaccinated group (n =5) that had a mean female tick engorgement weight of 175.4 ± 63.0, mean tick egg weight of 99.7 ± 39.4, mean egg hatchability rate of 100 ± 9.6 and mean days ticks took to drop off of 6.4 ± 0.4. Upon exposure to natural tick challenge over a period of 61 days, there was no difference in total tick counts between the vaccinated group (mean tick count= 2-9) and non-vaccinated group (Mean tick count = 2-9) for the first 38 days. However, thereafter over a period of 23 days, tick counts for the vaccinated group (mean tick count = 3) were consistently much lower than those of the non-vaccinated group (mean tick count = 6) on exposure to natural tick challenge. In conclusion, the study revealed that a cocktail of recombinant RAS-1-2 and RIM 36 antigens had effect against tick feeding on Zebu cattle under both experimental and natural conditions with an efficacy rate of 65 % against R. appendiculatus under experimental conditions.

Key words: Anti-tick vaccines; RAS-1-2; RIM 36; *R. appendiculatus*; Zebu cattle; Uganda

EFFETS DES VACCINS ANTI-TIQUES, DES INHIBITEURS RECOMBINANTS DE LA SERINE PROTEASE (RAS-1-2) ET DES ANTIGENES RIM 36 CONTRE LES TIQUES RHIPICEPHALUS APPENDICULATUS SE NOURRISSANT DE SANG DE BOVINS ZEBUS EN OUGANDA

Résumé

Un essai préliminaire d'un cocktail d'antigènes recombinants RAS-1-2 et RIM 36 a été mené en Ouganda dans le but d'évaluer les effets des vaccins anti-tiques contre les tiques Rhipicephalus appendiculatus se nourrissant de sang de bovins zébus, à la fois dans des conditions expérimentales et naturelles. Dans les conditions expérimentales, sur une période de 28 jours, le groupe vacciné (n = 5) a enregistré un poids moyen d'engorgement de tiques femelles de 147,7 ± 41,8, un poids moyen d'œufs de tiques de 90,5 ± 37,9, un taux moyen d'éclosion d'œufs de 87,0 ± 12,1 et une moyenne de jours que les tiques restaient sur les zébus avant de tomber d'elles-mêmes de 6,4 ± 0,4. Par contre le groupe non vacciné (N = 5) a montré un taux moyen d'engorgement de femelles de 175,4 ± 63,0, un poids moyen d'œufs de tiques de 99,7 ± 39,4, un taux moyen d'éclosion d'œufs de 100 ± 9,6, et un nombre moyen de jours pris par les tiques pour tomber d'elles-mêmes de 6,4 ± 0,4. Après une exposition à des sources naturelles de tiques sur une période de 61 jours, aucune différence n'a été notée au niveau du nombre total de tiques entre le groupe vacciné (nombre moyen de tiques = 2-9) et le groupe non vacciné (nombre moyen de tiques = 2-9) pendant les premiers 38 jours. Cependant, sur une période de 23 jours, le nombre de tiques du groupe vacciné (nombre moyen de tiques = 3) était constamment beaucoup plus bas que celui du groupe non vacciné (nombre moyen de tiques = 6) exposé aux sources naturelles des tiques.

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En conclusion, l'étude a révélé qu'un cocktail d'antigènes recombinants RAS-1-2 et RIM 36 avait un effet contre les tiques se nourrissant de sang de bovins zébus dans des conditions expérimentales et naturelles, avec un taux d'efficacité de 65% contre R. appendiculatus dans des conditions expérimentales.

Mots-clés : vaccins anti-tiques ; RAS-1-2 ; RIM 36 ; R. appendiculatus ; bovins zébus ; Ouganda

Introduction

Over 90% of the cattle population in Uganda are at constant risk of tick-borne diseases and the overall loss of the calf crop in indigenous cattle due to tick-borne diseases is estimated to be 30% (Anon., 1997). The cumulative mortality of 13.5% due to East Coast fever in indigenous calves up to 1 year old has been reported in ranch cattle in Uganda (Magona et al, 2011). Of the deaths attributable to tick-borne diseases, East Coast fever is responsible for 79%, anaplasmosis 11%, cowdriosis 5.6% and babesiosis 4.4% (Anon., 1992). The annual cost of imported acaricides in Uganda is estimated at US$ 10 million (Okello-Onen *et al.,* 1998).

Conventionally, control of tickborne diseases is by means of tick control using dipping or spraying animals with chemical acaricides. The cost of acaricides is prohibitive to most farmers, especially those keeping a few local cattle and those with smallholder dairy farms. Studies show that smallholder dairy farmers are constantly worried about the introduction of ticks on their property, especially by the untreated indigenous cattle and wild animals. As a result they pay too much attention to ticks and tick-borne disease control, which is estimated, at over 60% of the animal health budget (Okello-Onen et al, 1998). Reports show that the use of synthetic acaricides has become un-sustainable due to high costs, development of acaricide resistance by ticks, environmental pollution and contamination of meat and milk with toxic residues (Okello-Onen et al, 1998).

Prohibitive cost and emergence of resistance against conventional insecticides justifies the search for alternative tick control options. Other approaches proposed for tick control include use of hosts with natural resistance to ticks, pheromone-impregnated decoys for attracting and killing ticks, biological control agents and vaccine (Graf *et al.,* 2004; Willadsen, P., 2006). Although these control methods proved effective, most of them have been discontinued because of falling short of practical tick control (Imamura *et al.,* 2008). On the contrary, the anti-tick vaccines have the advantages of being specific to target species and being environmentally safe. In addition, they have no human health risk, no residues in food products, and are easy to administer and are affordable (Wikel, S. K., 1988). The immunological protection strategy is based on the fact that the host can naturally develop resistance against repeated tick infestation. Acquired immunity to tick infestation is expressed as an increase in feeding duration, reduced engorgement weight, inhibition of molting, high tick mortality and impaired reproduction and viability of ova (Willadsen, P. 1997; Willadsen, P. and Jongejan, F. 1999).

Commercialization of the first anti-arthropod vaccine in Australia against the cattle tick Boophilus microplus (B. microplus) demonstrated the feasibility of practical control of tick infestations with an immunological strategy (Willadsen et al. 1995). However, the efforts to develop more effective anti-tick vaccines have continued (Labuda *et al.,* 2006). Serine protease inhibitors (serpins) have been identified and characterized as vaccine candidates from hard tick strains (Sugino *et al.,* 2003).). The present study was one of the few trials so far conducted on anti-tick vaccines recombinant serine protease inhibitors (RAS-1-2) and RIM 36 antigens to establish their effects against *R. appendiculatus* feeding on indigenous Zebu cattle under African conditions in Uganda.

Materials and Methods

Purchase of calves

Between 11th and 18th May 2006, 10 calves consisting of 5 males and 5 females were purchased. According to records, five

calves were aged 4 months, 4 calves were aged 3 months and 1 calf was aged 2 months. The calves came from a farm where cattle were being dipped to control ticks. All the calves were housed and fed indoors.

Allocation of calves to groups

All calves were allocated randomly to either experimental or control group. Three males and 2 females eartagged 252 (M), 257 (F), 258 (M), 259 (F) and 260 (M) were allocated to the experimental group. Two males and 3 females eartagged 251(M), 253 (F), 254 (F), 255 (M) and 256 (F) were allocated to the control group. Calves belonging to different groups were kept in separate rooms.

Prior treatment

On 19th May 2006 all calved were dewormed and treated with antibiotics and multivitamins to improve their health status.

Blood sampling

Five (5) ml of blood were taken from each calf before the first vaccination. Two (2) ml were placed in a tube with anticoagulant and frozen, while the remaining 3 ml were used for separation of sera. A thin blood film was made for each sample, stained with Giemsa and examined microscopically for piroplasms.

Commencement of vaccination

The first vaccination was carried out on 24th May 2006. On the day of vaccination, one tube containing the mixture of the antigen (RAS-1-2 + RIM 36) and another containing a control antigen were removed from the deep freezer (-20 °C) and allowed to thaw in a Waterbath. Then they were vortexed to allow uniform mixture. For the experimental group, 6.5 ml of antigen mixture was withdrawn and administered through deep intramuscular injection into the neck muscles of each calf in the experimental group. The vaccine was delivered to two different sites on either side of the neck; one sites receiving 3.0 ml and another 3.5ml. For the control group, 6.5 ml of control antigen was withdrawn and administered by IM into the neck muscles at two different sites in a similar manner to calves in the experimental group.

Collection of tick

Engorged nymphs and adult ticks were collected from the field and used to challenge both the experimental and control calves.

Post-experiment tick count

After 28 days, all calves were released to pastures in the paddocks and exposed to natural tick challenge.

Results

The results regarding the effects of the anti-tick vaccine on tick feeding are shown in Table 1. Under experimental conditions, over a period of 28 days, the vaccinated group (n

Dated experimental schedule

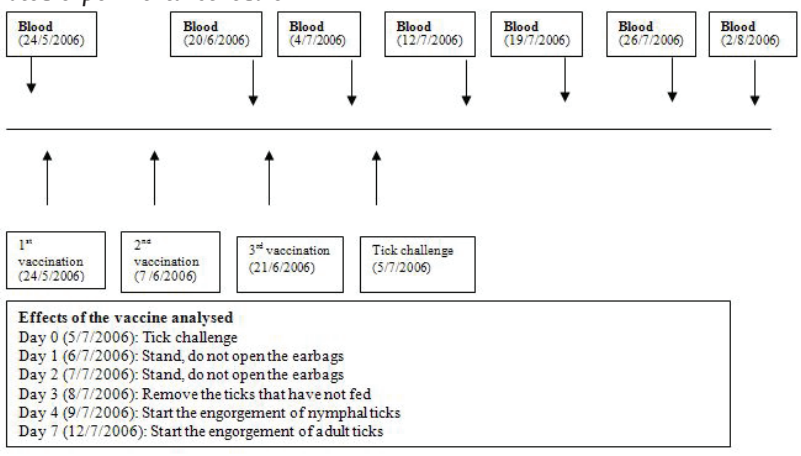

Table 1: Mean values of *Rhipicephalus* spp ticks on vaccinated and control Zebu calves

Tick parameter	Vaccinated calves	Unvaccinated calves	% reduction
Mean engorgement weight (mg) ± 95% CI	147.7 ± 41.8	175.4 ± 63.0	15%
Mean days taken to drop ± 95% CI	6.4 ± 0.4	6.4 ± 0.3	0%
Mean egg wt (mg) ± 95% CI	90.5 ± 37.9	99.7 ± 39.4	9%
Mean egg percentage hatchability ± 95% CI	87.0 ± 12.1	100 ± 9.6	13%

*CRW = WTV/WTC, CRO = PATV/PATC, CRF = PPLOV/PPLOC; Vaccine Efficacy = 100(1-(CRW*CRO*CRF) = 65%*

= 5) registered a higher mean female tick engorgement weight, higher mean tick egg weight and higher mean egg hatchability rate than the unvaccinated group. However, there was no difference between the vaccinated and non-vaccinated groups regarding the number of days ticks took to drop off.

Comparison between the vaccinated group and unvaccinated group regarding tick infestation under exposure to natural tick challenge is shown in Figure 1. During a period of 61 days, there was no difference in total tick counts between the vaccinated group (mean tick count= 2-9) and non-vaccinated group (Mean tick count = 2-9) for the first 38 days. However, thereafter over a period of 23 days, tick counts for the vaccinated group (mean tick count = 3) were consistently much lower than those of the non-vaccinated group (mean tick count = 6).

Discussion

In the present study, a preliminary trial was conducted to assess the effect of anti-tick vaccines composed of a cocktail of recombinant serine protease inhibitors (RAS-1-2) and RIM 36 antigens against *R. appendiculatus* tick feeding on indigenous Zebu cattle under African conditions in Uganda.

Tick serpins are known to play an important role in the homeostasis of organisms by targeting the enzyme active site or the enzyme active loop in addition to regulating serine proteases (Bode and Huber 1992). Some of the tick serpins are known to play regulatory roles in tick-host interactions (Prevot et al 2007). Moreover, tick serpins are considered to be potential candidates as vaccinal immunogens (Mulenga et al, 2001). RIM36 is an immuno-dominant protein, a component of the cement cone considered to be highly immunogenic (Trimnell et al, 2005). RIM36 (*Rhipicephalus* immunodominant molecule 36) was identified by Bishop et al, 2002 in e-cell granules of the type III salivary gland acini of *Rhipicephalus* appendiculatus. RIM36 could be used as a vaccine candidate in the control of *Rhipicephalus* appendiculatus tick population in cattle.

For over a period of 28 days under in-door experimental conditions, the vaccinated group had higher mean female tick engorgement weight, higher mean tick egg weight and higher mean egg hatchability rate than the unvaccinated group, suggesting that the anti-tick vaccines reduced the tick engorgement weight, egg weight and egg percentage hatchability. Unfortunately, data on tick attachment rate, period to attachment and tick mortality was not available for analysis. Similar studies were conducted using a cocktail including recombinant proteins of RAS-3 (rRAS-3), RAS-4 (rRAS-4) and RIM36 (rRIM36) against *R. appendiculatus* infestation on Friesian cattle in Zambia (Imamura *et al.,* 2008). Tick infestation challenge demonstrated protective immunity against female ticks, resulting in mortality rates of 39.5 and 12.8 % for the vaccinated and control groups, respectively (Imamura *et al.,* 2008). Other previous studies on a cocktail vaccine combining two serpins (rRAS-1 and rRAS-2) from *R. appendiculatus* demonstrated a significant reduction in the number of engorged nymphal ticks and a higher mortality rate of adult ticks that fed on

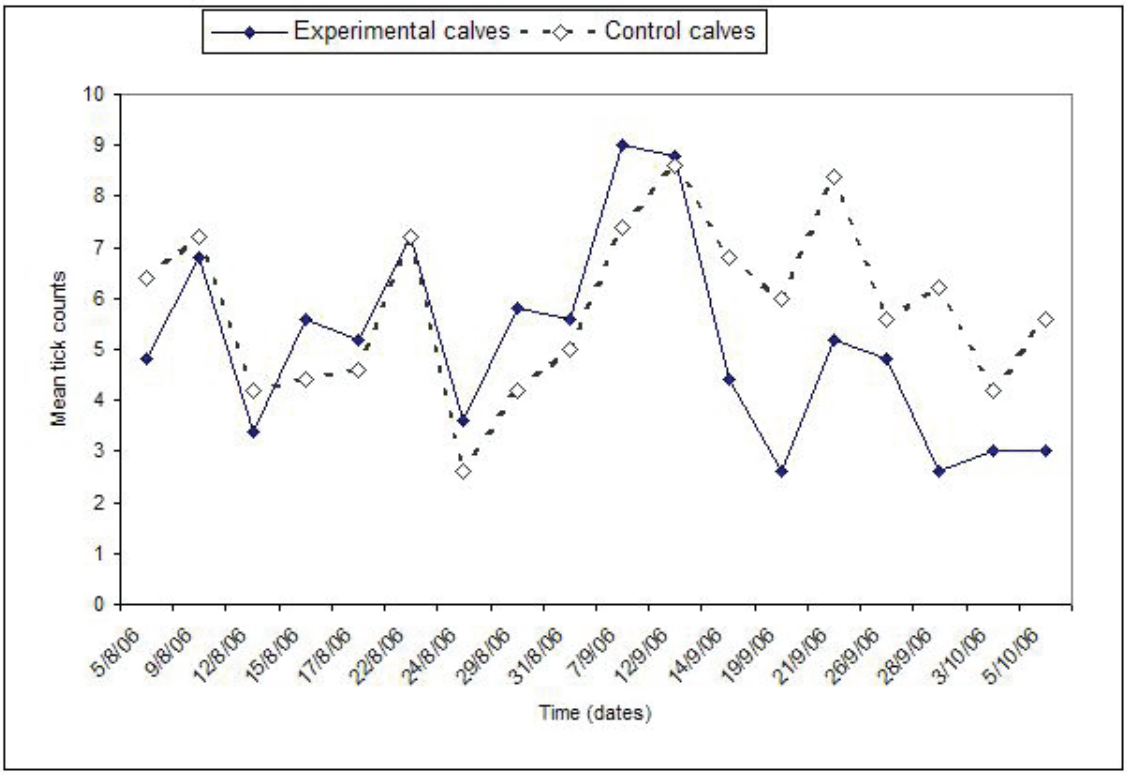

Figure 1: Mean Rhicephalus spp tick counts on calves exposed to natural tick challenge after release into paddocks

immunized cattle as compared those of ticks that fed on the control group (Imamura et al. 2006)).

Interestingly, upon release of calves from indoors on completion of experiment and exposing them to natural tick challenge, initially there was no difference in tick counts on the vaccinated group for first 38 days. However, thereafter, over a period of 23 days, tick counts on the vaccinated group was consistently lower than those of the non-vaccinated group. Exposure of vaccinated calves to natural tick challenge seemed to have had a boosting effect on the immunity leading to vaccinated group having lower tick counts than those of the non-vaccinated group. Other studies have equally demonstrated that a cocktail involving recombinant proteins of RAS-3 (rRAS-3), RAS-4 (rRAS-4) and RIM36 (rRIM36) does induce a partially protective effect against *R. appendiculatus* (Imamura *et al.,* 2008). In conclusion, the study revealed that a cocktail of recombinant RAS-1-2 and RIM 36 antigens had effect against tick feeding on Zebu cattle under both experimental and natural conditions with an efficacy rate of 65 % against *R. appendiculatus* under experimental conditions.

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INDIRECT EFFECT OF MORINGA OLEIFERA SUPPLEMENTED DIET ON GROWTH RATES OF PRE-WEANING BOER GOAT KIDS

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Abstract

The objective of this study was to evaluate the indirect effects of feeding *Moringa oleifera* (M. oleifera) supplemented diet on growth rates in pre-weaning kids. Namibia being a semi-arid and driest country in Africa south of the Sahel, lactating does are challenged with acquiring the required amount of forage in the rangelands to meet milk production and nutritional needs for their kids. This scarcity of forage along with the low nutritional quality of the available grasses and/or browses creates the need for supplementing lactating does with nutritionally-rich fodders. A completely randomized block design (CRBD) was used with four inclusion levels of M. oleifera supplemented diets and four replicates of does in each level to determine if the growth parameters differ with levels of M. oleifera. A total of 16 lactating does were used for this study with 20 kids since four does had twins. The present study revealed that there were significant differences ($P < 0.05$) in heart girth, body length and weight of kids which were measured as growth rate parameters along with body condition scores (BCS). Although Boer goats are known for their fast growth under favorable conditions, feed supplementation of pregnant and lactating does could be advantageous for maximum milk production to support their kids' healthy early growth and development especially under unfavorable conditions such as during winter and drought. Therefore, in a semi-arid drought persistent country like Namibia, M. oleifera would bring a possible solution for animal supplementation during drought and winter periods since M. oleifera grows very fast and produces more biomass per hectare; thus, alleviating farmer's stress of purchasing feed-supplement during pregnancy and lactation period.

Key words: Growth rates, kids, lactating does, *Moringa oleifera*, supplemented diet

EFFET INDIRECT DU REGIME SUPPLEMENTE EN *MORINGA OLEIFERA* SUR LE TAUX DE CROISSANCE DES CHEVREAUX BOER AVANT LE SEVRAGE

Résumé

L'objectif de cette étude était d'évaluer les effets indirects du régime supplémenté en *Moringa oleifera* (*M. oleifera*) sur les taux de croissance des chevreaux avant le sevrage. La Namibie étant un pays semi-aride et le plus sec d'Afrique subsaharienne, les animaux en lactation souffrent du problème d'insuffisance des quantités de fourrage nécessaires dans les parcours pour satisfaire aux besoins de production laitière et aux besoins nutritionnels de leurs chevreaux. Cette pénurie de fourrage ainsi que la faible qualité nutritionnelle de l'herbe et / ou feuilles engendrent une nécessité de compléter le lait par des fourrages riches en nutriments. L'étude a utilisé un modèle de blocs entièrement aléatoire (CRBD) avec quatre niveaux d'inclusion de régimes alimentaires supplémentés en *M. oleifera* et quatre répétitions pour chaque niveau, en vue de déterminer si les paramètres de croissance diffèrent selon les niveaux d'inclusion de *M. oleifera*. Au total, 16 chèvres en lactation ont été utilisées pour cette étude (avec 20 chevreaux puisque quatre chèvres avaient des jumeaux). L'étude a révélé des différences significatives (P

<0,05) concernant le tour de poitrine, la longueur du corps et le poids des chevreaux, qui ont été mesurés en tant que paramètres de croissance, ainsi que les notes d'état corporel (BCS). Bien que les chèvres Boer soient connues pour leur croissance rapide dans des conditions favorables, la supplémentation en aliments pour les chèvres gestantes et allaitantes pourrait être avantageuse pour une production laitière maximale à même de soutenir la croissance et le développement sain de leurs chevreaux, en particulier dans des conditions défavorables comme l'hiver et la sécheresse. Par conséquent, dans une région semi-aride persistante comme la Namibie, *M. oleifera* apporterait une solution possible pour la supplémentation de l'alimentation animale pendant les périodes de sécheresse et d'hiver puisque M. oleifera croît très vite et produit plus de biomasse par hectare, atténuant ainsi les problèmes rencontrés par les agriculteurs dans la recherche de suppléments alimentaires pour les chèvres gestantes et allaitantes.

Mots-clés : taux de croissance, chevreaux, chèvres allaitantes, *Moringa oleifera*, régime supplémenté

Introduction

Goats are very important in areas where feed resources are limited because they can consume a wide variety of plant species and parts and have a great ability to select high quality diets in these circumstances (Huston & Hart, 2002; Mpofu, 2004). Namibia has about 2 million goats nationwide, most of which are found in communal areas and play an important economic role for sustainability of subsistence farming. Officially 200,000 to 250,000 goats are marketed annually, of which 95 percent is exported to South Africa. Boer goat breed is one of the breeds found in Namibia and considers as most resilient small stock breed with a great capacity for adaptation (Boer Goat Breeders' Society of Namibia (BGBSN, 2008)). It is however of paramount importance to avail easily adaptable nutrient-rich plant species for maximum production and growth of goats; one of such plants is *Moringa oleifera* (M. oleifera) tree. M. oleifera leaves are readily eaten by cattle, sheep, goats, pigs and rabbits (United Caribbean, 2013). A feeding trial conducted with West African dwarf goats in Nigeria shows that M. oleifera leaves supplementation resulted in an average weight gain of 20.83 gram/animal/day (Asaolu *et al.,* 2012). A similar feeding trial revealed that supplementation of beef and dairy cows' diet with 40-50 percent of M. oleifera leaves led to an increase in milk yields for dairy cows and daily weight gains for beef cattle by 30 percent. Birth weight increased by 3-5 kg (Price, 2007). M. oleifera is well known for its enormous biomass production and it promises to be the plant of the future in ruminant animal supplementation strategy. Under high density cultivation, it yielded biomass in excess of 15 tonnes dry matter/hectare (DM/ha) in a 60-day growing cycle under the International Trypanotolerance Centre conditions in Banjul (Akinbamijo *et al.,* n.d.).

Research shows that every 100 grams of M. oleifera contain protein, vitamins, minerals, carbohydrates, fats, etc. in the following amounts: 6.7 g of protein in leaves, 2.5 g of protein in pods, 27.1 g of protein in leaf powder, 259 mg of potassium (K) in leaves, 259 mg of K in pods, 1,324 mg of K in leaf powder and 6.8 mg of vitamin A (β-carotene is precursor to Vitamin A) in leaves, 0.11 mg in pods and 16.3 mg in leaf powder (Price, 2007). M. oleifera has proven to be a valuable supplement for animals in other countries (Mendieta *et al.,* 2007); this means that feeding it to goats at the appropriate period of nutritional needs especially during pregnancy for proper foetus development and during lactation for kids development is necessary. M. oleifera leaves have a high potential as a protein source supplement for ruminants and their feeding value is similar to that of the widely used soybean meal and rapeseed meal (Soliva, 2005).

In Namibia, M. oleifera has not found much use as human food or feed for ruminants in comparison to other regions such as Asia and Western Africa (Radovich, 2007; Adegun *et al.,* 2011). In addition, the time of kidding determines the period of highest nutritional demand, as late pregnancy and early lactation are critical times for the does and kids (Coffey, 2006). Hence, the need for nutritional supplementation of does during this critical

period, as well as in winter and drought when rangelands are less productive with less nutritional value. It was hypothesized that M. oleifera supplemented diet has no indirect effect on growth rate parameters of pre-weaning Boer goat kids. Therefore, this study was aimed at evaluating the use of M. oleifera as a nutritional supplement for lactating does to meet their milk production requirement for proper early growth and development of their kids as suggested by Soliva et al. (2005).

Materials and Methods

Study area

This study was conducted at the Neudamm Experiment Farm, one of the campuses of the University of Namibia, about 30 km east of Windhoek with an area of 10, 187 hectares of land. Neudamm Campus is located at 22° 30' 07' latitude South and at 17° 22' 14' longitude East, and at an altitude of 1762 meter above sea level. The farm receives an annual average rainfall of 360 mm which is higher than the national annual average of 270 mm. The temperature ranges between a minimum of -7°C and a maximum 44°C (University of Namibia, 2011). The study was conducted during the period stretching from October, 2015 to January, 2016.

Study design

A completely randomized block design (CRBD) consisting of a one-way treatment structure was used in this trial. The treatment consisted of M. oleifera supplement at 4 inclusion levels (0 percent, 10 percent, 20 percent, and 30 percent) in isocaloric and isonitrogenous diets. Taking into consideration an average weight of 36 kg per goat, the percentages of inclusion translated into 0 percent (0 g), 10 percent (150 g), 20 percent (300 g) and 30 percent (450 g) of M. oleifera dry leaves per day as described by Gebregiorgis, et al. (2011). Alfalfa or Lucerne (Medicago sativa) was used as a basal diet for all goats supplemented with 300 g Ram-Lamb-Ewe pellets. A control group (0 percent M. oleifera) was only fed with lucerne and pellets while three groups were supplemented with M. oleifera leaf at three inclusion levels (10 percent, 20 percent and 30 percent). All goats were fed twice a day (8:00 am and 3:00 pm) for 74 days and clean water was available ad libitum in all cages. Sixteen lactating Boer goats were randomly allocated to each treatment (4 per treatment) to assess the effect of M. oleifera leaf supplemented diet on growth rates of their pre-weaning kids through their mothers' increased milk production. The goats were housed in individual wire mesh cages and introduced to M. oleifera supplemented diet two weeks to one month after birth. Both does and kids were weighed prior to the introduction of M. oleifera supplemented diet. An adjustment period of 14 days as suggested by Sarwatt et al. (2002) was observed to get animals used to the new diet, after which a 60-day trial period commenced. Feed given and refusals were recorded daily for the determination of daily feed intake (Gebregiorgis, *et al.,* 2011; Pulina *et al.,* 2013). Body weights, body length and heart girth of kids were measured weekly throughout the 60-day trial period; however, only body weights and body condition scores of does were taken weekly.

Experimental animals

Lactating goats were purposefully targeted in the current study. Among these, 16 lactating Boer goats from a population of 51 total lactating goats at Neudamm Experimental Farm were randomly sampled for the present feeding trial since most nutritional deficiencies are common among lactating does and their kids (Coffey, 2006). The trial had 4 treatment levels with 4 goats in each treatment as replication. The animals were penned in individual pen with their kids. All the 16 pens had equal size of 430

Table 1: Experimental design of M. oleifera supplementation to lactating does

Treatment, M. oleifera: percent (g)	0 (0)	10 (150)	20 (300)	30 (450)	Total
Replications	4 goats	4 goats	4 goats	4 goats	N = 16 goats

cm x 13 cm square with taps providing water ad libitum and trace mineral salt blocks throughout the research period discussed by Juhnke (2011). Kids were separated from their mothers every day during M. oleifera feeding that lasted for 10-20 minutes and were re-united with their mothers afterward. The separation was meant to test for the indirect effect of M. oleifera on kids' growth. Kids were weighed and measured weekly to assess body weight gain, increase in body length and girth as well as body condition scores (BCS). Boer goats are more sensitive to external parasites than internal parasites (Agra Professional Services, 2013). At the beginning of this research, both does and kids were treated against ectoparasites (Gebregiorgis *et al.,* 2011) using COOPERS SUPADIP (500 mL) at a dilution ratio of 50 mL: 10 L of water. At Neudamm Experimental Farm, Ivermectin (1 mL: 50 kg) and Dectomax (1 mL: 50 kg) are used to treat both ectoparasites and endoparasites, while Ecomectin (1 mL: 10 kg) is used to treat endoparasites.

Data collection

Lactating Boer goats with their kids and M. oleifera leaves were used as instruments for data collection. Collected data included weekly growth rates of kids and M. oleifera leaf supplemented ration as suggested by Abdulrazak, et al (2001); Sanchez, et al. (2005). Also, weekly body condition scores were assessed and recorded as described by Frost et al. (2008) for both does and kids. A weighing scale and tapeline were used to measure weekly growth rates (heart girth, body length and weight) of kids as demonstrated by Olatunji-Akioye and Adeyemo (2009). Lactating goats were fed with M. oleifera leaf supplemented ration twice a day by 8:00 a.m. and 3:00 p.m. as discussed by Yayneshet, et al. (2008) using 4 percent body weight, which considered the wastes during feeding as deliberated by Coffey (2006). Daily rations were divided into two equal parts and given one portion by 8:00 a.m. and the other by 3:00 p.m. as described by Gebregiorgis et al. (2011). During the feeding of M. oleifera leaf supplemented ration, kids were separated from their mothers to avoid them directly consuming it. The separation was done to observe the indirect effect of M. oleifera on the kids. *Moringa oleifera* leaf ration was then given and consumed by the mothers within 10–20 minutes. Upon completion of the M. oleifera ration, kids were re-united with their mothers and given lucerne and pellets together while suckling at the same time.

Data analysis

The linear regression model and general linear model (GLM) were used to analyse data to establish if the different diets significantly affected the kids' growth rates at the 5 percent level of significance (Olatunji-Akioye & Adeyemo, 2009; Gebregiorgis *et al.,* 2011). Kids' body weights, length, heart girth and body condition scores served as dependent variables while M. oleifera supplement served as explanatory (factor) variable. The Statistical Package for Social Sciences (SPSS® version 23) and Microsoft Office Excel® program were used for all data analyses.

Results

The results obtained from this study include growth performance parameters (heart girths, body lengths, and weights as well as body condition scores) of kids used in the study that suckled from does fed M. oleifera supplemented rations compared to a control group taken weekly for the 74 days period. Figures and tables were used to compare growth parameters of kids both descriptively and statistically. The results of statistical analyses reveal significant differences of growth parameters of kids whose mothers were fed with M. oleifera supplemented diet compared to the control (kids not indirectly fed M. oleifera).

Adjustment period

The goats were gradually introduced to M. oleifera supplement 14 days prior to the actual start of the feeding experiment as part of adaptation/adjustment period as suggested by Yayneshet et al. (2008). During this period, it was observed that 15 out of the 16 (93.8

percent) goats liked M. oleifera leaves in less than a week and consumed their entire ration. This confirms the view of Mpofu (2004) who discussed that goats feed on a wide variety of feeds ranging from tree and shrub leaves and grasses; thus, making them hardy to survive under difficult conditions. However, one of the goats could not adjust immediately, but gradually did. The quick adaptation of goats to M. oleifera leaf supplemented diet serves as an advantage of using it as hay, nutritional supplement or anthelmintic drug for goats and other livestock species as reported in a number of studies (Sanchez *et al.,* 2005; Hiawacha Bey, 2010; Jain *et al.,* 2013; Akinbamijo *et al.,* n.d.).

Kids' birth types and sex

Does used in the study had single and twins birth types with kids comprising of male and female sex. Figure 1 shows the birth types and sex of kids. A total of 20 kids were used in this research in which 12 (60 percent) were of single birth, and eight (40 percent) were twins with 11 (55 percent) males and nine (45 percent) were females. Thus, from the 16 lactating does used in the study, 12 had single births while four had twins.

Research goats were divided into four groups according to M. oleifera supplemented diet inclusion levels, which were zero percent (control), 10 percent (150 g), 20 percent (300 g) and 30 percent (450 g). The figures and tables present comparisons of differences in the four groups' growth rate parameters of kids.

Average and statistical differences of kids' girth

The comparison of kids' heart girth growth based on M. oleifera inclusion levels is shown in Figure 2. The result revealed that 10 percent M. oleifera inclusion level had the highest growth in kids' girth (48.55 cm, 49.67 cm, 50.36 cm, 51.54 cm and 52 cm). The second highest in kids' girth growth was 20 percent inclusion level (44.56 cm, 46.45 cm, 50.82 cm, 51.27 cm and 51.45 cm), which was followed by 30 percent inclusion level (44.27 cm, 44.82 cm, 49.64 cm, 50.36 cm and 51.36 cm); and the least was the control (0 percent) inclusion level (42.64 cm, 46.55 cm, 49.90 cm, 49.36 cm and 49 cm).

Table 2 presents the linear regression

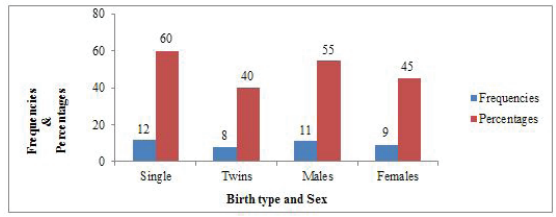

Figure 1: Birth type and sex of kids

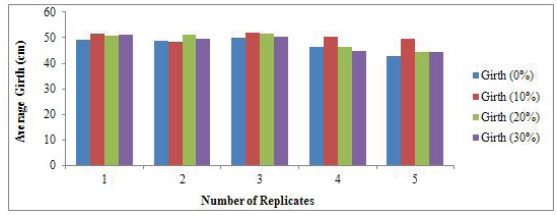

Figure 2: Comparison of average kids' girth at different M. oleifera inclusion levels

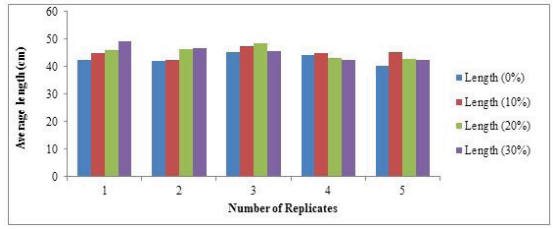

Figure 3: Comparison of average kids' length at different M. oleifera inclusion levels

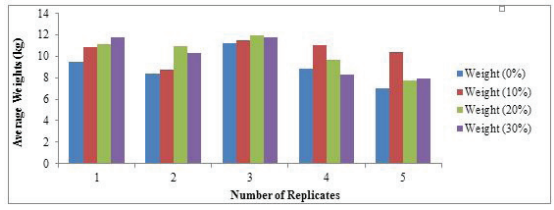

Figure 4: Comparison of average kids' weight at different M. oleifera inclusion levels

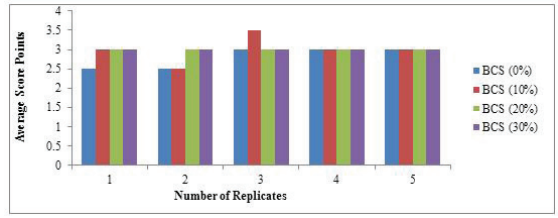

Figure 5: Comparison of kids' average BCS at different M. oleifera inclusion levels

Table 2: Regression analysis of kids' Girth (cm) over time and M. oleifera levels

	Unstandardized Coefficients		Standardized Coefficients		
Model	**B**	**Std. Error**	**Beta**	**T**	**Sig.**
(Constant)	40.527	0.828		48.930	0.000
Dum level 10 percent	2.091	0.626	0.165	3.339	0.001
Dum level 20 percent	1.109	0.626	0.087	1.771	0.078
Dum level 30 percent	0.691	0.626	0.054	1.103	0.271
Week1	0.600	1.038	0.031	.578	0.564
Week2	2.050	1.038	0.107	1.974	0.050
Week3	4.050	1.038	0.212	3.901	0.000
Week4	6.000	1.038	0.314	5.779	0.000
Week5	7.700	1.038	0.403	7.416	0.000
Week6	8.550	1.038	0.447	8.235	0.000
Week7	9.250	1.038	0.484	8.909	0.000
Week8	10.700	1.038	0.559	10.305	0.000
Week9	12.000	1.038	0.627	11.557	0.000
Week10	13.700	1.038	0.716	13.195	0.000

Dependent Variable: Kid girth (cm); F-statistics = 31.608; R-square = 0.666; N = 220; & Durbin-Watson Test= 1.028

Table 3: Regression analysis of kids' length (cm) over time and M. oleifera levels (page 10)

	Unstandardized Coefficients		Standardized Coefficients		
Model	**B**	**Std. Error**	**Beta**	**T**	**Sig.**
(Constant)	37.409	0.733		51.060	0.000
Dum level 10 percent	1.218	0.554	0.108	2.200	0.029
Dum level 20 percent	2.145	0.554	0.190	3.874	0.000
Dum level 30 percent	2.200	0.554	0.194	3.972	0.000
Week1	0.400	0.918	0.023	.436	0.664
Week2	0.300	0.918	0.018	.327	0.744
Week3	2.650	0.918	0.155	2.885	0.004
Week4	2.900	0.918	0.170	3.158	0.002
Week5	4.100	0.918	0.240	4.464	0.000
Week6	5.950	0.918	0.349	6.478	0.000
Week7	9.050	0.918	0.531	9.854	0.000
Week8	8.800	0.918	0.516	9.582	0.000
Week9	10.100	0.918	0.592	10.997	0.000
Week10	10.650	0.918	0.625	11.596	0.000

Dependent Variable: Kid girth (cm); F-statistics = 31.608; R-square = 0.666; N = 220; & Durbin-Watson Test= 1.028

Table 4: Regression analysis of kids' weight (kg) over time and M. oleifera levels

	Unstandardized Coefficients		Standardized Coefficients		
Model	**B**	**Std. Error**	**Beta**	**T**	**Sig.**
(Constant)	4.873	0.449		10.847	0.000
Dum level 10 percent	1.127	0.340	0.145	3.320	0.001
Dum level 20 percent	1.295	0.340	0.167	3.812	0.000
Dum level 30 percent	1.007	0.340	0.130	2.966	0.003
Week1	.440	0.563	0.038	.781	0.435
Week2	1.365	0.563	0.117	2.424	0.016
Week3	2.030	0.563	0.174	3.605	0.000
Week4	3.030	0.563	0.260	5.381	0.000
Week5	4.370	0.563	0.374	7.761	0.000
Week6	5.095	0.563	0.437	9.048	0.000
Week7	5.730	0.563	0.491	10.176	0.000
Week8	6.620	0.563	0.567	11.756	0.000
Week9	7.740	0.563	0.663	13.745	0.000
Week10	8.780	0.563	0.752	15.592	0.000

Dependent Variable: Kid girth (cm); F-statistics = 31.608; R-square = 0.666; N = 220; & Durbin-Watson Test= 1.028

Table 5: Regression analysis of kids' body condition scores over time and M. oleifera levels

	Unstandardized Coefficients		Standardized Coefficients		
Model	**B**	**Std. Error**	**Beta**	**T**	**Sig.**
(Constant)	2.473	0.084		29.302	0.000
Dum level 10 percent	0.127	0.064	0.120	1.995	0.047
Dum level 20 percent	0.109	0.064	0.103	1.710	0.089
Dum level 30 percent	0.173	0.064	0.163	2.708	0.007
Week1	0.375	0.106	0.235	3.545	0.000
Week2	0.475	0.106	0.298	4.490	0.000
Week3	-0.425	0.106	-0.266	-4.018	0.000
Week4	0.300	0.106	0.188	2.836	0.005
Week5	0.450	0.106	0.282	4.254	0.000
Week6	0.625	0.106	0.391	5.908	0.000
Week7	0.600	0.106	0.376	5.672	0.000
Week8	0.450	0.106	0.282	4.254	0.000
Week9	0.575	0.106	0.360	5.436	0.000
Week10	0.775	0.106	0.485	7.326	0.000

Dependent Variable: Kid girth (cm); F-statistics = 31.608; R-square = 0.666; N = 220; & Durbin-Watson Test= 1.028

analysis of M. oleifera inclusion levels on the kids' girth growth and time in weeks. The result reveals that M. oleifera levels and weeks had positive effect ($P < 0.05$) on the kids' girth increase at the 10 percent (150 g) and 20 percent (300 g), but this was not the case at the 30 percent (450 g) inclusion level and week one ($P > 0.05$).

Average and statistical differences of kids' length

The mean comparison of kids' length growth based on the four M. oleifera inclusion levels are shown in Figure 3. The analysis shows that 30 percent inclusion level had the highest increase in length (42.45 cm, 42.18 cm, 45.36 cm, 46.64 cm, and 49 cm). Twenty percent had the second highest increase in length (42.55 cm, 42.18 cm, 45.81 cm, 46.18 cm and 48.27 cm), follow by 10 percent inclusion level (42.45 cm, 44.81 cm, 44.81 cm, 45.06 cm and 47.45 cm); and, the control (zero percent inclusion level) had the least increase in length (40.09 cm, 41.91 cm, 42.27 cm, 44.18 cm and 45.09 cm), respectively.

Table 3 presents the linear regression analysis of M. oleifera inclusion levels on the kids' length growth over time in weeks. The result shows that M. oleifera inclusion levels had significant effect ($P < 0.05$) on kids' length growth at all three inclusion levels over time (weeks), although 10 percent was less significant than 20 and 30 percent's. Weeks were also had significant differences ($P <0.05$) on kids' length growth except for weeks one and two that had no differences ($P >0.05$).

Average and statistical differences of kids' weights

The mean comparison of kids weight gain based on M. oleifera inclusion levels is found in Figure 4. The analysis confirms that 20 percent inclusion level had the highest kids' weight gain (7.76 kg, 9.7 kg, 10.92 kg, 11.09 kg and 11.91 kg). The 30 percent inclusion level had the second highest weight gain (7.91 kg, 8.27 kg, 10.27 kg, 11.73 kg and 11.76 kg,), which was followed by 10 percent inclusion level (8.78 kg, 10.38 kg, 10.80 kg, 11.00 kg and 11.47 kg). Finally, the zero percent inclusion level (control) had the least weight gain (6.98 kg, 8.35 kg, 8.84 kg, 9.49 kg and 11.25 kg). The zero percent inclusion level (control) having the least kids' weight gain demonstrates the effect of M. oleifera on growth parameters.

Table 4 presents a linear regression analysis result of kids' weight gain as explained by M. oleifera inclusion levels and time. The result shows that M. oleifera and time had significant differences ($P < 0.05$) on kids' weight gain, except for week one that had no effect statistically.

Average and statistical differences of kids' body condition scores

Figure 5 show kids' average body condition scores (BCS). The analysis of BCS indicates that 10 percent M. oleifera inclusion level led to the highest BCS of 3.5 points but had one that was 2.5 points, while 20 percent and 30 percent had equal BCS of 3 points, and the zero percent (control) had two kids with 2.5 BCS, which means that zero percent had the highest number of kids with 2.5 points.

Table 5 presents a linear regression analysis of the body condition scores (BCS) of kids indirectly fed M. oleifera over time. The result indicates that M. oleifera had significant effects ($P < 0.05$) on the BCS of kids over time in weeks. Thirty percent had more effect on kids' growth compared to 10 and 20 percent.

Average and statistical differences of kids' growth by sex and birth types

Table 6 presents the average growth parameters of kids which reveal that males grew faster and bigger than females in heart girths, body lengths, body weight gains and even had better body condition scores. As per birth types, single males had the highest average growth parameters (heart girth 54.24 cm, body length 48.72 cm and body weight 13.07 kg), followed by twin males in girth (51.99 cm) and weight gains (11.04 cm) but not length (44.31 cm). Conversely, single females had higher length (45.67 cm) and body condition scores (3.18), while twin females had the least growth parameters of all the groups. Individually, the heart girth ranges between 50 cm to 60 cm, length 45 cm to 54 cm and weight 11.2 kg to 20 kg.

Table 6: Average growth parameters of kids by birth types and sex

Birth	Sex	No. of kids	Heart Girth (cm)	Length (cm)	Weight (kg)	Body Condition Scores
Single	Male	6	54.24	48.72	13.07	3.43
Single	Female	6	49.98	45.67	10.84	3.18
Twin	Male	5	51.99	44.31	11.04	2.73
Twin	Female	3	50.74	43.69	9.45	2.47
Single	Aggregate	12	52.11	47.19	11.96	3.90
Twin	Aggregate	8	51.36	44.00	10.25	2.60

Table 7: GLM Pairwise comparisons of kids' growth parameters on sex

Dependent Variable	(I) Sex	(J) Sex	Mean Difference (I-J)	Std. Error	Sig.c	95 percent Confidence Interval for Differencec	
						Lower Bound	Upper Bound
Kid girth	Female	Male	-3.072*	0.385	0.000	-3.836	-2.309
	Male	Female	3.072*	0.385	0.000	2.309	3.836
Kid length	Female	Male	-2.276*	0.372	0.000	-3.012	-1.540
	Male	Female	2.276*	0.372	0.000	1.540	3.012
Kid weight	Female	Male	-2.197*	0.196	0.000	-2.586	-1.809
	Male	Female	2.197*	0.196	0.000	1.809	2.586
Kid BCS	Female	Male	-0.116*	0.041	0.006	-0.197	-0.035
	Male	Female	0.116*	0.041	0.006	0.035	0.197

Based on estimated marginal means
**. The mean difference is significant at 0.05 level.*
a. Weighted Least Squares Regression - Weighted by MOL consumed
c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 8: Pairwise comparisons of M. oleifera effect on kids growth parameters on birth types (page 12)

Dependent Variable	(I) birth type	(J) birth type	Mean Difference (I-J)	Std. Error	Sig.c	95 percent Confidence Interval for Differencec	
						Lower Bound	Upper Bound
Kid girth	Single	Twins	-1.811*	0.385	0.000	-2.575	-1.047
	Twins	Single	1.811*	0.385	0.000	1.047	2.575
Kid length	Single	Twins	-1.483*	0.372	0.000	-2.220	-0.747
	Twins	Single	1.483*	0.372	0.000	0.747	2.220
Kid weight	Single	Twins	-0.520*	0.196	0.009	-0.909	-0.132
	Twins	Single	0.520*	0.196	0.009	0.132	0.909
Kid BCS	Single	Twins	0.113*	0.041	0.007	0.032	0.195
	Twins	Single	-0.113*	0.041	0.007	-0.195	-0.032

Based on estimated marginal means
**. The mean difference is significant at the 0.05 level.*
a. Weighted Least Squares Regression - Weighted by MOL consumed
c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 7 presents a General Linear Model (GLM) multivariate pairwise comparisons of M. oleifera effects on kids' heart girth, length, weight and BCS using a least significant difference (LSD) post hoc analysis with weeks as covariate on the male and female kids. The LSD result reveals that there were significant differences ($P < 0.05$) between males and females kids' growth rates over time (weeks).

Table 8 presents GLM multivariate pairwise comparisons of M. oleifera effects on kids' heart girth, length, weight and BCS using a least significant difference (LSD) post hoc analysis with weeks as covariate on birth-types (single and twin birth) of kids over time in weeks. The LSD multiple comparisons results indicate that kids' growth parameters were significantly different ($P < 0.05$) by birth-types; that is, between single and twin births.

Table 8: Pairwise comparisons of M. oleifera effect on kids growth parameters on birth types

Discussions

Figures 2 to 5 compared kids whose mothers were fed with M. oleifera supplemented diet with the control (kids not indirectly fed M. oleifera) growth parameters (heart girth, body length and weight as well as body condition scores) individually. Table 2 to 8 compared those growth parameters statistically to determine significant differences, except for Table 6 which compares kids by birth types and sex.

The comparison of kids' heart girth growth indicates that 10 percent (150 g) and 20 percent (300 g) M. oleifera inclusion levels can meet the nutritional needs of lactating Boer goats ceteris paribus, while 30 percent (450 g) resulted into diminishing returns to scale (Figure 2). Thus, it means that it is no longer economical to feed more than 20 percent inclusion level of M. oleifera. This result confirms findings obtained by Lu (2002) that Boer goats have gained worldwide recognition for excellent fast growing rate. The linear regression analysis was conduct to test significant differences of M. oleifera inclusion levels on the kids' girth growth and time in weeks (Table 3). It was noticed that feeding 150 g and 300 g of M. oleifera to lactating goats would economically result into maximum productivity of does and increase of kids' girth. Contrary, any rate of increase beyond these inclusion level would result into economic loss since M. oleifera is costly, scarce and in demand for human use as emphasized by Radovich (2007) and Edward, et al. (2014). Furthermore, girth growth over time was also significantly different ($P<0.05$) except in week one where there was no statistically significant increase in girth at the different M. oleifera inclusion levels and the control.

The mean comparison of kids' length growth revealed that 30 percent (49 cm) was highest, followed by 20 percent (48.27 cm) and 10 percent (47.45 cm) with the least zero percent (45.09 cm). This implies that kids' length growth depended on M. oleifera inclusion levels sequentially; that is, as the level of M. oleifera supplemented diet increases, the kids responded simultaneously (Figure 3). The linear regression analysis of M. oleifera inclusion levels on the kids' length growth over time in weeks was employed to statistically test the significant differences of growth. It was observed that 10 percent (150 g) M. oleifera inclusion level had less effect on growth in length compared to 20 percent (300 g) and 30 percent (450 g) inclusion levels that are statistically more conspicuous (Table 4). This less effect may have been attributed to less intake of milk as the kids grew bigger towards weaning period and exposed to consuming concentrates and folders by themselves as suggested by Mpofu (2004).

The average comparison of kids weight gain shows that 20 percent had the highest, followed by 30 percent and 10 percent with the least of zero percent which clearly indicates that the control (zero percent) had least weight gains among all kids (Figure 4). Lu (2002) reported that among all superior traits, heavier body weight and faster growth rate are the most notable of Boer goat kids. In a research with West African Dwarf goat kids, Asaolu et al. (2012) concurred that supplementing M. oleifera resulted into a significant weight gain

compared to Gliricidia sepium and Leucaena leucocephala fodders. In the present study, M. oleifera supplement diet increased weight gain than those fed with only lucerne and pellets diet. The linear regression analysis result of kids' weight gain over time in weeks shows significant differences of kids by treatments. It was shown that M. oleifera inclusion levels (treatments) had positive effect on weight gain of kids over time (weeks), which indicates that all inclusion levels were economically potential contributors to weight gain as time progressed (Table 5). Gebregiorgis et al. (2011) affirmed that feeding Moringa leaf to sheep increased body weight gain ($P < 0.05$) with increasing levels of Moringa leaf (300 g and 450 g), but not in the control group.

Just as the kids' girth, length and weight had the least value among the zero percent M. oleifera inclusion levels, it also applies to body condition scores (BCS) with the least being more among the zero percent (Figure 5). Body condition scores range from 1 to 5 with 0.5 increments and were assessed by visual observation and tactile as suggested by Frost et al. (2008). In the present study, kids' BCS lie between 2.5 to 3.5 points. This suggests that most of the kids were between moderate to good BCS as below 2 is considered thin (Mellado, 2002). These good BCS of kids under the drought conditions the present research was conducted is an indication that kids had sufficient milk from their mothers which sustained proper early growth and development. Villaquiran et al. (2015) explained that in most cases, healthy goats should have BCS of 2.5 to 4.0. The BCS of 1.0, 1.5, or 2.0 indicate a management or health problem. A BCS of 4.5 or 5 is almost never observed in goats under normal management conditions; however, it can sometimes be observed in show goats. On the other hand, a BCS of 1 is extremely thin and 5 is considered as obese or very over-conditioned (Mellado, 2002; Villaquiran *et al.,* 2015). The linear regression analysis of the BCS of kids indirectly fed M. oleifera over time was used to test the significant differences in kids. The BCS were best at 30 percent inclusion level (450 g) compared to 10 percent (150 g) and 20 percent (300 g) levels respectively (Table 6). This is a clear indication that M. oleifera supplemented diet contributed significantly to the nutritional needs of the does; thus, having direct effects on kids' BCS. It implies that the demand for the quantity of milk needed by kids whose mothers were not supplemented with M. oleifera ration was unmet.

The average growth parameters of kids reveals that males grew faster and bigger than females in heart girths, body lengths, body weight gains and even had better body condition scores (Table 6). This confirms the findings by Lu (2002) who stated that Boer goats male kids have higher body weight and post-weaning growth rate under standardized conditions. Weight range in the present study is lower than what was estimated by Agra Professional Services (2013) which reported that 100 days–old kids weigh between 25 kg to 32 kg. The aggregate for birth types shows that single-births grew faster and bigger than twins. The GLM multivariate pairwise comparisons of M. oleifera effects on kids' heart girth, length, weight and BCS using a least significant difference (LSD) post hoc analysis results indicate that male kids were significantly different in all growth parameters over time (weeks) regardless of the M. oleifera level on kids growth in girth, length, weight gains and BCS between (males and females). This implies that the growth parameters of male kids measured in this study were better than that of the female kids fed with M. oleifera at all levels (Tables 6 and 7). The GLM multivariate pairwise comparisons of M. oleifera effects on kids' heart girth, length, weight and BCS using a LSD post hoc analysis with weeks as covariate on birth-types (single and twin birth) of kids over time in weeks was used to test kids' statistical differences (Table 8). These differences might be attributed to the fact that singles were born bigger and heavier and suckled their mothers alone no matter how much milk they produced while twins were smaller and lighter and had to share the milk produced by their mothers. This offers chances for single kids to grow faster and bigger than twins. Zhang et al. (2008) concurred that singles have the heaviest birth weight and

largest body size. Although in this study, the result indicated that twins had greater growth parameter values than single births contrary to the expectation, this could be supported by the fact that males grow faster and bigger than females as pointed out in these results where there were more twin males than twin females and single births.

Conclusion

The supplementation of M. oleifera to lactating Boer does at all three different inclusion levels had positive indirect effect on all growth parameters of their kids which included heart girth, body length and weight as well as body condition scores. They were all statistically significant. Although Boer goats are known for their fast growth under favorable conditions, feed supplementation of pregnant and lactating does could be advantageous for maximum milk production to support their kids' healthy early growth and development especially under unfavorable conditions such as during winter and drought. This study was done under severe drought conditions after 2015 winter season. Therefore, in a semi-arid, driest and drought persistent country like Namibia, M. oleifera would bring a possible solution for animal supplementation during drought and winter periods since M. oleifera grows very fast and produces more leaf biomass per hectare; thus, alleviating farmer's stress of purchasing feed-supplement during pregnancy and lactation period. This was demonstrated by the present study where M. oleifera leaf supplemented diet of lactating Boer goats had positive effect on growth parameters (girth, length and weight) of their kids.

Acknowledgement

This study was financially supported by Namibia Students Financial Assistance Fund (NSFAF) and South Collaborative Research Programme through the National Commission on Research and Technology (NCRST). Komeho Namibia Development Agency provided the M. oleifera used for this research through its Moringa Rural Livelihood Project. Also thanks to the Liberian Government that provided this bilateral scholarship. This article is part of Morlu Korsor's PhD research at the University of Namibia, Department of Animal Science.

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ROLE OF SMALL RUMINANTS IN THE EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN SUDAN

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Summary

Anti-3ABC activity was studied, using the Priocheck ELISA, in 1195 sheep and goats sera from four different states in Sudan; South and North Kordfan, Blue Nile and Kassala. Positive sera were screened by serum neutralization test (SNT) for serotype specific antibodies against known active FMD infections in the country; "O", "A" and "SAT2". Results were compared with those of 119 cattle sera; tested simultaneously.

Regardless of the locality and the test system used, much lower activity was detected in sheep and goats than in cattle. Almost in every case, sero-prevalence of serotype specific antibodies in sheep and goats followed the same order that in cattle. Excluding the Blue Nile State, sero-prevalence of anti-3ABC antibodies in small ruminants (n=870) was 14.1% (C. I. 11.79%-16.41%) whereas that in cattle (n=89) was 53.9% (43.54%-64.26%). Unlike cattle, around 20% of small ruminants Priocheck positive sera failed to react in combined SNT while, concurrently, strong positive Priocheck reactors represented only 8.61% (n=1195). Apart from the Blue Nile state, sero-prevalence of serotype specific antibodies in small ruminants, in any instance, did not exceed 11%. Results were in agreement with the field observation in Sudan describing no clinical FMD in small ruminants. Infection of FMD in sheep and goats seemed largely secondary to that in cattle i.e. transmission within small ruminants' herds was likely less significant than transmission from cattle. Sero-prevalence's in goats, which generally reared more close to cattle, surpassed that in sheep (generally more susceptible), only, in Kassala state, where little grazing of cattle was practiced indicating also the insignificance of transmission within small ruminants herds. Diminished role of small ruminants in the epidemiology of FMD in Sudan is conceivable.

Where higher FMD activity was detected, in the Blue Nile state, sero-prevalenc of anti-3ABC antibodies mounted to 37.8% (C. I. 32.53%-43.07%) in small ruminants (n=325) and to 83.3% (C. I. 69.95%-96.65%) in cattle (n=30). Sero-prevalence's of the mostly predominant type "O" reached 64% and 24% in cattle and small ruminants respectively.

The no very good match between Priocheck ELISA and combined SNT positive reactors in small ruminants was likewise observed previously in wild life, in East Africa, when sero-prevalence estimates in the two test systems were low. Sheep, in Blue Nile and South Kordfan states where more multi-serotype conditions prevailed, showed statistically significantly different proportions (p= 0.0036) of such reactors. Likely, mild exposure (limited virus multiplication) to different serotypes resulted in boosting immune response to NSP but not to SP.

ROLE DES PETITS RUMINANTS DANS L'EPIDEMIOLOGIE DE LA FIEVRE APTHEUSE AU SOUDAN

Résumé

La présente étude a examiné, en utilisant l'ELISA Priocheck, l'activité anti-3ABC, dans 1195 sérums

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de moutons et de chèvres provenant de quatre États différents du Soudan : Kordfan Sud et Nord, Nil Bleu et Kassala. Les sérums qui se sont révélés positifs ont été examinés en utilisant le test de séroneutralisation (SNT) afin de détecter les anticorps spécifiques aux sérotypes contre des infections évolutives de fièvre aphteuse connues dans le pays : "O", "A" et "SAT2". Les résultats ont été comparés avec ceux de 119 sérums de bovins examinés simultanément.

Indépendamment de la localité et du système de test utilisé, une activité beaucoup plus faible a été détectée chez les ovins et les caprins par rapport aux bovins. Dans la quasi-totalité des cas, la séroprévalence d'anticorps spécifiques aux sérotypes touchant les ovins et les caprins a suivi le même ordre que chez les bovins. À l'exclusion de l'État du Nil bleu, la séroprévalence des anticorps anti-3ABC chez les petits ruminants (n = 870) était de 14,1% (IC 11,79% -16,41%) alors que chez les bovins (n = 89) elle était de 53,9% (43,54% -64,26 %). Contrairement aux bovins, près de 20% de sérums des petits ruminants testés positifs par Priocheck n'a pas réagi au SNT combiné alors que, en même temps, les réacteurs Priocheck fort positifs représentaient seulement 8,61% (n = 1195). En dehors de l'Etat du Nil bleu, la séroprévalence d'anticorps spécifiques aux sérotypes chez les petits ruminants (dans tous les cas) n'a pas dépassé 11%. Les résultats concordent avec les observations faites sur le terrain au Soudan n'évoquant pas de cas de fièvre aphteuse (FA) parmi les petits ruminants. Il semble que l'infection FA chez les ovins et les caprins ait été en grande partie secondaire à celle des bovins, c'est-à-dire que la transmission au sein des troupeaux de petits ruminants était probablement moins importante que la transmission dans les populations bovines. La séroprévalence chez les caprins, généralement élevés plus près des bovins, a dépassé celle des ovins (généralement plus sensibles) seulement dans l'État de Kassala qui pratiquait le pâturage modéré, une indication de l'insignifiance de la transmission chez les petits ruminants. Il est possible que le rôle des petits ruminants dans l'épidémiologie de la fièvre aphteuse au Soudan soit insignifiant.

Dans les cas où une forte activité FA a été détectée, la séroprévalence des anticorps anti-3ABC a atteint 37,8% (IC 32,53% -43,07%) chez les petits ruminants (n = 325) et 83,3% (IC 69,95% - 96,65%) chez les bovins (n = 30). La séroprévalence du type «O» prédominant atteint 64% et 24%, respectivement chez les bovins et les petits ruminants.

La faible concordance entre les tests ELISA Priocheck et les réacteurs combinés SNT positifs chez les petits ruminants avait également été observée auparavant dans la vie sauvage, en Afrique de l'Est, avec de faibles estimations de séroprévalence dans les deux systèmes de test. Dans le Nil Bleu et le Sud Kordfan, les ovins présentaient des conditions de multisérotypes plus élevées, avec des proportions significativement (p = 0,0036) différentes de ces réacteurs au plan statistique. Il est probable qu'une exposition modérée (multiplication limitée du virus) aux différents sérotypes ait entraîné une stimulation de la réponse immunitaire à NSP mais pas à SP.

Introduction

Among animal viral diseases foot-and-mouth disease (FMD) is the one with the most complex epidemiology. The disease is caused by an apthovirus of seven immunologically distinct serotypes; "O", "A", "C", Asia1 and the three South African Types (SAT1, 2 and 3). Natural infection of FMD is known in a wide range of domestic and wild animal species. In Africa, enzootic situations and great populations of susceptible domestic and wild species are expected to add further to this complexity. Already, control of FMD in South Africa addresses control in wild African buffalo, impala and kudu by fencing (Vosloo et al, 2002), whereas control elsewhere was largely consequent to vaccination in cattle. Domestic animals species which could play a significant role in the natural epidemiology of FMD, beside cattle and pigs, include sheep and goats (Alexandersen and Mowat, 2005). The latter two species were incriminated as responsible for introduction of FMD infection in countries in North Africa (Donaldson, 1999; Samuel et al, 1999), Europe (Tsaglas 1995) and South East Asia (Gleeson et al; 2003). Markers for such involvement include clinical disease, significant positive serology and/or significant incidence of carriers.

In Sudan sheep and goats mount to 70 millions heads, more than twice the number of cattle, mostly of indigenous breeds. In the extended area of the country (1.6 million Km2), different eco- and animal management systems prevails (Anon, 2009). Sheep, goats

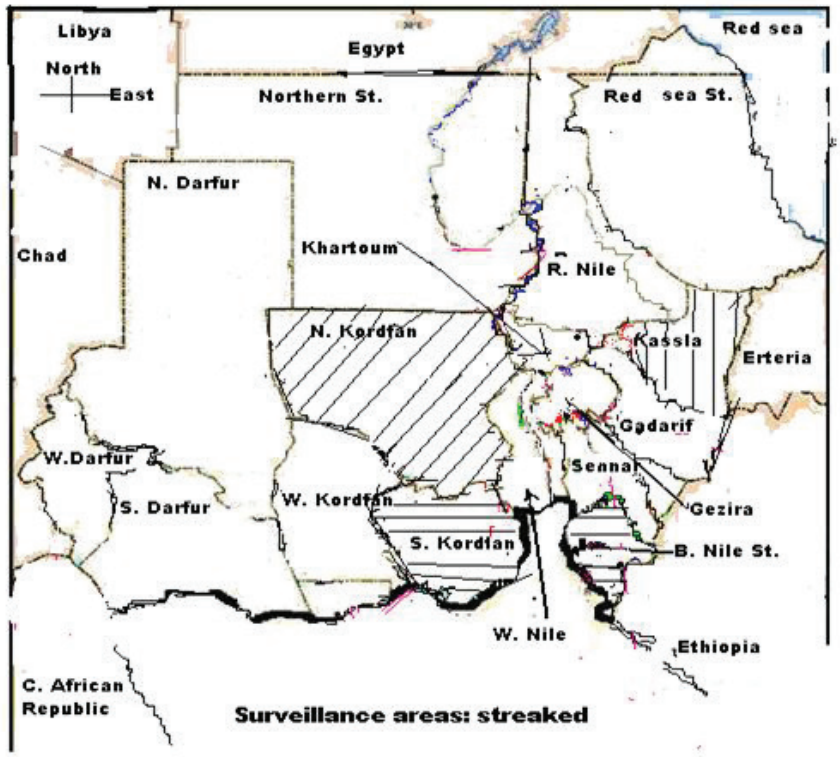


Figure 1: The study area

and cattle share common grazing fields in rural areas or closely kept together in urban or peri-urban areas. Earliest report of FMD in the country was in 1903. Currently, 3 serotypes of FMD virus are showing maintained activity, "O", "A" and "SAT2" (Raouf et al, 2010; Habeila et al, 2010), apart from "SAT1" which is showing insignificant activity (Raouf et al, 2009). Vaccination against FMD and restriction of animals' movement are not practiced to any appreciable extent in the country. In Sudan, the disease occurs annually in cattle but no confirmed report described clinical disease in small ruminants. Nonetheless, since early, serological evidence for FMD infection in sheep and goats was obtained. Not that merely, early data could suggest separate cycle of infection in these species apart from cattle (Abu Elzein et al, 1987). However, the latter suggestion was largely ruled out, since higher sero-prevalence's of antibodies to FMD viruses, were consistently detected in cattle rather than in sheep and goats (Habiela et al, 2010; Raouf et al, 2012; Raouf, 2015: a). Yet, the extent of involvement of small ruminants in the perpetuation cycle of FMD in the country remains largely to be clearly defined; either small numbers of small ruminants were studied or in different instances different levels of involvement were observed. Sero-prevalence's of type "O" and "A" antibodies in small ruminants (n=212), were approaching 50% in Khartoum state, at the centre of Sudan (Habiela et al 2009), whereas prevalence of anti-3ABC activity in sheep (n=118) in South Darfur mounted only to 8.47% (Raouf, 2015: a). Prevalence of type "O" antibodies in small ruminants from different areas in the country was 27.5% (Habiela et al, 2010) and 9.16% (Raouf et al, 2012) using the liquid-phase blocking ELISA (LPBE) and the serum neutralization test (SNT) respectively.

A technical corporation programme, TCP/SUD/3401, which was a FAO/government of Sudan joint effort was designed to promote surveillances and diagnosis of FMD in the country. One of its objectives was to determine the role of small ruminants in the epidemiology of FMD in Sudan. In the presented work, results of testing 1195 sheep and goats sera from four different Sudanese states, using the Priocheck

ELISA and combined SNT, is described. Further, results were compared with results of testing 119 cattle sera collected from three of these states during the same period of time.

Materials and Methods

Study area:

The study area is shown in Fig (1). It includes 4 states; South and North kordfan states from Western Sudan, the Blue Nile state in the South East corner of the country and Kassala state in Eastern Sudan. These states fall between 10 °N and 17 °N and 27 °E and 36 °E. The semi-desert prevails in kassala in the North while the 3 other states fall within the low rainfall savannahs belt. Livestock density, particularly cattle, is high in the latter ecological zone but low in the semi-desert zone (FAO, 2005). Sheep and goats in the study area represent about 40% of the country 60-70 million heads.

Tested sera and sample size:

Simple random serum samples were collected early in January/2013 from healthy animals older than 6-8 (sheep and goats) or 12 (cattle) months with no history of vaccination against FMD. The sampling frame and numbers of sheep and goats sampled from each state are shown in table (1). Cattle sera were selected from sample lots collected from the same areas, apart from North Kordfan, using the same sampling frame. A minimum sample size that allows a valid interpretation was employed; 30 from the Blue Nile, 49 from South Kordfan and 40 from Kassala.

The number of sheep and goats sampled in each state was determined using the formula described by Putt et al (1987):

$$N = \frac{P(100-P)}{SE2}$$

Where
N= sample size
P= expected prevalence
SE= Standard error as an absolute accuracy

The expected prevalence (P) was assumed to be 50%, the least favorable, to maximize the number sampled in case of sheep but 10% in case of goats according to previous findings of prevalence's in small ruminants (Raouf et al 2012; Raouf, 2015: a). Precision of 7% as an absolute accuracy (SE) was considered not to affect our interpretation of result at the 95% confidence level. The obtained sample size, 51 in case of sheep and 19 in case of goats, should be multiplied by 4 to compensate for error in random sampling from a wide geographical area (Putt et al, 1987). In each state, around 280 animals (around 200 sheep and 80 goats) or more were sampled (table 1).
Protocol of serum testing:

All serum samples were tested for 3-ABC antibodies using the Prio-check ELISA. Positive sera were inactivated at 56 °C for 30 minutes, cooled, received 5 µl of penicillin/ streptomycin mixture then further tested for type "O", "A" and "SAT2" antibodies using SNT. In both assays, sera from different species and from different states were tested simultaneously.

Priocheck ELISA:

The Priocheck ELISA (Sørensen et al, 2005) tests sera singly (i. e. at one well) in 100 µl volumes. All procedures, calculations and result interpretations were done as described by the manufacturers. In brief, 1/5 dilutions of test sera, negative, positive and weak positive controls were prepared in respective wells in the ELISA plate. Control sera were tested in duplicates. ELISA plates were sealed and incubated at 22± 3 °C for 16-18 hours. In the second day, plates were washed, received the proper conjugate dilution and incubated as before for one hour. At the end of the incubation time, the ELISA plates were washed then the TMB substrate solution was added. The reaction was stopped after 20 minutes of similar incubation by addition of stop solution. The OD values were measured at 450 nm using Elx808 plate reader (BIO-TEC). The average OD value of the two negative control wells was calculated (OD450 max). Results were expressed as percentage inhibition (PI) values calculated according to

the equation:

$$PI = 100 - \{OD450\ test\ sample\} \times 100 \over OD450\ max$$

The ELISA plate considered valid when the OD450 max >1.0, the mean PI of the weak positive control >50% and the mean PI of the positive control >70%. Test PI values < 50% were negative and ≥ 50% were positive.

SNT:

A screening format of SNT (Raouf et al, 2012) was used throughout the work. Viruses were recent Sudanese FMD virus isolates of the serotype "O", "SAT2" (Raouf et al, 2010) and "A"; referred to as O-Jaz 1/08 and SAT2-Kh 2/08 and A-Kh 1/011. Viruses were adapted to grow through serial passages in cultures of calf kidney cells (CKC) and baby hamster kidney (BHK) cells (O-Jaz 1/08 and SAT2-Kh 2/08) or BHK-21 cells (A-Kh 1/011). For SNT, viruses were grown in BHK-21 cells, clarified by centrifugation at 2000 rpm for 10 minutes, distributed in 2 ml aliquots and stored in liquid nitrogen vapor. Titres were determined in microtitre system (Raouf et al, 2010) using BHK-21 cells, calculated according to the method of Kärber (1931) and exactly 100 TCID50 in 50 µl volumes were used for the screening format of SNT.

Control sera were homologous positive bovine sera and known negative newborn calf serum (NBCS) (Sigma). All control sera were inactivated at 56 °C for 30 minutes.

The screening format procedure was similar to the microtest quantification of antibodies against FMD in serum described in the OIE manual (2012) except that sera were tested at final dilutions of 1/32 (10-1.5) and 1/64 (10-1.8) only. Diluents for sera and virus were complete Glasgo minimum essential medium (GMEM) {GMEM containing 10% tryptose phosphate broth (V/V), 10% tris buffer (0.05 M) and 0.0487% Na HCO3 (W/V)}. Growth media for BHK-21 cells contained in addition 10% NBCS (Sigma). Each microtitre plate tested 20 test sera in addition to cell, virus, positive and negative serum controls. The format discriminates positive sera with titres as low as 1.5 log10.

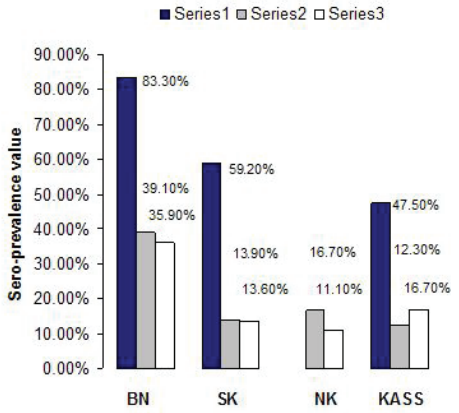
Table 1: Sampling frame and sample size

State	No. of provinces/ districts*	No. of collection sites (herd)	No. of sheep sampled	No. of goats sampled	Total No. of small ruminants sampled
Blue Nile	3	16	197	128	325
South Kordfan	8	15	194	132	326
North Kordfan	4	13	198	81	279
Kassala	7	24	187	78	265
Total	**22**	**68**	**776**	**419**	**1195**

**Two districts in Blue Nile were not included in the sample frame; Giasan and Kurmuk.*

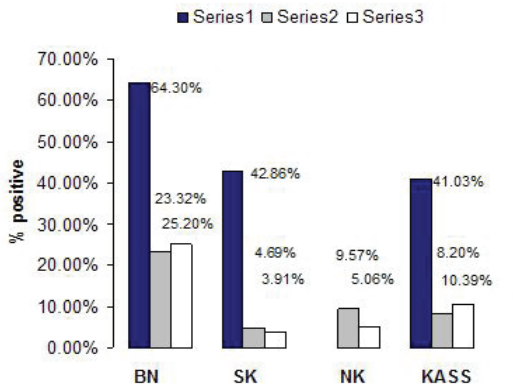
Table 2: Anti-3ABC activity in cattle and small ruminants

State / Species	Blue Nile		S. Kordfan, N. kordfan and Kassala	
	Detected anti-3ABC activity 39.1%	C. I.	Detected anti-3ABC activity	C. I.
Sheep	39.1%	32.29%-45.91%	14.3%	11.45%-17.15%
Goats	35.9%	27.59%-44.21%	13.7%	9.75%-17.65%
Sheep and Goats	37.8%	32.53%-43.07%	14.1%	11.79%-16.41%
Cattle	83.3%	69.95%-96.65%	53.9%	43.54%-64.26%



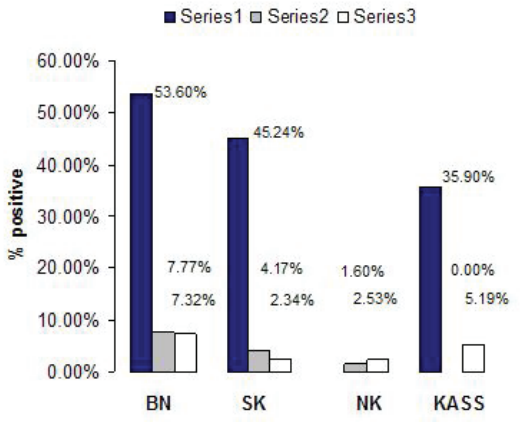
Series 1, 2 and 3= cattle, sheep and goats BN=Blue Nile, SK= South Kordfan, NK= North Kordfan and Kass= Kassala

Figure 2: Anti-3ABC activity in cattle and small ruminants



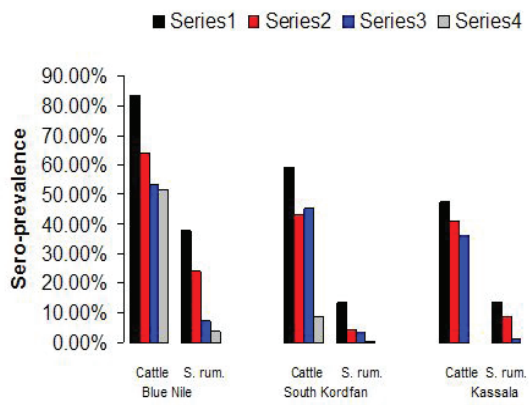
Series 1, 2 and 3= cattle, sheep and goats

Figure 3: Percentage positive of type "O" antibodies in cattle and small ruminants



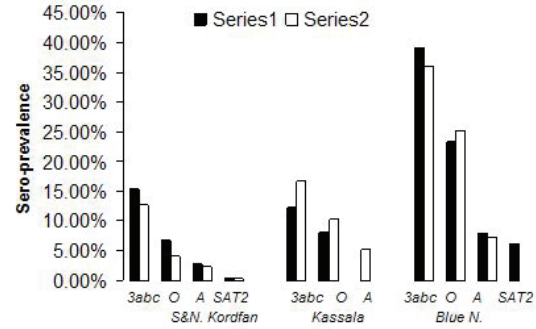
Series 1, 2 and 3= cattle, sheep and goats

Figure 4: Percentage positive of type "A" antibodies in cattle and small ruminants



Series 1, 2, 3 and 4 = anti-3ABC, type "O", "A" and "SAT2" antibodies

Figure 5: Order of sero-prevalence's in small ruminants followed that in cattle



Series 1 and 2 = sheep and goats

Figure 6: Comparison between sheep and goats in sero-prevalence's of different types of antibodies to FMD virus

Statistical analysis:

Prevalence rates of anti-3ABC antibodies were determined by dividing the number of positive serum samples by the total number of samples tested in each sub-population. A 95% confidence interval (C. I.) from a simple random sample was derived using the formula, $P \pm 1.96\sqrt{p(1-p)/n}$, described by Thrusfield (2005) for calculation of a confidence interval for a proportion; based on the Normal approximation to the binomial distribution. Where P is the estimated prevalence, n is the number of samples tested and 1.96 is the appropriate multiplier for the selected level of confidence. When C. I. values did not overlap then the statistics will always be statistically significantly different (StatNews # 73).

Table 3: Whole result data*

Anim. species	Blue Nile						South Kordfan						North Kordfan						Kassala					
	No. tested	Result	3ABC	O	A	SAT2	No. tested	Result	3ABC	O	A	SAT2	No. tested	Result	3ABC	O	A	SAT2	No. tested	Result	3ABC	O	A	SAT2
Sheep	197	No. +ve	77	45	15	12	194	No. +ve	27	9	8	1	198	No. +ve	33	18	3	1	187	No. +ve	23	15	Nil	Nil
		% +ve	39.1	23.3	7.8	6.2		% +ve	13.9	4.7	4.2	0.5		% +ve	16.7	9.6	1.6	0.5		% +ve	12.3	8.2	Nil	Nil
Goats	128	No. +ve	46	31	9	Nil	132	No. +ve	18	5	3	Nil	81	No. +ve	9	4	2	1	78	No. +ve	13	8	4	Nil
		% +ve	35.9	25.2	7.3	Nil		% +ve	13.6	3.9	2.3	Nil		% +ve	11.1	5.1	2.5	1.3		% +ve	16.7	10.4	5.2	Nil
Sheep & goats	325	No. +ve	123	76	24	12	326	No. +ve	45	14	11	1	279	No. +ve	42	22	5	2	265	No. +ve	36	23	4	Nil
		% +ve	37.8	24.1	7.6	3.8		% +ve	13.8	4.4	3.4	0.3		% +ve	15.1	8.2	1.9	0.7		% +ve	13.6	8.8	1.5	Nil
Cattle	30	No. +ve	25	18	15	15	49	No. +ve	29	18	19	4	ND						40	No. +ve	19	16	14	Nil
		% +ve	83.3	64.3	53.6	51.7		% +ve	59.2	42.9	45.2	8.7								% +ve	47.5	41	35.9	Nil

*Numbers missed testing in any system were not included in the calculation of that particular system

Proportions of positive reactors in SNT were determined by dividing the numbers of positive reactors, identified by the test, by the number of samples tested in each sub-population added to the numbers of negative 3ABC reactors in that sub-population.

To determine whether proportions of different sub-population units were statistically significantly different, P-values were calculated using the Fisher exact test for 2x2 Contingency Table or the Chi-squared test for 5x5 (or less) Contingency Table available at (www.socscistatistics.com/) and (http://epitools.ausvet.com.au/) respectively. The Fisher exact test was employed for smaller sample sizes. If $P < 0.05$, it indicates either one value (Fisher exact test) or that one or more values (Chi-squared test) are significantly different.

Results

Anti-3ABC activity in small ruminants:

Two levels of anti-3ABC activity in small ruminants were detected; one was in the Blue Nile state (n=325) of 37.8% sero-prevalence and the other of 14.13% sero-prevalence in South Kordfan, North Kordfan and Kassala states (n=870) (table 2). In no one instance, in the latter 3 states, anti-3ABC activity exceeded 16.7% (table 3; Fig. 2). Coinciding with that, strong anti-3ABC activity (PI≥70%) represented 8.61% of the whole examined lot; 16.92% in the Blue Nile and 4.9% to 6.09% in South Kordfan, North Kordfan and Kassala states (table 4).

Serotype specific antibodies activity in small ruminants:

Excluding the Blue Nile state, sero-prevalence of serotype specific antibodies ("O", "A" and "SAT2") in small ruminants in any instance did not exceed 11% (table 3; Fig. 3 and 4) highest for type "O" antibodies in goats in Kassala (10.4%). Only 3 animals of the small ruminant species, outside the Blue Nile state, showed positive serology to type "SAT2" (table 3).

Type "O" antibodies showed the highest sero-prevalence in all areas (Fig. 3 and 4). The high anti-3ABC activity in small ruminants

Table 4: Strong anti-3ABC activity in cattle and small ruminants

	Blue Nile	South Kordfan	North Kordfan	Kassala	Totals
Sheep	17.25% (34/197)	6.18% (12/194)	6.56% (13/198)	3.74% (7/187)	8.5% (66/776)
Goats	16.4% (21/128)	3.03% (4/132)	4.93% (4/81)	10.25% (8/78)	8.83% (37/419)
Sheep and Goats	16.9% (55/325)	4.9% (16/326)	6.09% (17/279)	5.66% (15/265)	8.61% (103/1195)
Cattle	70% (21/30)	48.97% (24/49)		25% (10/40)	46.21% (55/119)

Table 5: Comparison of proportions of "A" and "SAT2" reactors to whole serotype specific activity in different areas using P-values (Fisher exact test)

		B. Nile	S. Kordfan	N. Kordfan
	Proportion of "A" and "SAT2" reactors to whole serotype specific activity	**0.375**	**0.5**	**0.182**
S. Kordfan	0.5	.4217		
N. Kordfan	0.182	.1218	.0457	
Kassala	0.0	.0040	.0039	.1314

Table 6: Matching results of the Priocheck ELISA with combined SNT

	No. of 3ABC+ve Combined serum neutralization test –ve reactors			P-value (Chi-squared test)
	Cattle	Goats	Sheep	
Kassala	0	4	4	
N. Kordfan	ND	2	4	
S. Kordfan	1	4	8	
Blue Nile	2	6	17	
% in 3ABC +ve group*	(3/66) 4.54%	(16/73) 21.91%	(33/142) 23.23%	.0037
% in 3ABC tested lot*	(3/112) 2.67%	(16/406) 3.94%	(33/758) 4.35%	.6949

Table 7: Relative proportions of 3ABC+ve combined serum neutralization test –ve reactors in sheep in different states

	Kassala	N. Kordfan	S. Kordfan	B. Nile	P-valuee (Chi-squared test)
No. of 3ABC +ve combined SNT –ve sera	4	4	8	17	
% in 3ABC +ve sera	20% (4/20)	17.39% (4/23)	30.76% (8/26)	23.28% (17/73)	.709
% in 3ABC tested sera	2.17%	2.12%	4.14%	8.8%	.0036

in the Blue Nile state was matched by high sero-prevalence of type "O" antibody to 24.1% (table 3). Generally, antibodies to the 3 serotypes were demonstrable in noticeable values in Blue Nile, in similar values in South Kordfan, whereas in Kassala and North Kordfan, antibodies against types "SAT2" and "A" were either indemonstrable or with insignificant proportions in comparison to type "O" (table 3). Apparently, more multi-serotype conditions prevailed in Blue Nile and South Kordfan than in North Kordfan and Kassala. The proportion of type "A" and "SAT2" reactors to whole serotype specific activity in South Kordfan, was the highest (0.5) and was statistically significantly different from that in North Kordfan and Kassala but not the Blue Nile state (table 5).

Comparison between cattle and small ruminants:

It was evident that Regardless of the locality (Kassala, South Kordfan, North Kordfan and Blue Nile) and the test system used (3ABC, type "O", "A" or "SAT2" antibodies) much lower activity was detected in sheep and goats sera than in cattle (table 2, 3 and 4; Fig. 2, 3 and 4). Not that merely, apart from one case in south Kordfan, sero-prevalence of serotype specific antibodies in sheep and goats followed the same order that in cattle (Fig. 5).

Comparison between sheep and goats:

In general, sero-prevalenc's detected in sheep and goats were similar but a little higher in sheep than in goats. Nonetheless, infection in goats seemed to exceed that in sheep in Kassala (Fig. 6).

Matching results of the Priocheck ELISA positive reactors with combined SNT:

Results presented in table (6) show that, within 3ABC positive reactors, proportions of positive 3ABC reactors that proved negative by combined SNT were statistically significantly higher in small ruminants than in cattle. Numbers of such reactors were higher in Blue Nile and South Kordfan (where multi-serotype infection prevailed) than in Kassala and North Kordfan. When sheep sera were examined, relative proportions of such reactors in 3ABC tested sera in different areas were statistically significantly different; higher in Blue Nile and South Kordfan (table 7).

Discussion

Non-structural proteins (NSP) serology is advantageous in detecting infection with any of the seven serotypes of FMD virus. Nevertheless, it lacks a golden standard. Not to wrongly estimate the true prevalence when testing field sera (target population), statistical analysis was applied (Goris et al, 2007; Bronsvoort et al, 2008) or confirmatory tests are to be used (OIE, 2012). The Priocheck ELISA, used in this work, was identified as with sensitivity level as that of the OIE index screening method for verification of absence of FMD infection (Brocchie et al, 2006). Approving its performance, the Priocheck ELISA was proposed as the primary screening test for verification of absence of FMD infection from New Zealand (Kittelberger et al, 2008). Raouf (2015: a) indicated the superiority of the Priocheck ELISA for surveillances in endemic areas; it detected 8/196 positive sheep sera whereas the Checkit ELISA detected 0/196. When its performance was evaluated (Raouf 2015: b) regarding the panel of reference bovine sera (Campos et al 2008), the Priocheck ELISA performed similar to the OIE index screening method but failed detection of 3 positive sera collected 2 weeks or earlier following exposure. Bronsvoort et al (2008) matched the performance of Priocheck ELISA and combined SNT in testing buffalo and non-buffalo species in wild life in East Africa. It was observed that the two tests match well in testing buffalo but not when sero-prevalence estimates in the two test systems were low in non-buffalo species. Similar observation could be made in this work regarding cattle and small ruminants (table 6); as sero-prevalence estimates were low in small ruminants (table 2 and 3). Around 22% of the 3-ABC positive small ruminants sera proved negative in the combined SNT whereas only 4.54% of 3-ABC positive bovine sera showed negative result in the combined SNT (P= 0.0037). However, in this work, more

such disagreement between the two tests was observed where and when multi-serotype infections prevail (table 5); in Blue Nile and South Kordfan states (table 6 and 7). Repeated infection with the same serotype is expected to boost immune response to structural protein (SP) but not to NSP because of limited virus multiplication (Brocchi et al, 2006). In similar manner, exposure to mild infections (limited virus multiplication) of different serotypes is to be expected to boost immune response to NSP but not to SP.

Two levels of FMD activity in small ruminants were detected; one in the Blue Nile and the other in South Kordfan, North Kordfan and Kassala states (Fig. 2, 3 and 4). The higher level of anti-3ABC activity in the Blue Nile state (39.1% C.I. 32.29%-45.91%), was similar to the highest serotype specific activity (around 50%) detected in small ruminants in Khartoum state (Habiela et al, 2009). The lower level of anti-3ABC activity (14.1% C.I. 11.79%-16.41%), in the other 3 states, was similar to that detected (8.47%) in South Darfur state (Raouf, 2015: a). The state of affair clearly associated differences in sero-prevalence's in small ruminant with different geographical distribution of FMD infections. That seem to be particularly true, since these two levels were recognizable in four different systems; anti-3 ABC activity, type "O", "A" and "SAT2" antibodies. Moreover, similarly two levels of activity were detectable in cattle; one in the Blue Nile and the other in South Kordfan and Kassala states.

Regardless of the locality (Kassala, South Kordfan, North Kordfan and Blue Nile) and the test system used (3ABC, type "O", "A" or "SAT2" antibodies) much lower activity was detected in sheep and goats than in cattle (Fig. 2, 3 and 4). Results were consistent with field reports in Sudan that described clinical FMD in cattle but not in small ruminants (Abu Elzein, 1983). In small ruminants, the highest anti-3ABC activity detected was about 39.1%, in the Blue Nile state, and the highest serotype specific detected outside the Blue Nile state was 10.4% (table 3). Comparable figures in cattle in and outside the Blue Nile state were 83.3% and 45.2% respectively (table 3). Strong anti-3ABC activity which could be consequent to vigorous virus multiplication reached in cattle to 70% but never exceeded 17% in sheep and goats and in many instances scored just around 5% (table 4). In the UK 2001 epidemic (Alexandersen et al, 2002), 5% sero-positive sheep (n=237) were detected in one farm whereas morbidity in cattle was 91% (n=75) and in another farm 24% virus-positive sheep (n=148) were detected whereas morbidity in cattle was 98% (n=100). These figures from the UK and Sudan were not much unalike though the disease in Sudan is without control and occurs annually what indicated the nature of limited FMD infections in small ruminants.

Results obtained in this work indicated, not merely limited spread of FMD viruses from cattle to small ruminants, but obviously that FMD infection in small ruminants was largely secondary to that in cattle. Beside the wide difference in sero-prevalence between cattle and small ruminants, sero-prevalence of serotype specific antibodies in sheep and goats followed generally the same order that was in cattle (Fig. 5); what could be indicative of limited cycle of infection within small ruminant flocks. The disease in small ruminants is largely unapparent which, unlike cattle, allow little or no intervention of herds men and, besides, the structure of herds differ from that of cattle, yet the order of FMD infections remain similar. It could be concluded that transmission of the infection from cattle to small ruminants as opposed to sheep-to-sheep or goats-to-goats transmission was likely more significant. Within small ruminants, sero-prevalence estimates were generally a little higher in sheep than in goats except at Kassala (Fig. 6). Sheep are usually considered more susceptible than goats while goats are reared more close to cattle. In Kassala, little or no grazing of cattle was practiced, because of poor pasture. Accordingly, cattle are kept in more close contacts with goats and graze less with sheep in the pasture. Concurrently, FMD infections in goats tend to surpass that in sheep what was, also, suggestive of the significance of transmission of the infection from cattle to small ruminants in comparison to sheep-to-sheep or goats-to-

goats transmission. Similarly, in Khartoum state, where little grazing of cattle is practiced; FMD infections in goats were found to surpass that in sheep (Habiela et al, 2009)

According to the presented results, diminished role of small ruminants in the epidemiology of FMD in Sudan is conceivable. Reduction of the infection in cattle by immunization would, likely, reduce FMD virus circulation in small ruminants. Higher proportions of seropositive small ruminants than those shown in Blue Nile state were detected in Kenya, yet follow up studies showed an almost complete absence of virus carrier in sheep and goats and concluded no indication to include these species in vaccination programs (Anderson et al, 1976). Nonetheless, it would be likely necessary to include small ruminants moving from heavily infected areas, like Blue Nile state, to lightly infected areas in control quarantine procedures.

Acknowledgments

Efforts of members of the Department of Animal Health and Epizootic Diseases Control-Ministry of Animal Resources-Sudan, members of the FAO office-Khartoum and the technical staff at the FMD Unit-VRI are greatly acknowledged. This work was funded by FAO and the Government of Sudan (TCP/SUD/3401). Support of the steering committee of the programme was indispensible and distinguished.

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PREVALENCE, FARMERS' KNOWLEDGE AND MANAGEMENT OF MANGE IN SMALL RUMINANTS IN RURAL HOUSEHOLD COMMUNITIES OF EJISU-JUABEN MUNICIPALITY, GHANA

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Abstract

The current study investigated, on the basis of farm and clinical records, the prevalence, knowledge of farmers and methods of management of mange in sixty small-scale sheep and goat farms in the rural areas of Ejisu-Juaben Municipality in the Ashanti Region of Ghana. Data was compiled with employment of descriptive statistics based on farm visit records and administration of specifically-structured questionnaires to farmers and veterinary service personnel. The study showed that small-scale sheep and goat breeding is mostly a subsistence occupation, engaged predominantly by males and senior citizens of over 40 years. According to the study, majority of the rural small-scale farmers had basic or no formal education, with scanty knowledge about mange as a disease condition. The results revealed a trend of inadequate housing and management practices, with no provision for isolation and separation of afflicted animals from healthy ones. A few of the farms investigated has facilities to handle an outbreak. The study indicated a higher incidence of mange in goats; and also revealed that even as goats were mostly afflicted with demodectic mange, the predominant causative mite in the case of sheep mange was sarcoptes spp. It showed that farmers were more accurate in identifying mange in sheep; which is evidenced by a higher rate of confirmation of suspected mange cases in sheep as compared with goats. The study also confirmed methods of "in-house" management of mange as mostly inappropriate.

This study showed that failure to identify and register cases of mange could be attributed to the farmers' inadequate knowledge on the causes and impact of the disease. It is, therefore, recommended that regular extension education programmes on prophylaxis, control and prevention of mange in sheep and goats should be available to small-scale farmers in rural areas.

Keywords: Ghana, mange, management, prevalence, Sheep and goats, small-scale farmer.

PREVALENCE, CONNAISSANCE DES EXPLOITANTS ET GESTION DE LA GALE CHEZ LES PETITS RUMINANTS DANS LES COMMUNAUTES RURALES DE LA MUNICIPALITE D'EJISU-JUABEN AU GHANA

Résumé

La présente étude a examiné, sur base des données agricoles et cliniques, la prévalence, la connaissance des agriculteurs et les méthodes de gestion de la gale dans soixante petites exploitations ovines et caprines des zones rurales de la municipalité d'Ejisu-Juaben, dans la région Ashanti au Ghana. Les données ont été compilées à l'aide de statistiques descriptives basées sur les enregistrements de données lors des visites à la ferme et l'administration de questionnaires spécifiquement structurés aux exploitants et au personnel des services vétérinaires. L'étude a montré que l'élevage artisanal de moutons et de chèvres est principalement une occupation de subsistance, pratiquée essentiellement par des hommes et des personnes âgées de plus de 40 ans. Selon l'étude, la majorité des petits exploitants avaient suivi une éducation de base ou informelle, avec peu de connaissances sur la gale comme maladie. Les résultats révèlent une tendance à des pratiques inadéquates de logement et de gestion, sans aucun arrangement pour l'isolement et la séparation des animaux malades et des animaux sains. Quelques-unes

des exploitations étudiées ont des installations leur permettant de prendre en charge un foyer. L'étude a révélé une incidence plus élevée de la gale parmi les chèvres. Elle a également indiqué que, même si les chèvres étaient principalement atteintes de démodécie, l'acarien responsable prédominant dans le cas de la gale de mouton était Sarcoptes spp. Selon cette étude, les exploitants étaient capables d'identifier avec plus de précision la gale chez les moutons, cette capacité étant attestée par un taux plus élevé de confirmation de cas soupçonnés de gale chez les moutons par rapport aux chèvres. L'étude a également confirmé que les méthodes de gestion interne de la gale étaient généralement inappropriées.

Cette étude a montré que la non-identification et le non-enregistrement des cas de gale pourraient être attribués à une insuffisance des connaissances des exploitants sur les causes et l'impact de la maladie. Il est donc recommandé de mettre à la disposition des exploitants des zones rurales des programmes de vulgarisation régulière sur la prophylaxie, le contrôle et la prévention de la gale chez les ovins et les caprins.

Mots-clés : Ghana, gale, gestion, prévalence, ovins et caprins, petit exploitant.

Introduction

Sheep and goats are predominant features of subsistence rural economy in most parts of West Africa. The goat is thought to have been the first animal to be domesticated for economic purposes (Peacock, 1996); and early man tamed, confined and protected sheep for meat, milk, wool, and skin (Mahanta, 1987)

In Ghana small ruminants are reared under either the extensive system where they move from pasture to pasture, or the semi-intensive system, whereby they graze and are also given supplementary feed (Faizal A. & Kwasi O., 2014). The meat of both sheep and goats is popular in all parts of West Africa just as statistics indicate that annual increase in sheep and goat population in most West African countries averages 18-20% ad 10-12% respectively (Tweneboah, 2000).

Mange, a class of skin disease caused by parasitic mites, affects many animals including sheep and goats. Mites affecting sheep and goats are obligate parasites which may be burrowing or non-burrowing. Transmission is by direct skin to skin contact and heavy infestation causes dermatitis (Mclachan, 2008). There are six stages in the life cycle of the mite: egg, larvae, three nymphal stages and adult. The adults live in close contact with the skin surface and lay their eggs under the dead scales on the skin (Cole, 1996)

The mange mites which are reported to affect small ruminants in Sub-Saharan Africa include *Sarcoptes* spp (*S. s scabei*), *Psoroptes* spp (*P. caprae*, *P. communis*, and *P. cuniculi*) , Chorioptes spp (*Chorioptes texanus*, *C. caprae*) ,and *Demodex* spp (*D. folliculorum*, *D. brevis*, D. aries, D. caprae). The prevalence of mange mites differs from region to region. *S. scabei*, *P. communis*, and *D. folliculorm* in goats in Nigeria are estimated to be 24-33%, 22% and 11% respectively, whilst 11% prevalence of Sarcoptic mange has been reported in Kenya (Kambarage, 1996)

Sarcoptic mange is caused by Sarcoptes scabei. The female mite burrows into the skin and forms tunnels in which she lays eggs. The burrowing and feeding of mites cause intense itching and scratching, thickening and wrinkling of skin and hair loss. Typically, in goats it starts in the less hairy areas around the udder, abdomen and between the front legs, and spreads over the body. The skin around the mouth becomes hard making it difficult for the animal to feed and the rate of mortality can be high (Peacock, 1996).

Demodectic Mange is caused by Demodex spp. They burrow into the hair follicles and sebaceous glands of the host forming characteristic small pustules which exude yellow pus (Peacock, 1996). Several distinct species of Demodex often parasitize the same host animal, but each species tends to be restricted to a particular habitat. *Demodex* aries is a type of *Demodex* Spp in sheep which is confined to areas with very large sebaceous glands such as vulva, prepuce and nostrils. *Demodex caprae* causes nodular dermatitis in milk goats. (Bowman, 2009).

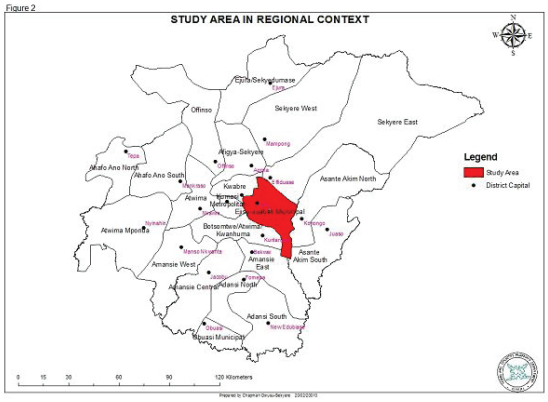
Chorioptic mange in sheep and goats is characterized by intense itching, which results in alopecia. The mites are non-burrowing and

feed on the skin surface. Scales, thickening and folding of skin are common features. In goats, lesions appears on the interdigital clefts, muzzle, eyelids, udder, scrotum, anal and tail regions, while in sheep lesions begin at the fetlock region and spread to the udder and scrotum (Kambarage, 1996). Psoroptic mange in sheep, caused by non-burrowing Psoroptes ovis, is a highly contagious infection characterized by intense pruritus, scratching, and raised tuff wools. (Bowman, 2009). Severely affected animals become emaciated, anaemic and may die due to exhaustion (Kambarage, 1996)

Animals that are infected with mites should be separated from other animals as much as possible during the healing period. Any shared living areas should be diligently cleaned and fresh bedding added before reusing the pens for new animals. Additionally, shared grooming tools must be disinfected before using them on uninfected animals. A foot bath must also be present at the entrance of the pen.

The main objective of the current study is to ascertain the prevalence of mange, ascertain the level of awareness of small ruminant farmers about the disease, identify the predominant types and causative agents of mange that affect small ruminants coupled with how the disease is managed by small-scale and household farmers in rural areas of the Ejisu Municipality with a view of recommending possible management and control measures.

Figure 2



Materials and Methods

Study Area

The Ejisu Juaben Municipality, an administrative district in the Ashanti Region borders with the Kumasi Metropolis to the west, Kwabre District to the northwest, Sekyere East to the northeast, Asante Akim North Municipal to the north and Bosomtwe Kwanwoma and Asante Akim South Districts to the south. The Municipality, with a total land area of approx. 640 sq.km, is endowed with varied natural resources including farming lands, forest reserves, water bodies, quarry, clay and sand deposits, etc.

The mean annual temperatures are 25°C – 32°C with relative humidity ranging from moderate in the dry season to high during the rainy season.

Scope of Study and Survey Methods

Sixty small-scale small ruminant farms with varying numbers of sheep or goats were investigated in the current study. Two approaches were involved in the survey method employed for collection of primary information.

1. Farm visit records were obtained through informal individual discussions with sheep and/or goat farmers and on site assessments
2. Formal conventional questionnaires were administered to the small-scale sheep and goat farmers, as well as to personnel of the veterinary services. The questionnaires were designed to be respondent-specific and enabled gathering of detailed information on sheep and goat breeding and disease management.

Information gathered from the farmers also included the length of years of existence of the respective farms, farmer's experience in sheep and goat breeding, frequency of registered new cases of mange, characteristics and notable symptoms of cases registered, handling of registered cases, medications administered and their sources of procurement, prophylactic, control and preventive measures

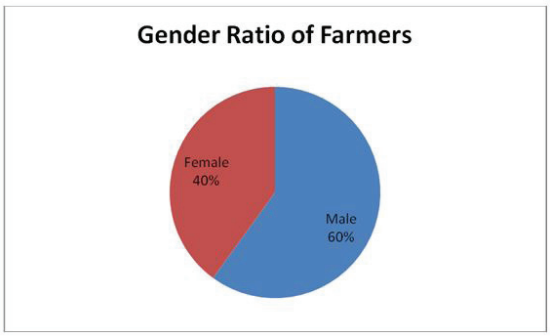


Figure 1: Pie Chart showing ratio of male farmers to female farmers

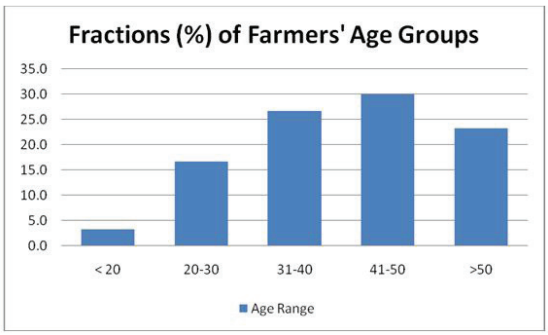


Figure 2: Bar chart showing age group Categories of Farmers

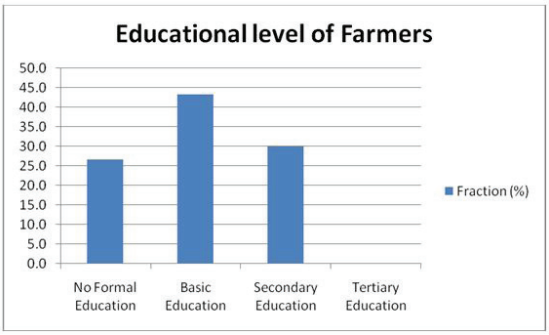


Figure 3: Bar Chart showing the fractions of farmers' levels of education

Figure 4: Bar Chart showing the fractions of farmers' status in their farms

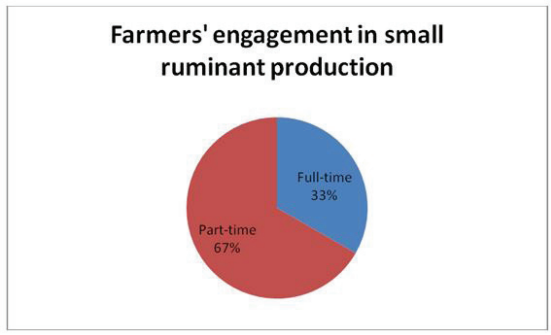


Figure 5: Pie Chart showing ratio of full-time vs. part-time engagement of farmers

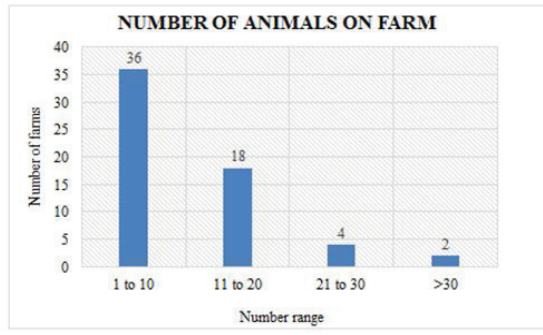


Figure 6: Bar Chart showing the sizes of animal populations in the farms investigated

practiced. Where required, veracity of data provided by a farmer was verified by inspection e.g. the number of infected animals, symptoms, medications given, etc.

Estimation of the data was by Statistical Package for Social Sciences: Version 16.0 (SPSSv16.0) using statistical tools to derived descriptive statistics like frequencies, and percentages

Results

Handlers of all the selected sheep and goat farms consented to participation in the study and provided answers to all questions or were guided to fill in the questionnaires. Categorization of interviewed farmers into age groups of <20, 21-30, 31-40, 41-50, and >50 gave a respective representation of 2 (3.3%), 10 (16.7%), 16 (26.7%), 18 (30%) and 14 (23.3%). Demographic analysis of the study results revealed that small-scale sheep and goat farmers were predominantly males, senior

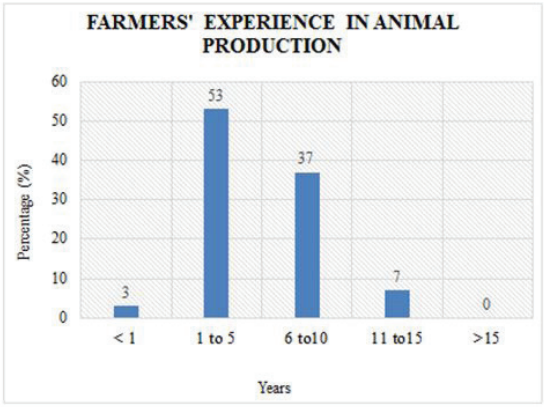


Figure 7: Number of Years of Experience of Farmer (Operation of sheep/goat Production Farm)

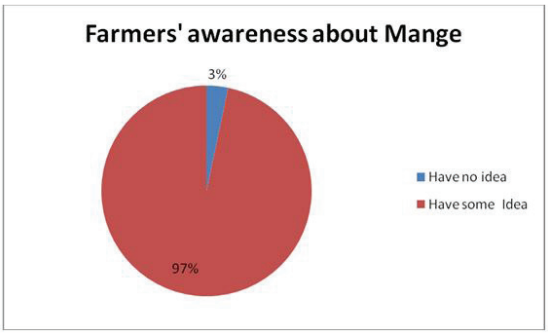
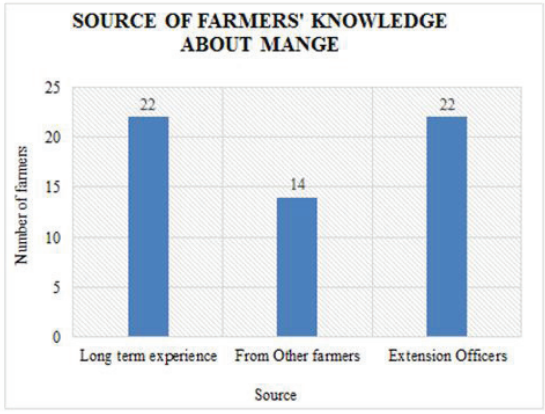
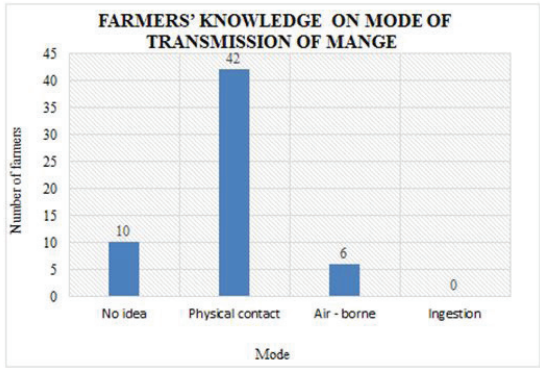
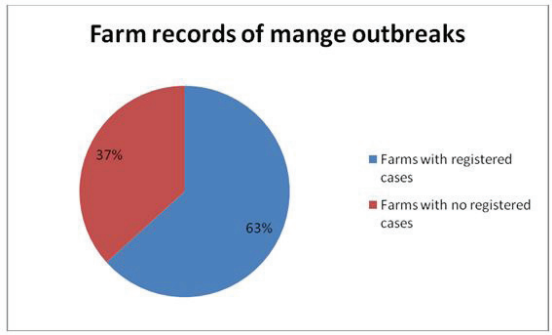
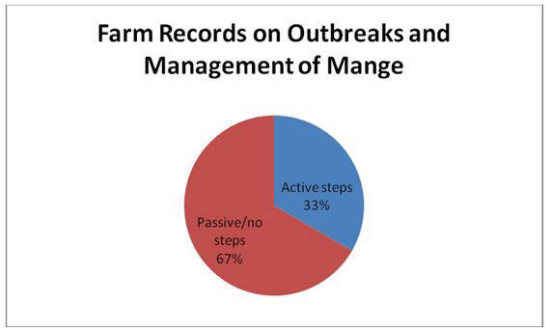


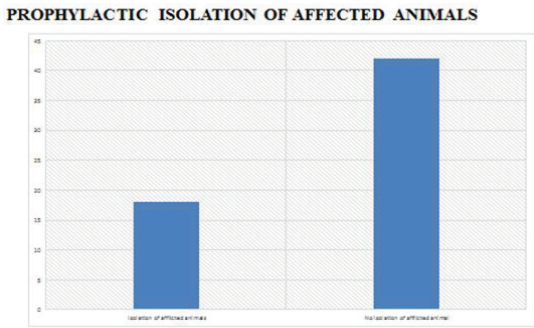
Figure 8: Pie Chart showing farmers' knowledge about mange

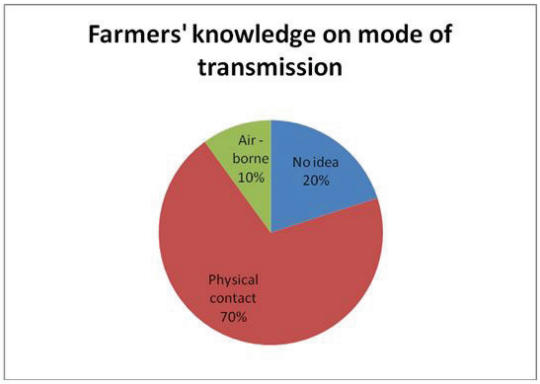












citizens of over 40 years and with mostly basic level of education. This is shown in Figs 1, 2 and 3.

Results of the current study revealed that the small ruminant farms in the study area are operated on small scale level, as a subsistence and secondary engagement by their owners who have other main businesses. Farm owners started their ventures with whatever resources available; hence most of the farms have small animal populations (mostly <25) bought from assorted markets with no special

Table 1

CLINICAL RECORDS OF REPORTS OF MANGE OUTBREAKS FROM FARMS

Species	Frequency		Fraction, %
	Monthly	Annual	
Goats	10	120	78.6
Sheep	4	48	21.4
Total	14	168	100

CLINICAL RECORDS OF REPORTED VS. CONFIRMED CASES OF MANGE

Species	Reported cases	Confirmed	Ratio of confirmed cases to reported cases
Goat	120	72	60
Sheep	48	48	100
Total	168	120	71

Table 3

CLINICAL RECORDS ON STAGE OF DISEASE AT PRESENTATION

Species	Condition of animal/ Stage of disease			Total
	Moderate	Severe	Advanced	
Goats	10	10	6	26
Sheep	4	4	0	8
Total	14	14	6	34

Table 4

CLINICAL RECORDS ON TYPES OF MANGE AND CAUSATIVE MITES IN CONFIRMED CASES

Species	Causative mites			
	Sarcoptes	Demodex	Fraction of Sarcoptes, %	Fraction of Demodex, %
Goats	6	10	38	62
Sheep	12	4	75	25
Total	18	12		

emphasis on health status of the source. This is shown in Figs 4, 5 and 6.

A third of the farmers had secondary education, with a majority two-thirds having either basic or no formal education.

The study revealed a growing interest in small-scale sheep and goat breeding. Analysis of the length of experience of farmers in the business shows the highest fraction of 53.3% was recorded for those with 1-5 years'

experience; whilst 90% of all farmers engaged in the study fell within 1-10 years' experience in sheep and goat farming. .

Pie Chart showing farmers' knowledge about mange

The study revealed an overall higher incidence of the disease in goats than in sheep; with similar trend registered in the number of cases reported to the veterinary clinic. It was, however, confirmed that mange in sheep was detected with more accuracy, and reported earlier to the veterinary clinic. This is evidenced in Tables 1, 2, 3 and 4.

Predominant causative mites of mange in the goats and sheep presented to the Municipal Veterinary Clinic were identified as mainly Sarcoptes scabei and Demodex folliculorum which causes Sarcoptic mange and Demodectic mange respectively. The study revealed that most cases of mange in goats were demodectic whilst mange in sheep was predominantly sarcoptic.

Discussion

This paper investigates the farm records, clinical records, sheep and goat farmers' knowledge and management of mange in small ruminants in the Ejisu-Juaben Municipality, Ashanti Region of Ghana. The study revealed a growing interest in small-scale sheep and goat production with a significant number of "new-comers" into the industry – up to 56% of animal handlers in the investigated farms have involved in sheep and goat farming for up to 5 years. This is a direct indicator of the significant role of small ruminant production in subsistence economy of rural folks and a means of poverty alleviation as well as a factor of youth deployment in Ghana. The study also revealed male domination of the small ruminant production industry with 60% of the 60 small-scale sheep and goat farmers being males.

The study revealed that most of the farms do not report any cases of mange to the Municipal Veterinary Clinic. Whilst some of the farms either do not take any action or resort to "in-house" symptomatic treatment of the affliction, a rather large portion of such "apathetic" farms claim to have never recorded any incidence of the disease.

The current study confirmed mange in small ruminants as one of the actual problems in local animal production characterized by a culture of unwillingness of the small-scale farmer to seek proper treatment from appropriate authorities. Many such farmers rather engage in "in-house" treatment and self-administration of medication and turn to seek expert advice from veterinary services only in exceptional or difficult cases. It was, however, revealed that most of the farmers had adequate idea about the mode of transmission of the disease. The study confirmed an overall higher incidence of the disease in goats than in sheep. However, whilst there were higher numbers of reported cases, to the veterinary clinic, of mange in goats, cases of suspected sheep scab were confirmed with much more certainty. Coupled with the fact that cases of sheep scab were seen to be reported at relatively earlier stages of development, it could be inferred that the farmers portrayed identified mange in sheep earlier and better than in goats. The study also evaluated the impact of improper, inadequate and/or lacking managerial factors on the occurrence and prevalence of mange in sheep and goats amongst small-scale farmers in rural communities and drew conclusions on measures that could be applied to prevent and/or control the disease.

In view of the inadequate knowledge on the part of most sheep and goat farmers and owners on proper management practices and biosecurity measures, the following recommendations are being made:

- Organization of regular extension education programmes for small-scale sheep and goat farm owners and operators;
- Cooperative efforts by veterinary, production and other specialists to design optimal methods of management of mange;
- Limiting population density and ensuring comfortable environment of sheep and goats;
- Design of appropriate housing conditions for sheep and goats;

- Adoption of appropriate feeds and feeding methods for sheep and goats;
- Isolation of affected animals and their separation from healthy ones;
- Other forms of required assistance, including vaccination and consultation are made available to farmers and farm workers.

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ANTIMICROBIAL RESISTANCE PROFILE IN BACTERIAL ISOLATES FROM SUBCLINICAL MASTITIC MILK SAMPLES IN DAIRY HERDS IN KENYA

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Abstract

This study was undertaken to investigate subclinical mastitis causing pathogens in dairy lactating cows and determine their antimicrobial susceptibility profile in rural and peri-urban areas of Thika, Mathioya and Kieni East Sub County. California Mastitis Test (CMT) was used to screen one hundred and sixteen lactating cows for subclinical mastitis. A total of 71 milk samples from cows that tested positive with CMT test were collected for bacterial isolation. The results show that *Staphylococcus aureus* was the most predominant bacterial isolates at 40.85%. This was followed by *Coagulase* negative *Staphylococcus* (21.13%), Negative growth (15.49%), *Streptococcus* spp (9.86%), *Streptococcus agalactiae* (8.45%), *Escherichia coli* (2.82%). and Klebsiella spp (1.41%). Antimicrobial susceptibility testing results shows that bacteria isolates were susceptible to *kanamycin, chloramphenicol* and *co-trimoxazole* drugs but resistant to *ampicillin* and streptomycin. The study shows *Staphylococcus aureus* in the most predominant bacteria and most bacterial isolates are resistant to ampicillin and streptomycin. The study recommends regular surveillance programs to detect the risk of spread of antimicrobial resistance

Key words: *Ampicillin*, antibiotic resistance profiles, dairy cows, *staphylococcus aureus*.

PROFIL DE RESISTANCE AUX ANTIMICROBIENS DANS LES ISOLATS BACTERIENS DES ECHANTILLONS LAITIERS DE VACHES ATTEINTES DE MAMMITE SUBCLINIQUE DANS LES TROUPEAUX LAITIERS DU KENYA

Résumé

La présente étude a été réalisée dans le but de déterminer les agents pathogènes responsables de la mammite subclinique chez les vaches allaitantes et déterminer leur profil de sensibilité aux antimicrobiens dans les zones rurales et périurbaines des sous-comtés de Thika, Mathioya et Kieni East. Le test de mammite de Californie (California Mastitis Test - CMT) a été utilisé pour le dépistage de la mammite subclinique chez cent seize vaches en lactation. Au total, 71 échantillons de lait provenant de vaches aux résultats positifs selon le CMT ont été recueillis pour isolement de bactéries. Les résultats ont montré que Staphylococcus aureus est l'isolat bactérien le plus prédominant au taux de 40,85%, suivi de Staphylocoque à coagulase négative (21,13%), croissance négative (15,49%), Streptococcus spp (9,86%), Streptococcus agalactiae (8,45%), Escherichia coli (2,82%), et Klebsiella spp (1,41%). Les résultats des tests de sensibilité aux antimicrobiens montrent que les isolats de bactéries sont sensibles à la kanamycine, au chloramphénicol et au co-trimoxazole, mais résistants à l'ampicilline et à la streptomycine. L'étude a révélé que Staphylococcus aureus est la bactérie la plus prédominante, et que la plupart des isolats bactériens résistent à l'ampicilline et à la streptomycine. L'étude recommande des programmes de surveillance régulière pour détecter le risque de propagation de la résistance aux antimicrobiens.

Mots-clés : ampicilline, profils de résistance aux antibiotiques, vaches laitières, *Staphylococcus aureus*.

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Introduction

Dairy farming is a major economic activity of majority of rural and peri-urban smallholder farmers in Kenya. Survey done by Smallholder Dairy (Research and Development) Project (SDP) shows that small holder dairy farmer are more than a million in the country. The study shows that they dominate at the production level and account for 70% of milk marketed from farms (SDP 2004, FAO 2011). Milk production has been increasing rapidly driven by high demand due to rising population in both urban and rural areas. Other factors contributing to the growth in dairy sectors include increased purchasing power due expansion of economy, improved breed of dairy cows, access to farm inputs, and well organized marketing structure such as dairy cooperative (FAO 2011). However dairy sectors is still facing a number of challenges such as low average milk production per cow, high cost of production, availability of feed throughout the year due to fluctuating weather conditions, high prevalence of infectious diseases such as tick borne diseases, mastitis which affect milk quality (Mureithi and Mukiria 2015).

Among these factors, infectious disease like mastitis is one of the most important diseases affecting dairy cows. It is a multifactorial disease accounting for major monetary losses in dairy farming due to the reduction in milk production, costs for treatments, discarding of contaminated milk after treatment and early culling of diseased animal (Hamidi and Sylejmani 2016). Subclinical mastitis is even more damaging economically due to its ability to maintain a reservoir of infection within the dairy herd and this increase the risk of exposure of uninfected cows to infectious pathogens. Bacterial contamination of milk from subclinical mastitis cows may render it unsuitable for human consumption by causing food intoxication or interference with manufacturing process (Amdhun et al 2016). Study in Kenya by Mureithi and Njuguna 2016 have shown that management practices such as improved udder hygiene in wet season, regular cleaning of floor or upgrading of floors to concrete may help in reducing prevalence of subclinical mastitis.

Even with best practice management of the dairy cow, it occasionally becomes indispensable to treat dairy cow for mastitis with antimicrobials.Antimicrobial agents are the main therapeutic tools for the treatment and control of mastitis. The cure rates for mastitis are not always satisfactorily. The success of bovine mastitis treatment depends on several factors such as the etiological agent, clinical manifestation, antimicrobial susceptibility of causative agent and the efficiency of body immune system (Gitau *et al* 2011). The abusive or incorrect use of antimicrobials has been implicated as the major selective force for the development of resistance. The indiscriminate or unwarranted use of antibiotics in dairy cows is a big concern with S. aureus being targeted in treating mastitis. This indiscriminate use of antibiotic may affect the human health in numerous ways such as accumulation of antibiotic residue in milk consumed, as well as the development and transfer of antibiotic-resistant bacteria through the food chain (Global Antibiotic Resistance Partnership—Kenya Working Group. 2011).

Studies on subclinical mastitis have been conducted in few rural, urban and peri-urban areas in past years in Kenya (Gitau *et al* 2014, Mureithi and Njuguna 2016) and showed prevalence ranging from 30 to 65% however; information on resistance pattern of major bacterial pathogen of subclinical mastitis in dairy farm is still scarce. The information is essential since antibiotics play a significant role in the control of mastitis. A good surveillance system for antibiotic resistance will ensure optimal result in the control of mastitis. It will also reduce the risk of development and spread of resistance in dairy farms. It is from the above that he study sought to address the paucity of data by investigating the antimicrobial resistance profile in bacterial isolates from subclinical mastitis milk samples in three central region of Kenya

Materials and methods

Study area

The study was carried out in Thika, Mathioya and Kieni East Sub-County from the Month of May to August 2016. Thika is one of the 12 Sub Counties in Kiambu County with an elevation of 1550 metres above sea level. The average annual rainfall ranges between 900 mm and 1,250 mm and an average annual temperature of 25 °C. Mathioya Sub-County is located in Murang'a County. Temperatures in this area are cool and warm, with rainfall between 1400 to 2000mm per year. Humidity is 90 to 96 with an altitude of 2000 m above sea level. Kieni East Sub County is located in Nyeri County. The average rainfall ranges between 550 to 950 mm per annum. The three regions have two rainy seasons with long rains in March and May and the short rains in September to November. There is a cold spell from the month of July to August. Dairy farming is an important economic activity in these areas.

Sample collection

Small holder dairy farms were listed with the assistance of the area administrative officers and veterinary Para-professionals in the three study area. For the farm to be included in the study it had to meet the inclusion criteria of being a dairy farm with no history of clinical mastitis or mastitis causing pathogens, a herd size ranging from 1 to 46 cows, and with no history of antibiotic therapy. A random number table was used to select 13 dairy farms proportionate to the total number of farms in each area. From these farms California Mastitis Test (CMT) was used to screen one hundred and sixteen lactating cows for subclinical mastitis. All the animals were first clinically and physically examined with more emphasis on the cow's udder. The dairy cows did not have any clinical symptoms, no clinical mastitis, and no recent treatment against mastitis and lived under close comparable condition of breeding environment and system of feeding. A total of 71 milk samples were aseptically collected from CMT positive lactating cows. Before sample collection teats were cleaned and dipped in a disinfectant and finally wiped with alcohol swabs and dried. The first few milk streams were discarded and then about 10ml of milk were collected into sterile test tubes. The samples were transported to the laboratory in ice box and stored at refrigerated temperature for a maximum of 24 hours until processing.

Identification of mastitis-causative micro-organisms

Following the protocol described by Mureithi and Njuguna 2016 the milk samples were examined for bacteriology. From every milk sample, 10µL was inoculated on 5% sheep blood agar (SBA) and MacConkey agar plates. The petri dish plates were then incubated at 37oC in aerobic incubators for 18 to 24 hours. Plates were examined for growth after 24 hours and those with no growth additionally incubated up to 4 days before the examination. The bacterial strains were identified using colony morphology, microscopic characteristics and gram staining. Then by use of biochemical tests the isolates were identified to the species level. Gram negative rod colonies after staining and using growth morphology were classified as lactose and non-lactose fermenters. Lactose fermenters were further tested by citrate fermentation to differentiate E.coli and Klebsiella, with citrate positive classified as Klebsiella and citrate negative as E.coli. Gram positive, small to medium sized cocci colonies that were hemolytic or no hemolytic on 5% SBA were tested on catalase test with catalase negative being categorized as streptococci. Catalase positive were further tested for coagulase activity with rabbit plasma with those with coagulase activity being classified as Staphylococcus aureus while those without as Coagulase Negative Staphylococcus (CNS). Catalase-Negative Streptococci were tested using Bacitracin and those testing negative categorized as Streptococcus agalactiae.

Antibiotic Susceptibility test

Antimicrobial susceptibility test was performed on isolated bacteria as per Kirby-Bauer Method following manufacturer's instruction. Four pure cultures of bacterial isolates were selected and inoculated into 5 ml

Tryptone Soya Broth and incubated at 37oC for 2-8 hours until light to moderate morbidity develops. The inoculum turbidity was then standardized to 0.5 McFarland. A sterile cotton swab on a wooden applicator was dipped into the standardized inoculum, soaked and held on to the upper side of the wall of the tube to remove excess fluid. The swab was then used to streak the entire surface of the Mueller Hinton (MH) agar plate. The discs containing ampicillin (25 µg), gentamicin (10 µg), tetracycline (25 µg), sulfamethoxazole (200 µg), streptomycin (10 µg), chloramphenicol (30 µg), Co-Trimoxazole (25 µg), Kanamycin (30 µg) (Himedia) were applied aseptically. The plates were then incubated immediately and examined after 24 hours for the zone of inhibition. Using a caliper, the zone showing complete inhibition was measured and the diameter of the zones recorded to the nearest millimeter. Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) tables, (CLSI 2012).

Data analysis

The collected data was recorded into Microsoft excel Spreadsheet. Descriptive statistics were used to present data. Proportion of bacterial isolates from subclinical mastitis milk samples were calculated using percentage value. Proportion of susceptible bacterial isolates to antibiotic was also calculated as percentage value.

Results

Bacterial isolates results

Table 1 provides summarized results of the bacteria isolated from cultured milk samples in the current study. Results from this study shows that Staphylococcus aureus was the most predominant bacterial isolates at 40.8%. This was followed by Coagulase negative Staphylococus (21.1%), Negative growth (15.5%), Streptococcus spp (9.86%), Streptococcus agalactiae (8.45%), Escherichia coli (2.82%). and Klebsiella spp (1.41%)

Antimicrobial profile results

The antimicrobial susceptibility and resistance profiles of bacterial isolates from subclinical cases of mastitis to antibiotics are shown in table 2. In this study Staphylococcus aureus isolates were found to be highly susceptible to kanamycin (96.6%), followed by co-trimoxazole (93.1%), gentamycin (93.1%), chloramphenicol (89.7%), tetracycline (75.9%) and Sulfamethoxazole (72.4%). However these isolates were highly resistant to ampicillin (62.1%), and streptomycin (58.6%). Coagulase Negative Staphylococci were mostly sensitive to co-trimoxazole (100%), Sulfamethoxazole (93.3%), chloramphenicol (86.7%), kanamycin (86.7%), tetracycline (73.3%), and gentamycin (66.7%) except ampicillin which showed resistance at 66.7%. Streptococcus agalactiae was found to be highly resistant to gentamycin (66.7%) and streptomycin (50%). Streptococcus spp isolate showed high susceptibility to chloramphenicol, co-trimoxazole, kanamycin and streptomycin and moderate resistance to the other antibiotic being tested. In general, it was found that for the bacterial isolates tested for antimicrobial susceptibility, kanamycin, chloramphenicol and co-trimoxazole were the

Table 1: Bacterial isolates from subclinical mastitis milk samples

Bacterial isolates	Number of isolates	Prevalence rate
Escherichia coli	2	2.82%
Negative growth	11	15.5%
Staphylococcus aureas	29	40.8%
Coagulase Negative Staphylococcus	15	21.1%
Streptococcus spp	7	9.86%
Klebsiella species	1	1.41%
Streptococcus agalactiae	6	8.45%

Table 2: Antimicrobial susceptibility of bacterial isolates from cows with subclinical mastitis to antibiotics

Bacterial isolates		**Tested antimicrobial drugs**							
		AMP	**GEN**	**TET**	**SMX**	**S**	**CMP**	**COT**	**KAN**
E. coli	S	50	100	50	50	100	100	100	100
n=2	R	50		50	50				
S. aureus	S	37.9	93.1	75.9	72.4	41.4	89.66	93.1	96.6
n=29	R	62.1	6.90	24.1	27.6	58.6	10.34	6.90	3.40
CNS	S	33.3	66.7	73.3	93.3	40	86.67	100	86.7
n=15	R	66.7	33.3	26.7	6.67	60	13.33		13.3
Streptococcus spp	S	57.1	71.4	57.1	57.1	71.4	100	100	71.4
n=7	R	42.9	28.6	42.9	42.9	28.6			28.6
Klebsiella spp	S	100	100		100		100	100	100
n=1	R			100		100			
S. agalactiae	S	66.7	33.3	66.7	83.3	50	83.3	66.7	83.3
n=6	R	33.3	66.7	33.3	16.7	50	16.7	33.3	16.7

Legend:AMP:Ampicillin;TET:Tetracycline; SMX: Sulfamethoxazole; GEN: Gentamicin; S: Streptomycin; COT: Co-Trimoxazole; CMP: Chloramphenicol; KAN: Kanamycin. S-% Susceptible R-% Resistant

drugs more active while the majority of isolates were resistant to ampicillin.

Discussion

In the current study, bacterial isolates were similar to those found by (Gitau et al 2011, Mureithi and Njuguna 2016). The main bacteria isolate (table 1) was S. aureus at 40.6%. The finding was lower than that found by Gitau et al 2014 of 72.9% in two regions of Kenya but higher that found in Kosovo by Sylejmani et al 2015, Bhat et al 2016 in India also isolated S. aureus at a higher prevalence of 66.67% than this study. Since the principle reservoir for Staphylococcus is the udder skin and infected gland milk, this may explain the high isolation of this organism in this study. Management practices such as poor udder hygiene, muddy soil floor and lack of regular cleaning of concrete floor may also explain the high frequency of staphylococcal mastitis.

Result of antimicrobial sensitivity tests of Staphylococcus aureus in this study agrees with past study by Marimuthu et al 2014, who reported an increased resistance of Staphylococcus aureus to streptomycin and ampicillin. Haftay et al 2016 also reported a similar resistance to penicillin and ampicillin in Ethiopia. The study found a high sensitivity for Coagulase Negative Staphylococcus to co-trimoxazole (100%), sulfamethoxazole (93.33%), chloramphenicol (86.67%), kanamycin (86.67%), tetracycline (73.33%), and gentamycin (66.67%). This finding agrees with Idriss et al 2014 who found similar results in Slovakia. The bacteria isolates were generally more sensitive to gentamycin and co-trimoxazole with increased resistance to ampicillin and streptomycin. The finding agrees with results by Gitau et al 2014 found majority of pathogen susceptible to gentamycin but they differ in that the results showed high susceptibility to co-trimoxazole. This can be explained by the fact that ampicillin and streptomycin intramammary infusion have been in use in the country for a while in control of mastitis while gentamycin and kanamycin were recently introduced. Chloramphenicol has been burned for use in food animal in the country hence the higher susceptibility to bacterial isolates.

Conclusions

The study concludes that Staphylococcus aureus is the most predominant bacterial isolates in subclinical mastitis. Ampicillin and streptomycin are the most

resistant antibiotic to the isolated subclinical mastitis bacteria. The study recommends regular susceptibility testing before treatment of mastitis is initiated.

Acknowledgment

The authors appreciate the dairy farmers for their participation this study. We are also grateful to the veterinary paraprofessional for their help in milk sample collection.

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

Conflict of Interest: The authors declare that they have no conflict of interest

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MOLECULAR DETECTION OF PROTOZOAN PARASITES IN TICKS INFESTING CATTLE ENTERING NIGERIA THROUGH A MAJOR TRANS-BOUNDARY ROUTE IN OGUN STATE.

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Abstract

There is a significant influx of cattle to Nigeria from neighbouring countries on daily basis along the trans-boundary areas without any form of veterinary inspection or quarantine. An assessment of protozoan parasite load in the ticks infesting cattle entering the country by hooves through a major trans-boundary route in Ogun State was carried out using Polymerase Chain Reaction and sequencing. The prevalence of *Babesia bovis* and *B. bigemina* in *Boophilus* ticks was 14/92 (15.2%) and 16/92 (17.4%) respectively. There was no detectable band for the *Trypanosoma species* and *Ehrlichia ruminantium* specific PCR. The prevalence of *B. bovis, B. bigemina* and *Theileria annulata* in *Rhipicephalus* ticks were 6/22 (27.3%), 4/22 (18.2%) and 4/22 (18.2%) respectively. The *Amblyomma* and *Hyalomma* ticks screened were not positive for any of the parasites. This is the first report on protozoan parasites detected in ticks infesting cattle entering Nigeria through a major trans-boundary route in Nigeria. The study shows that cattle entering Nigeria from Burkina Faso, Benin Republic, Niger Republic, Mali, Togo and Cote d'Ivoire are infested with adult ticks of various genera harbouring protozoan parasites that are pathogenic to cattle. Hence, we recommend that quarantine centres be established by government to screen and treat infected animals entering the country.

Keywords: Cattle, Nigeria, PCR, Protozoans, Ticks, Trans-Boundary Route

DETECTION MOLECULAIRE DE PARASITES PROTOZOAIRES DES TIQUES INFESTANT LES BOVINS ENTRANT AU NIGERIA PAR UNE VOIE TRANSFRONTALIERE MAJEURE DANS L'ÉTAT D'OGUN

Résumé

Le Nigeria enregistre chaque jour un afflux significatif de bovins en provenance de pays voisins, le long des zones transfrontalières, sans aucune forme d'inspection vétérinaire ou de quarantaine. Une évaluation de la charge parasitaire de protozoaires dans les tiques infestant les bovins entrant dans le pays au sabot par une route transfrontalière majeure dans l'État d'Ogun a été effectuée en utilisant la réaction en chaîne par polymérase et le séquençage. Le taux de prévalence de Babesia bovis et de B. bigemina chez les tiques Boophilus était respectivement de 14/92 (15.2%) et 16/92 (17.4%). Il n'y avait pas de bande détectable pour la PCR spécifique aux espèces Trypanosoma et Ehrlichia ruminantium. La prévalence de B. bovis, B. bigemina et Theileria annulata pour les tiques Rhipicephalus était respectivement de 6/22 (27,3%), 4/22 (18,2%) et 4/22 (18,2%). Les tiques Amblyomma et Hyalomma n'étaient positives pour aucun parasite. Il s'agit du premier rapport sur les protozoaires détectés chez les tiques qui infestent les bovins entrant au Nigeria par une voie transfrontalière majeure. L'étude montre que les bovins entrant au Nigeria en provenance du Burkina Faso, de la République du Bénin, de la République du Niger, du Mali, du Togo et de la Côte d'Ivoire sont infestées de tiques adultes de divers genres, hôtes de parasites protozoaires pathogènes pour les bovins. Ainsi nous recommandons au Gouvernement de mettre en place des centres de quarantaine pour examiner et traiter les animaux infectés entrant dans le pays.

Mots-clés : bovins, Nigeria, PCR, protozoaires, tiques, route transfrontalière

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Introduction

Livestock sector contributes significantly to the growth of Nigerian economy. It accounts for 4.5% to 5.0% of the Gross Domestic Products (GDP) (Shaw and Hoste, 1987). Among these livestock, cattle contribute more than 50% of meat needs (Obi, 1997) and virtually all milk. The livestock population of Nigeria consists of 13.9 million cattle, 22 million sheep, 34 million goats, 3.4 million pigs and over 150 million poultry (RIM, 1993).

The major constraints to profitable livestock production in sub-Sahara African include low productivity of indigenous stock, inadequate nutrition and high incidence of animal diseases (Obi, 1997) and the greatest constraint being the losses as a result of animal diseases (Itard, 1989). Among the diseases, haemoprotozoan parasitic diseases contribute significantly to this problem.

Generally, more than 90% of livestock, especially cattle is in the hands of pastoralists. They respond to the dictates of their immediate environment in terms of food and water availability for their livestock, sometime, they also avoid disease epidemic. This has led to animals being grazed over varying distances, even across countries borders, depending on availability of fodder and water. This system of grazing is commonly referred to as nomadism. The nomads move from one place to another in search of greener pasture and also carry with them the diseases the animals may be incubating and their vectors especially ticks. Such increase in worldwide movement of animals and people is responsible for the globalization of pathogens (De Deken *et al.,* 2007).

In Nigeria, three major diseases are given particular attention. These include Foot-and-Mouth Disease, Trypanosomosis and Dermatophilosis (Talabi *et al.,* 2014) but less attention is paid to tick-transmitted diseases. Among these parasitic diseases, babesiosis, theileriosis and anaplasmosis cause morbidity and mortality in domestic animals especially, cattle. The presence of these diseases also constrains the introduction of exotic breeds of cattle with a view to improving the livestock industry in Nigeria (Talabi *et al.,* 2011).

Nigeria experience significant influx of cattle from neighbouring countries on daily basis by hoof along the trans-boundary areas (Talabi *et al.,* 2011) without any form of veterinary inspection or quarantine. But to meet the growing demand for animal protein in Nigeria, there is need for concerted effort towards controlling and eradication of animal diseases especially those animals coming into the country on hoof along the trans-boundary areas. In this report, we carried out preliminary assessment of protozoan parasite load in the tick infesting cattle that are entering Nigeria on hooves through a major trans-boundary route in Ogun State using Polymerase Chain Reaction (PCR) technique.

Materials and methods

Location of study: The study was carried out along a major trans-boundary route, the Imeko-Atokun route in Yewa division of Ogun State, Nigeria. Imeko is located at a coordinate of 7026'10.14" North and 2050'20.85" East while Atokun is located at a coordinate of7018'43.17" North and 3004'06.41" East and bound to the west by the Republic of Benin, with which the region shares 155 kilometresof International boundary, within latitude 60 15' N and 70 58' N in a deciduous/derived Savannah zone of Nigeria (Onakomaiya *et al.,* 1992).

Ticks collection and identification: A total of 62 herds of cattle entering Nigeria from neighbouring West African Countries (Burkina Faso, Benin Republic, Niger Republic, Mali, Togo and Cote d'Ivoire) by hoof along the Imeko-Atokun route were examined for the presence of adult engorged ticks in October and November 2014. Four animals with ticks per herd of cattle were restrained and some engorged adult ticks on each of the selected animals were collected as described by Okello-Onen et al. (2006) into labelled universal bottles with the permission of the owners and care was taken to minimise discomfort for the animals. Collected tick samples were sorted and identified using MAFF (1977) identification

keys in the Veterinary Parasitology Laboratory at the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria.

Molecular detection of parasites in Tick DNAs

DNA extraction: Three engorged ticks of the same species collected from the same animal were crushed in sterile mortar with a pestle. The debris of the ticks was removed and the haemolymph expressed in the mortar was thoroughly mixed with 400µl of distilled water. Aliquot of the mixture (400µl) was then added to 1.5ml eppendorf tube. Genomic DNA was extracted using Quick-gDNA™ MiniPrep (Zymo Research Corporation, Irvine, CA 92614, U.S.A) as described by the manufacturer. The eluted DNA was stored at -200C until use.

In all, DNA was extracted from 160 tick samples, *Amblyomma* spp. (N=34), *Boophilus* spp. (N=92), *Rhipicephalus* spp. (N=22) and *Hyalomma* spp. (N=12) from 120 heads of cattle coming into Nigeria from Burkina Faso, Benin Republic, Niger Republic, Mali, Togo and Cote d'Ivoire.

Primer sets optimization and parasites detection by Polymerase Chain Reaction: Different sets of primers (Table 1) were selected for optimization based on published evaluations. These sets of primers were optimized with DNA extracted from the blood of cattle and ticks that have being proven in the molecular laboratory of the College of Veterinary Medicine, FUNAAB to be positive for *Babesia bovis*, Babesia bigemina, *Ehrlichia ruminantium*, Trypanosoma spp., Theileria spp. and *Theileria annulata* using PCR.

Amplification was performed in a 20µl final reaction volume containing equivalent of 20ng of genomic DNA, 10mM Tris-HCl, pH 8.3, 1.5mM MgCl2, 50µM KCl, 200µM each of dNTPs, 40ng of each of the primers and 1unit of Thermus aquaticus DNA polymerase (Bioneer USA). The reactions were placed in MJ MiniTM personal cycler (BIORAD, USA). The reaction conditions were as follow: *Babesia bovis* and *B. bigemina* detection involved an initial denaturation at 94oC for 30sec followed by 45 cycles of denaturation at 94oC for 15sec, annealing temperature at 60oC/ 56oC for 15sec, and extension at 68oC for 2 min. This was then followed by a final extension at 68oC for 7 min. E. ruminantium detection involve a nested PCR reactions, the primary and secondary reactions were carried out using primer sets (U24 & L24) and (AB128 & AB129) in reaction conditions; initial denaturation at 94oC for 1 minute, annealing temperature at 550C for 1 minute and extension at 72oC for 2 minutes. For the nested reaction, 5µl of the PCR product from the primary reaction was used as the template under the same PCR conditions as the primary reaction. Trypanosoma spp. detection involved an initial denaturation at 94oC for 5min, followed by 35 cycles of denaturation 94oC for 30sec, annealing at 58oC for 30sec, and extension at 72oC for 30sec. This was then followed by a final extension 58oC for 5 min. Theileria spp. detection involved initial denaturation at 94oC for 5mins followed by 35 cycles of 94oC for 45s, 55oC for 45s and 72oC for 45s and final extension at 72oC for 5mins.

Gel electrophoresis: Ten microliters of the PCR products were electrophoresed through 1% agarose gel in 1 x TAE (40 mM TRIS-acetate and 1 mM EDTA) at 90 V for 45 min. along with 5µl of biological marker, GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, UT, USA). Gels were stained with GelRed(R) Nucleic Acid Stain (PHENIX Research Product, Candler, NC, U.S.A) at 5µl/100ml of the agarose gel suspension. After electrophoresis, the PCR products were visualized using ultra violet trans-illuminator (SpectrolineR TC 312 E).

Data Analysis: The prevalence protozoan parasites detected in ticks were expressed in percentages.

Results

Parasites detection in ticks using PCR: The protozoan parasites detected in ticks using PCR is presented in Table 2. The 160 extracted DNA samples analyzed by 1% agarose gel electrophoresis showed that all yielded strong DNA products. Amplified DNA samples using

the set of primers listed in Table 1 and their analysis on 1% agarose gel electrophoresis revealed 44 (27.5%) out of the 160 tick samples examined to have band sizes of about 1587 bp, 1467 bp, 193 bp and 233 bp which corresponded with the expected band sizes of *B. bovis, B. bigemina*, Theileria spp. and *Theileria annulata* respectively. There was no detectable band for the *Trypanosoma species* and *Ehrlichia ruminantium* specific PCR.

Only the sequences obtained from the *B. bigemina* and Th. annulata positive samples were readable and used for analysis while those of B. bovis were noisy and discarded. Homology search revealed the sequences of *B. bigemina* to have 95% to 98% homologies with those sequences (Accession number: DQ785311 and KU206297) available in GenBank and Th. annulata sequences had 99% homology with sequence (Accession number: KM288517) deposited in the GenBank.

The prevalence of B. bovis and *B. bigemina* in *Boophilus* ticks was 14/92 (15.2%) and 16/92 (17.4%) respectively. The prevalence of *B. bovis, B. bigemina* and Th. annulata in *Rhipicephalus* ticks were 6/22 (27.3%), 4/22 (18.2%) and 4/22 (18.2%), respectively. The *Amblyomma* and *Hyalomma* ticks screened in this study were not positive for any of the parasites.

Discussion

Tick-borne diseases especially babesiosis and anaplasmosis and trypanosomosis are the major parasitic diseases limiting profitable livestock production in Nigeria (Pukuma *et al.,* 2011). These parasitic diseases are majorly transmitted by arthropod vectors such as ticks and haematophagus flies.

The *Amblyomma* spp., *Boophilus* spp., *Rhipicephalus* spp. and *Hyalomma* spp. that were detected on cattle entering Nigeria through trans-boundary route in this study have been reported by Talabi et al. (2011) on cattle reared around major trans-boundary areas of Ogun State. This may be an indication that genera of ticks infesting cattle entering Nigeria through these trans-boundary routes are the major source of tick infestation to indigenous cattle.

The detection of Babesia and Theileria species in engorged ticks infesting cattle entering Nigeria through a major trans-boundary route in Ogun State underscore the importance of animal movement as a major source of spreading parasitic diseases. Though, this study only assessed the protozoan pathogens in fully engorged ticks, it is imperative to check the questing and unfed ticks to ascertain the diversity of protozoan and rickettsial in both the fed and the unfed. This may also shed light on the probable transmission mode of the detected parasites. Though our result could not be compared due to paucity of report on pathogen of ticks infesting cattle entering Nigeria through a major trans-boundary routes, but similar work carried out on tick infesting cattle in Nigeria by Reye et al. (2012) reported Rickettsia, Coxiella, Anaplasma, Ehrlichia, Th. mutans in the ticks sampled but Babesia spp. and Th. annulata were not detected.

The detection of B. bovis and *B. bigemina* in *Boophilus* ticks is in agreement with the reportsof Oliveira-Sequeira et al. (2005) and Oliveira et al. (2008). Contrary to the belief that *Theileria annulata* is vectored by *Hyalomma* ticks alone (Urquhart *et al.,* 1996), we detected the DNA of Th. annulata in the *Rhipicephalus* sampled, though the ability of this tick genus to act as a vector of Th. annulata still needs further investigation.

Detection of Babesia and Th. annulata in the sampled tick is an evidence that trans-boundary incursion of ixodid ticks in to Nigeria may significantly increase the risk of animal tick-borne diseases especially babesiosis and theileriosis which are the major cause of morbidity and mortality in the Nigerian livestock industry.

The *Amblyomma* and *Hyalomma* species screened in this study did not yield DNA for any of the tested primers. Though their vectoral ability for heartwater disease and Mediterranean theileriosis, respectively have been well documented (Peter *et al.,* 2000; Abdigoudarzi, 2013; Kifle and Sori, 2014; Meng *et al.,* 2014).

In conclusion, this is the first report on protozoan parasites detected in ticks infesting cattle entering Nigeria through a major trans-boundary route in Nigeria. The study shows that cattle entering Nigeria from Burkina Faso, Benin Republic, Niger Republic, Mali, Togo and Cote d'Ivoire are infested with adult ticks of various genera. These ticks carry protozoan parasites that are pathogenic to cattle. To reduce the risk of tick borne disease of cattle, it is recommended that government establish effective quarantine centres to screen and treat infected animals entering the country.

Acknowledgements

Funding: Financial support was not received from any funding body for this study. We appreciate the technical support of Mr E. I. Olugbogi of the Department of Veterinary Parasitology and Microbiology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Nigeria

Conflict of interest: The authors declare that they have no conflict of interest.

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SOCIOECONOMIC ROLES AND WELFARE ASPECTS OF DONKEY (EQUUS ASINUS) IN ETHIOPIA

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Abstracts

Given the fact that donkeys contribute to valuable socio-economic activities in small holders agricultural sectors, they are under low welfare condition in developing countries. This is need assessment of the profiles, socioeconomic roles and welfare of donkeys in developing countries like Ethiopia. A total of 384 randomly selected donkeys in selected sites of Ethiopia were assessed for socioeconomic activities and their welfare issues including working day-length. The majorities of donkeys used in the study area are 6-10 year (70.8%), and are male (94.3%), and in moderate body condition (59.1%). Here, they are frequently used for transportation of animal feed, water, constriction material, market and mill, charcoal and fire wood, and salt ban. In addition to these 48.7% of the studied donkeys were also used for ploughing. Average mean ± SD working-hours/day was 11.00±1.00 and days/week was 6.56±0.68. Feed shortage, unfair communication, watering once per day, and outdoor keeping are the commonly observed. However, majorities (86.6%) were kept off-pray-dieter and 72.1% receive Tigray Donkey Health and Welfare Project health services. Donkeys' contribution in farmers' livelihood for poverty alleviation in developing countries worth a lot. They reduces burden failed on the local society mainly on woman's and children but, welfare issues need to do lot in Ethiopia.

Key words: Donkey profile, socioeconomic activity, welfare, transportation, Ethiopia

ROLES SOCIOECONOMIQUES ET ASPECTS DE BIEN-ETRE DE L'ANE (EQUUS ASINUS) EN ÉTHIOPIE

Résumé

Étant donné que l'âne contribue à des activités socio-économiques fort utiles dans les petits secteurs agricoles, il est en très mauvais état de santé dans les pays en développement. Par conséquent, il est nécessaire d'évaluer les profils, les rôles socio-économiques et le bien-être de cet animal dans les pays en développement comme l'Éthiopie. Au total, 384 ânes sélectionnés de manière aléatoire dans certains sites de l'Éthiopie ont été évalués au regard des activités socio-économiques pour lesquelles ils sont utilisés, leurs problèmes de bien-être, y compris la longueur de la journée de travail. La majorité des ânes utilisés dans la zone d'étude sont âgés de 6 à 10 ans (70,8%), et sont mâles (94,3%) et de condition corporelle modérée (59,1%). Dans le pays, ils sont fréquemment utilisés pour le transport des aliments pour animaux, de l'eau, du matériel de construction, d'articles destinés au marché et au moulin, du charbon de bois, du bois de chauffage et du sel. Outre ces activités, 48,7% des ânes étudiés ont également été utilisés pour tirer les charrues. La moyenne ± écart-type d'heures de travail / jour était de 11,00 ± 1,00 et la moyenne de jours / semaine était de 6,56 ± 0,68. La pénurie de nourriture, la communication inadéquate, l'abreuvement une seule fois par jour, et le logement en plein air sont les problèmes couramment observés. Cependant, la majorité (86,6%) a été kept off-prey-dieter et 72,1% bénéficient des services de santé du projet Tigray Donkey Health and Welfare. La contribution de l'âne aux moyens de subsistance des petits exploitants pour la réduction de la pauvreté dans les pays en développement a de la valeur. L'âne allège un fardeau principalement porté par les femmes et les enfants dans la société locale, mais beaucoup reste à faire en ce qui concerne l'amélioration du bien-être de cet animal en Éthiopie.

Mots-clés : profil de l'âne, activité socio-économique, bien-être, transport, Éthiopie

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Introduction

Donkeys are highly appreciated due to their valuable contribution to development strategies (Fernando and Starkey, 1996) for socioeconomic activities of small scale farmers' livelihoods, mainly for transportation of commodity (FAO, 1995) and income-generation (Fernando and Starkey, 1996) through renting, breeding or petty trade. They are cheaper (Admassu and Shiferaw, 2011) and easily thrive on minimally supplemented feeds (Smith and Pearson, 2005). Their affordability, docile nature and better abilities to survive drought in arid areas of tropical Africa and Asia suited them for developing countries (Smith and Pearson, 2005). Ethiopian households keep donkeys mainly for pack services (56%), cart use (26%), homestead pack only (14%) and exclusively for renting, breeding or petty trade 4% (Admassu and Shiferaw, 2011). Fernando and Starkey, (1996) also showed importance of donkey for transportation of commodity in Tigray. Thus, geographic land escape of rural areas of Ethiopia made them the main means of livelihood items transportation (Hassen, 2000). However, in Ethiopia, they receive less attention in terms of feeding, health and other management cares (Catley and Blakeway, 1997) which expose them to a number health (Kumar *et al.,* 2014; Sevendsen, 1997) and welfare problems (Kumar et al, 2014). Marshall and Ali (2004) and Gebreab et al. (2004) also identified the roles of donkeys and challenges they are facing in the Eastern Harerge, Central Tigray and Eastern Shewa parts of Ethiopia. But, no studies were conducted at the Tigray Donkey Health and Welfare Project (TDHWP) converge areas in Adigudem and Quiha sitesof Tigray Regional State, North Ethiopia. Thus, the aims of this study was to assess the profiles, socioeconomic roles and welfare of donkeys in the selected areas Tigray in northern Ethiopia.

Materials and Methods

Description of Study Areas and Population

The study was conducted at Adigudem and Quiha sites in Hintalo Wajirat and Inderta districts, respectively in the North of Tigray Regional State, North Ethiopia (CSA, 2005; TRARDO, 1999). The sites were selected in that they have common boundary (TRARDO, 1999), and are in the area converge of The Tigray Donkey Health and Welfare Project (TDHWP). The areas receive 11.3-39.1.mm3 annual rainfall, have 12-27oC temperature (CSA, 2005), and have a total of 15,224 - 16,358 donkeys. The TDHWP clinic was established and provide services in the areas.

Methods Used

Sampling and sample size determination

The sample size of the study animal was calculated according to Thrusfield (1995) with 50% expected population at 95% of confidence interval and 5% desired precision. Thus, a total of 348 donkeys were randomly selected. In Ethiopia donkeys are owned by private individuals (Curran and Smith, 2005; Gebreab *et al.,* 2004). Thus, in parallel, a total of 384 questionnaires and interview were presented to respective owner of donkey.

Factors of the study

Factors such as study sites, served family size, and factors related to donkeys' such as sex, age using dentition and body condition (Sevendson, 1997) are considered. Considering local conditions of the study area, owners were interviewed for the socioeconomic contribution and animal welfare issues like feed availability, watering, housing, fare communication, keeping from pray-dieters and health care were assessed. Working length of hour per/day and days per/week was also assessed for the socioeconomic contributions (the functional activities) of donkeys in their family livelihood.

Data analysis

Data obtained from the investigation was analyzed using SPSS 16 and STAT 11 (Texas 77845 USA). The percentage and confidence interval were used to determined significances of factors at p-values <0.05 for determining socioeconomic roles and welfare aspects of

donkeys in the area.

Result and Discussion

Donkey Profiles

As shown in Fig.-1, significantly higher number of donkeys in 6-10 year age group (70.8%), male (94.3%) ($p < 0.05$), moderate body condition (59.1%) were used for livelihood activities. Differences between the studied sites within ages, sex and body condition profiles of donkeys were not observed ($p>0.05$). This could be due to similar agro ecologies of the studied sites with common boundary (TRARDO, 1999). The present survey shows 59.1%, 34.6% and 6.3% of donkeys have moderate, ideal and poor body conditions, respectively indicating their better adaptation in the studied site. McLean (2010) also reported the mean body condition score of donkeys 3.6, 5.3, and 8.0 ± 0.3, and mean ages of 9, 4, and 7 ± 3 years for thin (poor), moderate and obese, respectively in Mali. These show the affordability, docile nature and better abilities of donkeys to survive drought in arid and semi arid areas of tropical African countries. Smith and Pearson (2005), NRC (1984), and Swai and Bwanga (2008) also reported such stability and adaptation of donkeys under low management conditions.

Of the total assessed donkeys, 18%, 47.1% and 34.9% assist household family sizes of 1-4, 5-8 and 9-12, respectively. Several studies over a period of years indicated the socioeconomic contribution of donkey in different continents (Fernando and Starkey, 1996; Catley and Blakeway, 1997; Hassen, 2000; Marshall and Ali, 2004; Gebreab *et al.,* 2004; Smith and Pearson, 2005; Admassu and Shiferaw, 2011; McLean, 2010; Kumar *et al.,* 2014). But, none of them indicated the household family size served by a donkey. In this study, regardless of age groups, sex and body condition. Donkeys provide function for different household family size in similar proportion (Fig.-2). But, the productive age groups (6 to 10 year olds) are much more used for livelihood activities. Males (94.3%) are most commonly used. This could be due to the fact that males are preferred for working while females are mostly for preproduction, and also presence of pregnancy and parturition period. Donkeys cannot work for the last five months of the gestation period (Blench *et al.,* 2004).

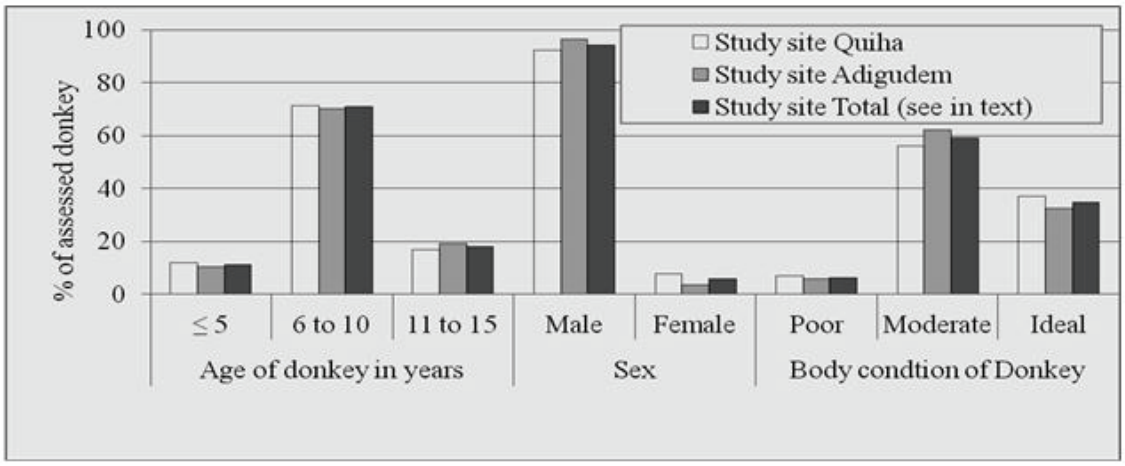

Figure 1: Profiles of the studied donkeys with some of inherent characteristics

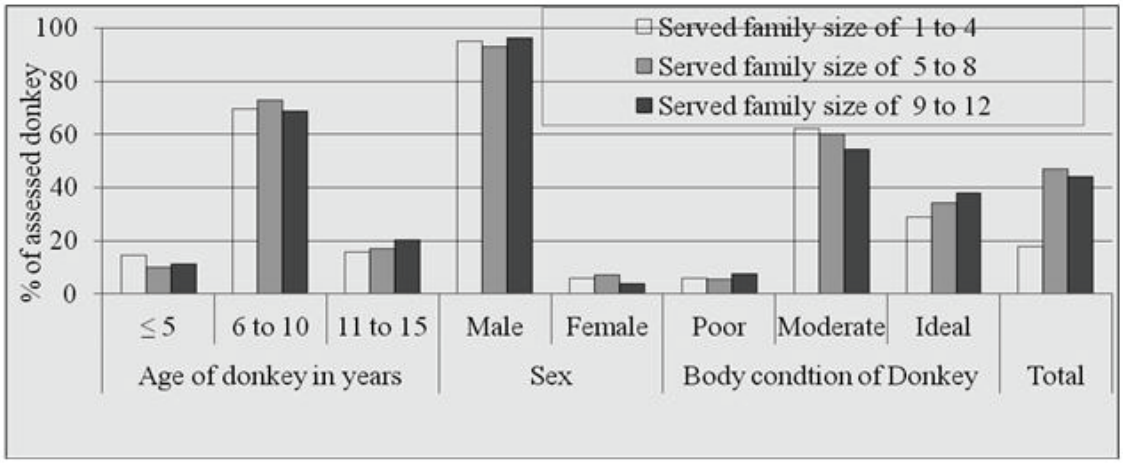

Figure 2: Household family size served by the studied donkeys in relation to inherent characteristics

Socioeconomic Roles of Donkeys

In both studied sites, donkeys were used for multiple activities (Table-1). All ,i.e., (100%) donkeys provided numerous socioeconomic activates in the area. Commonly and frequently, they were used for transportation of animal feed, water, construction material, market and mill, charcoal and fire wood, and salt ban. They are also used for ploughing and renting. Most of these contributions of donkeys for rural development were similar with reports of Fernando and Starkey (1996), FAO (1995) and Hassen (2000). In many African countries like Botswana, Eritrea, Ethiopia, Djibouti, Somalia and others (Rubenstein, 2011; Gebreab *et al.,* 2004; Marshall and Ali, 2004; Mrema, 2004), donkeys are the best alternatives for farm power and transport for low income farmers with poor resource and unable to afford oxen or tractors. Through renting, donkeys provide daily income of the families. Similarly, Gebreab *et al.,* (2004) indicated that trading and renting of donkeys in some other part of Ethiopia is a common practice. Unlike others report in Ethiopia, 48.7% of the studied donkeys were also used for ploughing. This shows extraordinary importance of donkeys for agricultural sectors. Oudman (2002) also indicated that donkey's are better draught resistant than other draught animals. Hence, besides reducing work burden for rural women and girls, donkeys can be used to replace draught oxen in arid and semi arid areas of developing countries like Ethiopia. However, they are an undervalued power source in large part of the world (Oudman, 2002).

Donkey's total mean ± SD working-hours per/day was 11.00±1.00 and working-days per/week was 6.56±0.68 with differences neither in working hours per/day between sites, sex, and among age groups, body conditions, and served sizes family, nor in days per/week for those variables (data is not in this article). Although, Gebreab et al. (2004) reported a relatively low utilization of donkey, the mean ± SD working-hours/day in the present finding showed improvement in the studied area. But, the present working-days/week (6.56±0.68) registered was similar with 5-6 working-days/week reported by Sisay and Tilahun (1997) in Ethiopia.

Welfare Aspect of the Donkeys

Modern donkey farms consisted of sheltered stables with nutritional feed supplements, grazing fields and unrestricted access to water for improving welfare (Meutchieye *et al.,* 2014)however, in Table-2, feed shortage, watering once/day and outdoor keeping in 85.9%, 83.8% and 70.1% of donkeys, respectively, were observed. But, 86.6% were kept from pray-dieter. Overall donkey

Table 1: Socioeconomic activities of donkey in the studies site

Study variables		No. (%) of assessed donkey	Socioeconomic activities of the assessed donkeys in the study area¶					
			Activity A No. (%)	Activity B No. (%)	Activity C No. (%)	Activity D No. (%)	Activity E No. (%)	Activity F No. (%)
Study sites	Quiha	192 (50)	0 (0)	23 (12.0)	12 (6.3)	87 (45.3)	54 (28.1)	16 (8.3)
	Adigudem	192 (50)	2 (1.1)	55 (28.7)	13 (6.8)	100 (52.1)	22 (11.5)	0 (0)
Sex	Male	362 (92.3)	2 (0.6)	70 (19.3)	25 (6.9)	178 (49.2)	71 (19.6)	16 (4.4)
	Female	22 (7.7)	0 (0)	8 (36.4)	0 (0)	9 (40.9)	5 (22.7)	0 (0)
Donkeys age (Yrs)	≤5	43 (11.2)	0 (0)	14 (32.5)	1 (2.3)	20 (46.5)	5 (11.6)	3 (7.0)
	6-10	272 (70.8)	2 (0.7)	56 (20.6)	21 (7.7)	132 (48.5)	49 (18.0)	12 (4.4)
	11-15	69 (18.0)	0 (0)	8 (11.6)	3 (4.4)	35 (50.7)	22 (31.9)	1 (1.5)
Body condition	Poor	24 (6.3)	0 (0)	3 (12.5)	2 (8.3)	8 (33.3)	9 (37.5)	3 (7.0)
	Moderate	227 (59.1)	1 (0.4)	38 (16.7)	5 (2.2)	123 (54.2)	54 (23.8)	12 (4.4)
	Ideal	133 (34.6)	1 (0.6)	37 (27.8)	18 (13.5)	56 (42.1)	13 (9.8)	1 (1.5)
Served family size	1 to 4	69 (18.0)	0 (0)	12 (17.4)	2 (2.9)	35 (50.7)	13 (18.8)	7 (10.1)
	5 to 8	181 (47.1)	2 (1.1)	41 (22.7)	8 (4.4)	90 (49.4)	33 (18.2)	7 (3.9)
	9 to 12	134 (34.9)	0 (0)	25 (18.7)	15 (11.2)	62 (46.3)	30 (22.4)	2 (1.5)
Total		**384 (100)**	**2 (0.5)**	**78 (28.7)**	**25 (6.5)**	**187 (48.7)**	**76 (19.8)**	**16 (4.2)**

¶Activity A = Animal feed, Water, Constriction material, Market and mill (AfWaCmMm), charcoal and fire wood, and salt ban transportation, ploughing and renting.
¶Activity B = AfWaCmMm, charcoal and fire wood, and salt ban transportation, and ploughing
¶Activity C = AfWaCmMm, charcoal and fire wood, and salt ban transportation
¶Activity D = AfWaCmMm, charcoal and fire wood transportation and ploughing
¶Activity E = AfWaCmMm, charcoal and fire wood transportation
¶Activity F = AfWaCmMm transportation

management in the area under observations is similar within study site, sex, age, body condition and serviced family size. Admassu and Shiferaw (2011) also reported shortage of fodder and grazing area as important limiting factors for equine production in selected woredas of Ethiopia.

Unlike others studies (Kumar *et al.,* 2014; Admassu and Shiferaw, 2011; Rubenstein, 2011; McLean, 2010; Smith and Pearson, 2005; Gebreab *et al.,* 2004; Marshall and Ali, 2004; Mrema, 2004; Hassen, 2000; Catley and Blakeway, 1997; Fernando and Starkey, 1996), menace of owners' communication with their donkeys were assessed. All were unfair communication, including biting only (69.0%), biting and verbal (10.9%), and biting, verbal and pocking (20.1%) in Ethiopia. These expose the donkeys to bruising and in sever conditions may open wound which predispose them to infectious diseases and insect bites. Moreover, donkeys may suffer from pain and stress condition of such unfair communication, Moreover, most of them (83.8%) are watered once per day and (70.1%) are kept outdoors. Catley and Blakeway, (1997) and Tegene and Crawford (2000) also indicated the less attention given to donkeys in terms of feeding, health and other management cares which prone them to a number of diseases, mal nutrition, early death, and wound due to absence of protection while using harness (Sevendsen, 1997).

Almost all of the studied working donkeys receive health care either from governmental clinic, traditional healers, and/or TDHWP. All interviewed owners in the area who provided responses for all of the questions raised the issue of health care of donkeys. But, 57.9% of the respondents said nothing in Mekelle City, north Ethiopia (Kumar *et al.,*

Study variables		Total	Food statues No. (%)		Pray dieter keeping No. (%)		Communication No. (%)			Watering per day No. (%)		Housing No. (%)		
			Shortage	Enough	Present	Absent	Biting	Biting and verbal	Biting, verbal and poking	Once	Twice	Indoor	Outdoor	In- and out - doors
Study sites	Quiha	192	149 (77.6)	43 (22.4)	160 (83.3)	32 (16.7)	83 (43.2)	32 (16.7)	77 (40.1)	161 (83.9)	31 (16.2)	34 (17.7)	148 (77.1)	10 (5.2)
	Adigudem	192	181 (94.6)	11 (5.7)	184 (95.8)	8 (4.2)	182 (94.8)	10 (5.2)	0 (0)	161 (83.9)	31 (16.2)	68 (53.4)	121 (63.0)	3 (1.6)
Sex	Male	362	311 (85.9)	51 (14.1)	322 (88.9)	40 (11.1)	252 (69.6)	40 (11.1)	70 (19.3)	304 (84.0)	58 (16.0)	96 (26.5)	253 (69.6)	13 (3.6)
	Female	22	19 (86.4)	3 (13.6)	22 (100)	0 (0)	13 (59.1)	2 (9.1)	7 (31.8)	18 (81.8)	4 (18.2)	6 (27.3)	16 (72.7)	0 (0)
Donkey age (Yrs)	<5	43	40 (93.0)	3 (7.0)	42 (97.7)	1 (2.3)	25 (58.1)	3 (7.0)	15 (34.9)	38 (88.4)	5 (11.6)	5 (11.6)	37 (86.1)	1 (2.3)
	6-10	272	239 (88.9)	33 (12.1)	245 (90.1)	27 (9.9)	184 (67.7)	30 (11.0)	58 (21.3)	225 (28.7)	47 (17.9)	76 (27.6)	186 (68.4)	10 (3.7)
	11-15	69	51 (78.9)	18 (26.1)	57 (82.6)	12 (17.4)	56 (81.2)	9 (13.0)	4 (5.8)	59 (85.3)	10 (14.5)	21 (30.4)	46 (66.7)	2 (2.9)
Body condition	Poor	24	19 (79.2)	5 (20.8)	22 (91.7)	2 (8.3)	19 (79.2)	3 (12.5)	2 (8.3)	20 (83.3)	4 (16.7)	6 (25.00	18 (75.0)	0 (0)
	Moderate	227	192 (89.5)	35 (15.4)	204 (89.9)	23 (10.1)	163 (71.8)	28 (12.3)	36 (15.9)	195 (85.9)	32 (14.1)	55 (24.2)	164 (72.3)	8 (3.5)
	Ideal	133	119 (89.5)	14 (10.5)	118 (88.7)	15 (11.3)	83 (62.4)	11 (8.3)	39 (29.3)	107 (80.5)	26 (19.5)	41 (30.8)	87 (65.4)	5 (3.8)
Family size	1 to 4	69	64 (92.7)	5 (3.7)	64 (92.7)	5 (3.7)	45 (65.2)	5 (7.2)	19 (27.5)	59 (85.5)	10 (14.5)	15 (21.7)	52 (75.4)	2 (2.9)
	5 to 8	181	150 (82.9)	31 (17.1)	164 ()90.6	17 (9.4)	122 (67.4)	15 (8.3)	44 (24.3)	150 (82.9)	31 (17.1)	47 (26.0)	126 (69.6)	8 (4.4)
	9 to 12	134	116 (86.4)	18 (13.4)	116 (86.6)	18 (13.4)	98 (73.1)	22 (16.4)	14 (10.4)	113 (84.3)	21 (15.7)	40 (29.8)	91 (68.0)	3 (2.2)
Total		**384**	**330 (85.9)**	**54 (14.1)**	**344 (89.6)**	**40 (10.4)**	**265 (69.0)**	**42 (10.9)**	**77 (20.1)**	**322 (83.8)**	**62 (16.2)**	**102 (26.6)**	**269 (70.1)**	**13 (3.4)**

Table 3: Health care service for donkey in the studied sites

Study variables		Total	Sources of health services No. (%)			
			Govr't clinic and TDHWP*	TDHWP and traditional healers	Govr't clinic, TDHWP* and traditional healers	TDHWP*
Study sites	Quiha	192	41 (21.4)	26 (13.5)	6 (3.1)	119 (62.0)
	Adigudem	192	18 (9.4)	16 (8.3)	0 (0)	158 (82.3)
Sex	Male	362	57 (15.7)	39 (10.8)	6 (1.7)	260 (71.8)
	Female	22	2 (9.1)	3 (13.6)	0 (0)	17 (77.8)
Age in years	<5	43	8 (18.6)	4 (9.3)	0 (0)	31 (72.1)
	6-10	272	35 (12.9)	28 (10.3)	6 (2.2)	203 (74.6)
	11-15	69	16 (23.2)	10 (14.5)	0 (0)	43 (62.3)
Body condition	Poor	24	5 (20.8)	4 (16.7)	2 (3.8)	13 (54.2)
	Moderate	227	38 (16.7)	26 (11.5)	3 (1.3)	160 (70.5)
	Ideal	133	16 (12.0)	12 (9.0)	1 (0.8)	104 (78.2)
Family size	1 to 4	69	13 (18.8)	3 (4.4)	1 (1.4)	52 (57.4)
	5 to 8	181	28 (15.5)	21 (11.6)	1 (0.6)	131 (72.4)
	9 to 12	134	18 (13.4)	18 (13.4)	4 (3.0)	94 (70.2)
		384	59 (15.4)	42 (10.9)	6 (1.6)	277 (72.1)

**TDHWP = Tigray Donkey Health and Welfare Project*
Govr't = Government

2014). The majorities of the donkeys (72.1%) were provided healthcare from TDHWP only (Table-3). However, Kumar et al. (2014) indicated that 31.6% of the total respondents take the diseased donkeys to veterinary clinic. Like the 10.5% reports by Kumar et al. (2014), 48 (12.5%) respondents uses traditional methods of donkey disease treatment in addition to using of TDHWP and governmental clinics.

In conclusion, numerous socioeconomic activities of donkey were observed in the studied area with especial role for ploughing as an extraordinary role for agricultural sectors. Hence, besides reducing work burden of household family livelihoods on rural women and girls in respect to types of activities and family size, donkeys can be used to replace oxen in arid and semi arid areas of developing countries like Ethiopia. However, the animal welfare practices of feeding, watering, and housing given to them in the sites are quit poor and could not cope up with their intensive contribution. Moreover, the unfared menaces of owner's communication with their donkeys prone to wound, predispose to diseases conditions and create pain stress. To this extent, most of the owners keeping from pray-dieter and TDHWP provides the majorities of health care services. Donkeys' contribution in farmers' livelihood for poverty alleviation in developing countries is immense. In developing countries, they reduce burden failed on the local society mainly on women's and children. But, animal welfare issues need to do lot in Ethiopia.

Acknowledgements

We would like appreciate the well-organized cooperation of donkey's owners in the studied sites for provision of all rounded information and the time they devoted during the interview. We would also like to express our gratitude to the TDHWP teams for creating

suitable study environment and unreserved assistance. Finally, Mr. Ahmed Dedo is highly appreciated for proof reading the manuscript to improve the English language.

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THE EFFECT OF FRESH LEAF *Ocimum gratissimum* AND DRIED BUDS Eugenia caryophyllata EXTRACTS ON THE TISSUES BACTERIOLOGICAL CHANGES OF Clarias gariepinus JUVENILES

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Abstract

The use of antibiotics has lead to antibiotic resistance and residual effects among others in fish tissue thus necessitate the advocate for alternative natural plants for antimicrobial activities in aquaculture. Effect of fresh leaf *Ocimum gratissimum* and dried buds *Eugenia caryophyllata* extracts on the tissues bacteriological changes of Clarias gariepinus juveniles were examined by dietary intake in 2016. Seven experimental diets: control (0.0%), OG1 (0.5%), OG2 (1.0%), OG3 (1.5%), EC4 (0.5%), EC5 (1.0%) and EC6 (1.5%) were formulated to 40% crude protein and fed to fish in triplicates. Fish (10.57±0.46g) were fed twice at 3% body weight for 84 days. Water and fish tissues were examined for total viable control (TVC) and total entearobactericea (TEB). The data were analysed using descriptive and ANOVA. The results revealed that fish fed control diet had higher TVC and TEB than other diet ($p<0.05$). The TVC and TEB also varies greatly among the organs with skin taken the lead, followed by the gill ($p<0.05$). It is therefore suggested that extracts of leaf *Ocimum gratissimum* and dried buds *Eugenia caryophyllata* in the diet of *C. gariepinus* significantly reduced TVC and TEB to acceptable values making it a promising immunostimulants candidate to promote organic aquaculture.

Keywords: Antimicrobial, aquaculture, *Eugenia caryophyllata*, extracts, *Ocimum gratissimum*

EFFET DES EXTRAITS DE FEUILLES FRAICHES d'Ocimum gratissimum ET DE BOURGEONS SECHES d'Eugenia caryophyllata SUR LES CHANGEMENTS DES TISSUS BACTERIOLOGIQUES DE *Clarias gariepinus* JUVENILES

Résumé

L'utilisation d'antibiotiques a entraîné une résistance aux antibiotiques et des effets résiduels entre autres dans le poisson Tissulaire nécessitent donc le plaidoyer en faveur d'autres plantes naturelles pour les activités antimicrobiennes en aquaculture. Effet de la feuille fraîche *Ocimum gratissimum* et des bourgeons séchés *Eugenia caryophyllata* extraits sur les tissus Les changements bactériologiques des juvéniles de Clarias gariepinus ont été examinés par apport alimentaire en 2016. Sept Des régimes expérimentaux: contrôle (0,0%), OG1 (0,5%), OG2 (1,0%), OG3 (1,5%), EC4 (0,5%), EC5 (1,0%) et EC6 (1,5%) ont été formulés à 40% de protéine brute et alimentés en poisson en triple. On a alimenté du poisson (10,57 ± 0,46 g) Deux fois à 3% de poids corporel pendant 84 jours. L'eau et les tissus de poissons ont été examinés pour le contrôle total viable (TVC) Et les entéarobactéries totales (TEB). Les données ont été analysées par analyse et ANOVA. Les resultats Ont révélé que le régime de contrôle alimenté par les poissons avait une TVC et une TEB plus élevées que les autres aliments ($p <0,05$). Le TVC et le TEB Varie également beaucoup parmi les organes avec la peau prise le plomb, suivie par la branchie ($p <0.05$). Il est donc A suggéré que les extraits de feuilles *Ocimum gratissimum* et les bourgeons séchés *Eugenia caryophyllata* dans l'alimentation de *C.gariepinus* réduit significativement TVC et TEB à des valeurs acceptables en faisant un immunostimulants prometteurs Candidat à la promotion de l'aquaculture biologique.

Mots clés: *Antimicrobien*, aquaculture, *Eugenia caryophyllata*, extraits, *Ocimum gratissimum*

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Introduction

Fish food supply has been reported to be capable of harbouring bacteria including both pathogenic and non-pathogenic bacteria. Pathogenic bacteria are receiving more attention from scientists resulting in increase in researches and publications. In recent times pathogenic bacteria like Aeromonas, Listeria, Streptococcus and Pseudomonas species have been isolated from raw fish (Al-Harbi 1994; Varvarigos 1997; Narvaez et al. 2010; Bello et al. 2012; Adeshina et al. 2016). Bacteria load in fish beyond acceptable values has become a burden in fish business resulting in product retention and rejection. The pattern of spread of pathogenic bacteria from fish and other seafood are a major concern/dangerous issue globally. The Rapid Alert System for Food and Feed (RASFF) reported that fish is second only to vegetable in terms of alerts recorded between 2009 and 2012, which place the fish on attention drawn list in Europe (Jami et al. 2014). RASFF in 2012 reported that import rejection of seafood was approximately 15% out of total product rejection (Anonymous 2013). In international trade, the major reason for product rejection is bacterial pathogens (Ababouch et al. 2005). It is worth noting that the proportion of fish and fish products that originate from developing countries is huge and most of these countries do not effectively monitor the bacteria load in product originated from its nation thus leading to product rejections in international trade and bankruptcy.

The chemicals and antibiotics used in the control of bacteria load are expensive, may/will leave residue and cause side effects. More so, residual effect caused by continuous use of chemicals raises a serious concern. It is also reported that bacteria develops resistant to these chemical as a result of continuous and indiscriminate usage. The need for concerted effort for immediate use and adoption of immunostimulants in the prevention, control and management of pathogenic organism cannot be overemphasized as a replacement for antibiotics bearing in mind the potential threat of diseases to human and animal health. The common say "you are what you eat" is a word of wisdom i.e the safety of human populace directly depends on the safety of what we consumed especially from fish and fish products is moving the farming business into the "new world" called organic aquaculture. In the previous studies, it has been reported that use of immunostimulants are capable of enhance and improving the health study of fish by catalyze the activities of non-specific defense mechanism (Li et al. 2004; Cerezula et al. 2009; Yin et al. 2009; Bello et al. 2012).

In Nigeria, use of medicinal plants in health management is very common locally, because traditional ways of treating diseases are well established. The healing ability of medicinal plants has contributed to the pharmaceutical industries. Plants from Lamiaceae family are commonly use in trado-medicine from time immemorial. *Ocimum gratissimum* L (Lamiaceae), commonly also known as "African Basil" and E. caryophyllata commonly also known as "clove" have been use in the treatment of different diseases in Nigeria. The plants contain phenolic compounds, which possess antimicrobial activity. These compounds were classified as generally recognized as safe (GRAS) as such could be used in the control and management of bacterial in food (Bayoub et al. 2012). Despite the promise potential of these plants there is paucity of information in uses of fresh leaf *Ocimum gratissimum* and dried buds *Eugenia caryophyllata* on bacteriological changes of *C. gariepinus*.

With a noticeable increase in the consumption pattern of fresh fish especially African Catfish (Clarias gariepinus) in Nigeria directly proportional to the increase in the number of restaurants and relaxation centres there is therefore, the needs for compounds that are naturally derived which have antimicrobial properties necessitate this study. This aim of this study was to evaluates the potential fresh leaf *Ocimum gratissimum* and dried buds *Eugenia caryophyllata* extracts on bacteriological changes of Clarias gariepinus juveniles.

Materials and Methods

Experimental procedure and design

A total number of four hundred and twenty (420) fish of average body weight of 10.57±0.46g were used for the experiment. The fish were acclimatized for 2 weeks in plastic tanks before the experiment. Fish were weighed and distributed in to twenty-one rectangular plastic tanks (60 x 38 x 27 cm) tanks in a completely randomized design. Each tank contained twenty fish. The experiment had six treatments and a control with three replicates. Water was replaced on three days interval, fed at 3% body weight daily (1.5 % was given in the morning by 8:00am and 1.5 % in the evening by 5:00pm) throughout the period of experimentation. Measurement of the weight changes were performed fortnightly and the feeding rate were adjusted accordingly with respect to the new body weight. The experiment lasted for 12 weeks from July to September 2016.

Plant collection and identification

The fresh leaves of O. gratissimum and E. caryophyllata L. were collected from a backyard at Agbowo and "Oja Oba" Ibadan respectively in the month of October, 2015. Ibadan is located on 10°23'0"N, 12°5'0"E. The plants were identified in the Herbarium Unit of Forest Research Institute of Nigeria (FRIN), Ibadan. The fresh O. gratissimum specimen was placed in Forest Herberium Ibadan under voucher registration FHI 110548 while E. caryphyllata specimen was placed under voucher registration FHI 110603.

Extraction of plants materials

The plants were immediately transported for extraction in the Department of Aquaculture and Fisheries Management, University of Ibadan in a sterilized nylon. The leaves were trimmed and cut into smaller pieces using scissors while the E. caryophyllata were breaking into smaller pieces in a hand-mortar and pestle. About 5g of O. gratissimum and E. caryophyllata were weighed on a digital ScoutPro sensitive scale, covered with sterilized cotton wool and transferred into Soxhlet apparatus. Exactly 170ml of Ethyl-acetate was poured in a spiral tube of the equipment. The extraction last for six hours until no further drop of extract in triplicates (Matasyoh et al. 2007; Hema et al. 2010; Bayoub et al. 2012). The filtrate were concentrated on a rotary evaporator at 30 - 45°C for chemicals elimination and stored until use.

Preparation of experimental diet

The experimental diets were prepared using standard method to formulate 40% crude protein containing 0.0%, 0.5% 1.5% of the extracts. The gross composition of the feed are: fish meal (30.83%), soybean (30.83%), maize (33.84, 33.34, 32.84, 32.34, 33.34, 32.84 and 32.34% for Control, OG1, OG2, OG3, EC4, EC5 and EC6 respectively), starch (1.00%), vegetable oil (1.50%) and premixes* (2.00%) and proximate composition of the experimental diets were shown in Table 1.

Microbiological analysis

Water and fish organs (liver, kidney, gill, intestine, flesh/muscle and spleen) were aseptically collected and weighed into sterile universal bottles while skin samples were collected using skin swab. The Samples were inserted into peptone water (0.1 %) and allowed to release the available bacterial for a period of 2-3 hours. One ml was taken from each sample bottles and diluted in ten folds and subsequently serially diluted with dilution f actor 10-4. Two ml were taken from each sample and dispensed into two petri dishes (1ml to each). The first dish received plate count agar (PCA) for Total Bacteria Count(LAB M, LAB149) while the second petri dish was treated with MacConkey agar (LAB M, LAB002) for total coliform count using the pure plate count method. The media were prepared according to manufacturers' instruction. Each dilution was overlaid with PCA and MacConkey respectively that has been cooled to 500C. At this temperature, agar is still in liquid form. The dishes were then gently swirled to mix the bacteria with the liquid agar (Nester et al. 2004). The mixtures were allowed to harden. When the mixture

Table 1: Proximate composition of experimental diets

Diets	Crude protein	Ether extract	Moisture	Ash	CHO	Fibre
Control	40.23±0.21^{ab}	9.27±0.15^{a}	9.17±0.12^{c}	8.27±0.06^{a}	20.17±0.61^{a}	12.90±0.56^{a}
OG1	39.97±0.16^{a}	9.50±0.10^{ab}	8.83±0.12^{ab}	8.56±0.16^{bc}	20.37±0.77^{a}	12.76±0.62^{a}
OG2	40.53±0.35^{ab}	9.63±0.15^{ab}	9.17±0.15^{c}	8.53±0.10^{bc}	20.00±0.92^{a}	11.97±1.46^{a}
OG3	40.80±0.56^{c}	9.50±0.17^{ab}	8.76±0.15^{a}	8.53±0.15^{bc}	20.13±0.96^{a}	12.90±0.50^{a}
EC4	40.27±0.15^{ab}	9.40±0.10^{ab}	8.53±0.15^{a}	8.50±0.10^{ab}	20.30±0.20^{a}	13.00±0.20^{a}
EC5	40.43±0.45^{ab}	9.37±0.15^{a}	8.83±0.31^{ab}	8.50±0.17^{ab}	20.40±0.17^{a}	12.47±1.04^{a}
EC6	40.97±0.68^{c}	9.40±0.10^{ab}	9.17±0.15^{c}	8.77±0.15^{c}	19.17±0.83^{a}	12.53±0.35^{a}

Note: CHO = Carbohydrate

Note: Premixes = Premixes = HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; Nicotinic acid 18000 mg; Pantothenic acid, 8000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Choline chloride 120,000 mg; Iron, 8000 mg; Copper, 800 mg; Manganese, 6000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C C(coated), 60,000 mg; Inositol, 10,000 mg; Colbat, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg.*

is hardened, the individual cells are fixed in place and incubated (Newlife Laboratory Incubator NL-9052-1) for 24 hours at 370C to allow a distinguish colonies to form. The colonies formed were counted using Wincom Colony Counter (16W,220V±10%, 50Hz). The experiments were replicated three times. The total viable count and total enterobacteriacea were expressed in Log10CFU/g (APHA 1985; Hitchins et al. 1995; Bello et al. 2012).

Statistical analysis

Data obtained were expressed in logarithm base ten (10) subjected to on-way analysis of variance (ANOVA) and the mean individual were separated using Duncan multiple range test using IBM SPSS package (version 20).

Results

Microbiological analyses of experimental water

The control diet recorded highest total viable bacteria count (6.23±0.31, 6.36±0.03 and 5.25±0.14 Log10CFU/ml) and enterobacteriacea (6.03±0.09, 6.11±0.10 and 5.19±0.60 Log10CFU/ml) at day 28, 56 and 84 respectively. The lowest TVC (5.82±0.03, 5.45±0.12 and 4.45±0.02 Log10CFU/ml) and TEB (5.43±0.16, 5.06±0.08 and 4.01±0.17 Log10CFU/ml) were recorded in EC6 at day 28, 56 and 84 respectively. The TVC and TEB recorded were statistically significant different ($p<0.05$) among the treatments (Table 2).

Microbiological analyses of C. gariepinus juveniles fed fresh O. gratissimum leaves extract for 84 days

Fish treated with the control diet had highest total viable count and total enteaerobacterica when compared with treatment OG1, OG2 and OG3 throughout the study period. There was also a progressive increase in the bacterial load in the group fed control diet. On the skin, the highest TVC were recorded in fish fed control (6.26±0.20, 6.30±0.01 and 6.45±0.15 Log10CFU/g on day 28, 56 and 84 respectively), gill (6.18±0.10, 6.23±0.09 and 6.34±0.05 Log10CFU/g on day 28, 56 and 84 respectively), intestine (5.82±0.03, 5.85±0.05 and 6.01±0.07 Log10CFU/g on day 28, 56 and 84 respectively), liver (5.66±0.06, 5.72±0.05 and 5.79±0.02 Log10CFU/g on day 28, 56 and 84 respectively), spleen (5.16±0.08, 5.31±0.05 and 5.33±0.03 Log10CFU/g on day 28, 56 and 84 respectively), kidney (5.43±0.13, 5.50±0.06 and 5.59±0.04 on day 28, 56 and 84 respectively) and muscle (5.19±0.09, 5.42±0.03 and 5.55±0.04 Log10CFU/g on day 28, 56 and 84 respectively) and TEB on skin was highest in control (5.76±0.04, 5.86±0.02 and 6.45±0.15 Log10CFU/g on day 28, 56 and 84 respectively), gill (5.75±0.05, 5.80±0.03 and 6.11±0.09

Table 2: Total viable and enterobacteriacea count of experimental water samples treated O. gratissimum leaves and E. caryophyllata buds extracts for 84 days

Treatments	Day 28 (Log_{10}CFU/ml)		Day 56 (Log_{10}CFU/ml)		Day 84 (Log_{10}CFU/ml)	
	TVC	TEB	TVC	TEB	TVC	TEB
Control	6.23±0.31^{c}	6.03±0.09^{a}	6.36±0.03^{d}	6.11±0.10^{a}	5.25±0.14^{a}	5.19±0.60^{a}
OG1	6.05±0.06^{b}	5.81±0.10^{a}	5.94±0.09^{c}	5.57±0.04^{a}	5.16±0.13^{b}	4.43±0.21^{b}
OG2	5.97±0.03^{b}	5.73±0.14^{a}	5.55±0.07^{a}	5.29±0.03^{b}	5.11±0.11^{b}	4.01±0.07^{c}
OG3	5.77±0.02^{a}	5.64±0.07^{a}	5.50±0.13^{a}	5.16±0.13^{b}	5.08±0.13^{c}	4.03±0.13^{c}
EC4	6.21±0.04^{c}	5.83±0.06^{a}	5.98±0.03^{c}	5.54±0.10^{a}	5.28±0.05^{a}	4.22±0.09^{d}
EC5	6.05±0.08^{b}	5.69±0.14^{a}	5.98±0.10^{b}	5.35±0.08^{b}	5.05±0.11^{c}	4.11±0.08^{d}
EC6	5.82±0.03^{a}	5.43±0.16^{b}	5.45±0.12^{a}	5.06±0.08^{a}	4.45±0.02^{d}	4.01±0.17^{c}

Values are represented as mean ± standard deviation; different superscripts letters in the same column are statistically significant different between means at P < 0.05.

Log10CFU/g), intestine (5.38±0.06, 5.62±0.05 and 5.66±0.11 Log10CFU/g on day 28, 56 and 84 respectively), liver (5.17±0.08, 5.43±0.06 and 5.79±0.02 Log10CFU/g on day 28, 56 and 84 respectively), spleen (4.52±0.24, 5.08±0.16 and 5.16±0.06 Log10CFU/g on day 28, 56 and 84 respectively), kidney (5.00±0.18, 5.26±0.06 and 5.35±0.03 Log10CFU/g on day 28, 56 and 84 respectively) and muscle (4.60±0.21, 4.99±0.13 and 5.12±0.07 Log10CFU/g on day 28, 56 and 84 respectively) while the least were observed in TVC and TEB were recorded in fish fed OG36. However, skin and gill had higher occurrence of TVC and TEB across the treatments. The results revealed that in the control the level of TVC and TEB were increase and reducing in other treatments. There were statistically significantly differences ($p<0.05$) in the TVC and TEB among the treatments (Table 3).

Microbiological analyses of C. gariepinus juveniles fed dried E. caryophyllata buds extracts for 84 days

Fish treated with the control diet had highest total viable count and total entearobacterica when compared with treatments EC4, EC5 and EC6 throughout the study period. There was stead decrease in bacterial load of the fish with respect to extract concentration in the diets. The highest TVC and TEB were observed on the skin, gill, intestine, liver, spleen, kidney and muscle in the group treated control diet while the least were observed in group fed EC6. The least TVC on skin (5.32±0.04, 4.49±0.20 and 4.62±0.15 Log10CFU/g on day 28, 56 and 84 respectively) gill (5.16±0.12, 4.93±0.03 and 4.62±0.15), intestine (4.96±0.10, 4.63±0.06 and 4.10±0.17), liver (5.06±0.12, 4.79±0.10 and 4.36±0.10), spleen (5.06±0.06, 4.84±0.11 and 4.26±0.24), kidney (4.83±0.04, 4.36±0.10 and 4.36±0.31) and muscle (4.83±0.16, 4.64±0.19 and 4.20±0.12) and least TEB on the skin (4.88±0.24, 4.26±0.24 and 4.00±0.00 Log10CFU/g on day 28, 56 and 84 respectively) gill (4.83±0.13, 4.42±0.10 and 4.10±0.17 Log10CFU/g on day 28, 56 and 84 respectively), intestine (4.55±0.30, 4.20±0.31 and 4.00±0.00 Log10CFU/g on day 28, 56 and 84 respectively), liver (4.67±0.60, 4.32±0.28 and 4.10±0.20 Log10CFU/g on day 28, 56 and 84 respectively), spleen (4.19±0.30, 4.36±0.10 and 4.00±0.29 Log10CFU/g on day 28, 56 and 84 respectively), kidney (4.20±0.17, 4.00±0.23 and 3.92±0.52 Log10CFU/g on day 28, 56 and 84 respectively) and muscle (4.26±0.24, 4.10±0.32 and 4.10±0.23 Log10CFU/g on day 28, 56 and 84 respectively). The results revealed that in the control the level of TVC and TEB were increase and reducing in other treatments. There were statistically significantly differences ($p<0.05$) in the TVC and TEB among the treatments (Table 4).

Table 3: Total viable and enterobacteriacea count of experimental water samples fed fresh O. gratissimum leaves extracts for 84 days

Treatments		Week 28 (Log_{10}CFU/g)		Week 56 (Log_{10}CFU/g)		Week 84 (Log_{10}CFU/g)	
		TVC	TEB	TVC	TEB	TVC	TEB
Control	Skin	6.26±0.20^{f}	5.76±0.04^{d}	6.30±0.01^{e}	5.86±0.02^{d}	6.45±0.15^{d}	6.00±0.03^{b}
	Gill	6.18±0.10^{e}	5.75±0.05^{e}	6.23±0.09^{a}	5.80±0.03^{e}	6.34±0.05^{a}	6.11±0.09^{e}
	Intestine	5.82±0.03^{c}	5.38±0.06^{c}	5.85±0.05^{e}	5.62±0.05^{c}	6.01±0.07^{e}	5.66±0.11^{c}
	Liver	5.66±0.06^{c}	5.17±0.08^{d}	5.72±0.05^{d}	5.43±0.06^{d}	5.79±0.02^{d}	5.47±0.05^{c}
	Spleen	5.16±0.08^{ab}	4.52±0.24^{a}	5.31±0.05^{bc}	5.08±0.16^{d}	5.33±0.03^{d}	5.16±0.06^{d}
	Kidney	5.43±0.13^{d}	5.00±0.18^{cd}	5.50±0.06^{d}	5.26±0.06^{d}	5.59±0.04^{d}	5.35±0.03^{d}
	Muscle	5.19±0.09^{b}	4.60±0.21^{bc}	5.42±0.03^{d}	4.99±0.13^{c}	5.55±0.04^{d}	5.12±0.07^{c}
OG 1	Skin	5.60±0.15^{e}	5.91±0.01^{d}	5.88±0.20^{d}	5.79±0.03^{d}	5.67±0.12^{c}	5.57±0.04^{c}
	Gill	6.13±0.25^{e}	5.96±0.03^{d}	5.80±0.04^{b}	5.64±0.07^{a}	5.71±0.08^{c}	5.42±0.07^{d}
	Intestine	5.79±0.13^{c}	5.30±0.07^{c}	5.63±0.08^{d}	5.13±0.08^{d}	5.31±0.08^{d}	5.02±0.80^{b}
	Liver	5.69±0.04^{c}	5.31±0.05^{d}	5.34±0.60^{b}	5.19±0.08^{c}	5.21±0.11^{c}	4.96±0.12^{b}
	Spleen	5.64±0.06^{c}	5.36±0.06^{d}	5.41±0.08^{c}	5.13±0.12^{d}	5.12±0.08^{cd}	4.72±0.22^{c}
	Kidney	5.46±0.20^{d}	5.23±0.21^{d}	5.31±0.03^{c}	5.13±0.04^{d}	5.02±0.02^{c}	4.91±0.06^{c}
	Muscle	5.25±0.02^{b}	4.93±0.08^{d}	5.18±0.06^{c}	4.90±0.50^{c}	4.96±0.07^{c}	4.09±0.09^{b}
OG 2	Skin	5.79±0.03^{d}	5.49±0.07^{c}	5.50±0.03^{c}	5.13±0.06^{c}	5.36±0.15^{b}	4.65±0.16^{d}
	Gill	5.55±0.08^{c}	5.32±0.05^{c}	5.27±0.06^{c}	4.83±0.13^{b}	5.12±0.05^{d}	4.42±0.10^{bc}
	Intestine	5.38±0.07^{b}	5.07±0.11^{b}	4.96±0.70^{b}	4.66±0.01^{b}	4.46±0.15^{b}	4.20±0.17^{a}
	Liver	5.25±0.07^{b}	4.95±0.14^{a}	5.00±0.03^{c}	4.37±0.12^{b}	4.82±0.10^{b}	4.10±0.17^{a}
	Spleen	5.31±0.11^{b}	4.83±0.16^{bc}	5.18±0.06^{b}	4.59±0.11^{b}	4.96±0.10^{c}	4.46±0.15^{b}
	Kidney	5.16±0.04^{bc}	4.78±0.16^{bc}	4.82±0.10^{b}	4.30±0.30^{ab}	4.36±0.10^{a}	4.00±1.30^{ab}
	Muscle	5.15±0.05^{a}	4.84±0.06^{cd}	4.78±0.08^{ab}	4.52±0.07^{b}	4.58±0.17^{b}	4.20±0.11^{a}
OG 3	Skin	5.41±0.05^{c}	5.12±0.07^{b}	4.86±0.09^{b}	4.62±0.15^{b}	4.64±0.19^{a}	4.10±0.17^{a}
	Gill	5.38±0.08^{b}	5.05±0.06^{b}	5.03±0.02^{d}	4.59±0.11^{c}	4.86±0.09^{b}	4.26±0.24^{ab}
	Intestine	5.05±0.09^{a}	4.64±0.19^{a}	4.86±0.90^{b}	4.37±0.01^{b}	4.42±0.10^{b}	4.02±0.71^{a}
	Liver	5.05±0.06^{a}	4.59±0.28^{b}	4.81±0.13^{a}	4.36±0.11^{a}	4.36±0.10^{a}	4.10±0.31^{a}
	Spleen	4.98±0.19^{a}	4.55±0.13^{ab}	4.81±0.13^{a}	4.16±0.28^{a}	4.55±0.12^{b}	4.10±0.12^{a}
	Kidney	4.98±0.07^{ab}	4.62±0.15^{b}	4.96±0.24^{b}	4.03±0.13^{ab}	4.63±0.013^{b}	4.20±0.15^{a}
	Muscle	4.84±0.06^{a}	4.20±0.17^{a}	4.16±0.10^{a}	4.10±0.78^{a}	4.36±0.01^{ab}	4.00±0.18^{a}

Values are represented as mean ± standard deviation; different superscripts letters in the same column are statistically significant different between means at $P < 0.05$.

Table 4: Total viable and enterobacteriacea count of experimental water samples fed dried E. caryophyllata buds extracts for 84 days

Treatments		Week 28 (Log_{10}CFU/g)		Week 56 (Log_{10}CFU/g)		Week 84 (Log_{10}CFU/g)	
		TVC	TEB	TVC	TEB	TVC	TEB
Control	Skin	6.26±0.20^{f}	5.76±0.04^{d}	6.30±0.01^{e}	5.86±0.02d	6.45±0.15^{d}	6.00±0.03^{b}
	Gill	6.18±0.10^{e}	5.75±0.05^{e}	6.23±0.09^{a}	5.80±0.03e	6.34±0.05^{a}	6.11±0.09^{e}
	Intestine	5.82±0.03^{c}	5.38±0.06^{c}	5.85±0.05^{e}	5.62±0.05c	6.01±0.07^{e}	5.66±0.11^{c}
	Liver	5.66±0.06^{c}	5.17±0.08^{d}	5.72±0.05^{d}	5.43±0.06d	5.79±0.02^{d}	5.47±0.05^{c}
	Spleen	5.16±0.08^{ab}	4.52±0.24^{a}	5.31±0.05^{bc}	5.08±0.16d	5.33±0.03^{d}	5.16±0.06^{d}
	Kidney	5.43±0.13^{d}	5.00±0.18^{cd}	5.50±0.06^{d}	5.26±0.06^{d}	5.59±0.04^{d}	5.35±0.03^{d}
	Muscle	5.19±0.09^{b}	4.60±0.21^{bc}	5.42±0.03^{d}	4.99±0.13^{c}	5.55±0.04^{d}	5.12±0.07^{c}
OG 1	Skin	5.93±0.21^{b}	5.52±0.04^{c}	5.51±0.06^{c}	5.32±0.78^{c}	5.48±0.05^{bc}	4.88±0.08^{e}
	Gill	5.78±0.06^{d}	5.41±0.05^{c}	5.60±0.03^{e}	5.06±0.06^{b}	5.31±0.30^{e}	4.62±0.15^{c}
	Intestine	5.85±0.04^{c}	5.49±0.04^{c}	5.52±0.50^{c}	5.35±0.06^{e}	5.32±0.09^{c}	4.99±0.05^{b}
	Liver	5.70±0.03^{c}	5.33±0.05^{d}	5.46±0.23^{b}	5.01±0.07^{c}	5.14±0.02^{c}	4.81±0.13^{b}
	Spleen	5.28±0.25^{b}	5.04±0.20^{c}	5.15±0.14^{b}	4.91±0.13^{a}	4.40±0.17^{ab}	4.00±0.10^{a}
	Kidney	5.52±0.05^{d}	5.06±0.21^{cd}	5.34±0.08^{c}	4.72±0.22^{c}	4.92±0.13^{c}	4.33±0.35^{b}
	Muscle	5.19±0.21^{b}	5.02±0.11^{d}	5.17±0.06^{c}	4.53±0.21^{b}	4.92±0.13^{c}	4.10±0.37^{a}
OG 2	Skin	5.35±0.04^{a}	5.01±0.17^{ab}	4.81±0.13^{b}	4.49±0.20^{ab}	4.83±0.12^{a}	4.10±0.17^{a}
	Gill	5.49±0.03^{bc}	5.09±0.06^{b}	5.11±0.04^{f}	4.73±0.05^{bc}	4.86±0.09^{b}	4.40±0.72^{bc}
	Intestine	5.23±0.16^{b}	5.95±0.17^{b}	4.83±0.32^{b}	4.55±0.13^{b}	4.36±0.10^{b}	3.10±0.32^{a}
	Liver	5.18±0.65^{ab}	4.88±0.03^{a}	4.82±0.07^{a}	4.36±0.11^{a}	4.49±0.10^{a}	4.10±0.22^{a}
	Spleen	5.07±0.17^{ab}	4.81±0.31^{abc}	4.93±0.10^{a}	4.20±0.17^{a}	4.05±0.22^{ab}	4.10±0.73^{a}
	Kidney	5.17±0.08^{c}	4.86±0.09^{bc}	4.94±0.10^{b}	4.59±0.29^{bc}	4.67±0.06^{b}	4.20±0.71^{ab}
	Muscle	5.08±0.12^{b}	4.40±0.14^{ab}	4.91±0.08^{b}	4.20±0.17^{a}	4.36±0.10^{ab}	4.00±0.35^{a}
OG 3	Skin	5.32±0.04^{a}	4.88±0.24^{a}	4.49±0.20^{a}	4.26±0.24^{a}	4.62±0.15^{a}	4.00±0.00^{a}
	Gill	5.16±0.12^{a}	4.83±0.13^{a}	4.93±0.03^{g}	4.42±0.10^{d}	4.62±0.15^{f}	4.10±0.17^{a}
	Intestine	4.96±0.10^{a}	4.55±0.30^{a}	4.63±0.06^{a}	4.20±0.31^{a}	4.10±0.17^{a}	4.00±0.00^{a}
	Liver	5.06±0.12^{a}	4.67±0.60^{b}	4.79±0.10^{a}	4.32±0.28^{a}	4.36±0.10^{a}	4.10±0.20^{a}
	Spleen	5.06±0.06^{ab}	4.19±0.30^{abc}	4.84±0.11^{a}	4.36±0.10^{ab}	4.26±0.24^{a}	4.00±0.29^{a}
	Kidney	4.83±0.04^{a}	4.20±0.17^{a}	4.36±0.10^{a}	4.00±0.23^{a}	4.36±0.31^{a}	3.92±0.52^{a}
	Muscle	4.83±0.16^{a}	4.26±0.24^{a}	4.64±0.19^{a}	4.10±0.32^{a}	4.20±0.12^{a}	4.10±0.23^{a}

Values are represented as mean ± standard deviation; different superscripts letters in the same column are statistically significant different between means at P < 0.05.

Discussion

The microbial load decreased in the treatments but increases in the control. The results followed the similar trend as recorded by Bello et al. (2012) which suggest the effectiveness of the plants extract as antimicrobial substances. This agree with Shalaby et al. (2006) who reported in his study using Orechromis niloticus fed diet fortified with garlic and chloraphenicol. The values recorded in this study falls within the observations of Sugita et al. (1989) and ICMSF (1982) that bacterial count in growing water ranges between 4.00 and 5.70 Log10CFU/ml. The results OG2, OG3, EC5 and EC6 were lower than the values recorded by McKeon et al. (2001) and Bello et al. (2012) on day 56 and 84 which could be attributed to the higher

phenolic compounds. Furthermore, the filtrate nature of the extract contrary to the residue used by Bello et al. (2012) easily gets absorb by the cell membrane of the bacterial hence has more inhibition or bacteriocidal effect on the organism.

TVC and TEB of the fish body tissues were significantly decreases among the treatments reflecting the effectiveness of the plants extract as antimicrobial agents except in control. Across the sampled organs (skin, gill, liver, spleen, kidney and muscle), fish treated with diet OG2, OG3, EC5 and EC6 had lower TVC and TEB similar to the values recorded by Salaby et al. (2006), and Bello et al. (2012) whose recorded values ranges between 4.78 – 5.69 log10CFU/g. In most organs, the values observed were lower than the values recorded by Al-Harbi and Uddin, (2003) who recorded up to 7.76 Log10CFU/g. The present of eugnol, thymol, p-cyeme etc in high percentages might be responsible for the difference. The presence of some metabolites such as tannin in high quantities could also be responsible for the ability to reduce bacteria growth in the fish body system.

The bacteriological study revealed fresh leaves of O. gratissimum and dried buds E. caryophyllata reduced the microbial load in water and fish tissues as the inclusion level increased with days. International Commission on the Microbiological Specification of Foods (ICMSF) in 1982 recommended that acceptable level of bacterial load in food should be between 102 – 107per gram or cm2 equivalent to about 5.70 Log10cfu/cm2 for skin while FAO, (1979) recommended 105 per gram for other part of fish as acceptable level thus extracts of fresh leaves of O. gratissimum and dried buds E. caryophyllata are said to be a good agent in control and management of bacteria load in fish tissues and promote organic aquaculture.

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TOXICITY EFFECT OF AFRICAN MESQUITE (PROSOPIS AFRICANA) SEED ON AFRICAN CATFISH (CLARIAS GARIEPINUS) JUVENILES

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Abstract

Toxicity test of African Mesquite (Prosopis Africana) seed on African Catfish (Clarias gariepinus) was carried out to determine the acute lethal concentration (LC50) in 24 – 48 hours. Two hundred and fifty (250) juveniles were acclimatized in the laboratory for 14days. Ten (10) fishes were placed in each of the experimental plastic containers, containing test concentrations of 0 ml, 10 ml, 20 ml, 40 ml, 80 ml, 160 ml, 320 ml and 640 ml/liter of water.

The 24 and 48 hours LC50 of Prosopis africana applied to Clarias gariepinus juvenile were 118.25ml/l and 53.70ml/l respectively. Toxic reactions exhibited by the fish include erratic movement, air gulping, and loss of reflex. Mortality increased with increase in concentration of Prosopis africana and time of exposure in Clarias gariepinus juveniles. The acute toxicity that killed all the fish within 24 hours was discovered to be the highest concentration (640ml/l) of the extract. It is then recommended that Prosopis africana plant should be used as organic piscicide and further study on the toxicity of Prosopis africana plant on other commercially viable fish be studied.

Keywords: Bioassay, Extract, Toxicity, Concentration, Mortality

TEST DE TOXICITE DE LA GRAINE DE MESQUITE AFRICAINE (PROSOPIS AFRICANA) CHEZ LES POISSONS-CHATS AFRICAINS (CLARIAS GARIEPINUS) JUVENILES

Résumé

Un test de toxicité de la graine de mesquite (Prosopis Africana) sur le poisson-chat africain (Clarias gariepinus) a été effectué en vue de déterminer la concentration à effet létal aigu (LC50) en 24 - 48 heures. Deux cent cinquante (250) juvéniles ont été acclimatés en laboratoire pendant 14 jours. Dix (10) poissons ont été placés dans chacun des récipients en plastique expérimentaux, contenant des concentrations d'essai de 0 ml, 10 ml, 20 ml, 40 ml, 80 ml, 160 ml, 320 ml et 640 ml / litre d'eau.

La LC50 de 24 et 48 heures de Prosopis africana appliquée aux Clarias gariepinus juvéniles était respectivement de 118,25 ml / l et 53,70 ml / l. Des réactions toxiques ont été observées chez les poissons, notamment des mouvements erratiques, l'aspiration d'air et la perte de réflexe. La mortalité s'est accrue avec l'augmentation de la concentration de Prosopis africana et du temps d'exposition des juvéniles Clarias gariepinus. La toxicité aiguë, qui a tué tous les poissons dans les 24 heures, s'est révélée être liée à la concentration la plus élevée (640 ml / l) de l'extrait. Il est alors recommandé d'utiliser la plante Prosopis africana comme piscicide biologique, et d'étudier davantage la toxicité de cette plante sur d'autres poissons intéressants du point de vue commercial.

Mots-clés : dosage biologique, extrait, toxicité, concentration, mortalité

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Introduction

Plant poisons called botanicals are extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant (Sirivam *et al.,* 2004). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Wang and Huffman, 1991). Toxic plants like Prosopis africana, Blighia vinifera, Parkia biglobosa are frequently in use by the fisherfolks because they are highly potent (Fafioye, 2001) due to the presence of active ingredients such as saponins and rotenone (Obomanu, 2007). These varieties of chemicals found in these plants stun or stupefy fish (Kritzon, 2003).

Fish toxicants (piscicides) can be herbal or synthetic. Synthetic piscicides are not degradable, pose the problems of environmental resistance, pest resurgence and have detrimental effects on non-target organisms (Fafioye, 2005). Herbal toxicants do not create hazards like those experienced in synthetic ones. Secondly plants are virtually an inexhaustible source of structurally diverse and biologically active substances (Batabyal *et al.,* 2007).

Herbal piscicides have been widely used by traditional societies all over the world as means of catching fish (Murthy *et al.,* 2010). The active ingredient known as alkaloids, resin, tannin, saponin, nicotine, diosgenin, etc (Wang and Huffman, 1991) is released by mashing the appropriate plant parts which are then introduced to the water environment. Poisoning can be generally done in stagnant pools or slow flowing rivers that allow the pounded seed, bark, leaf, root, and fruit, to concentrate its power without being washed away or diluted by a strong current. Most fish poisons, also called icthyotoxin or piscicide occur in several related plants will stun fish when it passes through the gills or in some cases ingested, the fish then floats to the surface for easy capture (Houerou, 2002). Even though the effects of the poison are powerful, they are not usually fatal. Fish that are washed away into untainted water revive, and can return to their pre-toxic condition. Because of this, the fishermen would have to gather the stunned fish quickly as they floated to the surface (Kritzon, 2003).

Contrary to synthetic fish toxicant, herbal toxicant is believed to be more environmental friendly, because they are easily biodegradable and leave no residues in the environment. Synthetic piscicides are not degradable, pose the problems of environmental resistance, pest resurgence and have detrimental effects on non-target organisms (Fafioye *et al.,* 2005). Herbal toxicant do not create hazards like those experienced in synthetic ones, apart from the fact that plants are virtually an inexhaustible source of structurally diverse and biologically active substances (Batabyal *et al.,* 2007). Based on the piscicidal properties of Prosopis africana which is embedded in the seed, there is the need to evaluate the aqueous extracts of this plant for its potentials as herbal fish poisons (Adesina and Omitoyin, 2011) to aid fish cropping greatly as it saves time of fishing and increase easy handling (Burkill, 1985). Therefore this work evaluates the piscicidal potential of aqueous extract Prosopis africana seed on African Catfish (Clarias gariepinus) juvenile.

Prosopis africana is a Leguminosae belonging to the family of Fabaceae. It reaches 4-20m in height; has an open crown and slightly rounded buttresses; bark is very dark, scaly, slash, orange to red-brown with white streaks. It was once said that Prosopis africana is the only tropical African Prosopis species, occurring from Senegal to Ethiopia in the zone between the Sahel and savannah forests. Due to extensive overexploitation, it has disappeared from extensive parts of the Southern Sahel and the adjacent Sudan savannah (Vogt, 1995). The trees of P. africana are common in the Middle belt and Northern parts of Nigeria. The local names are "Kiriya" and "Okpehe" in Hausa and Idoma/Tiv languages in Nigeria respectively.

EIFAC (1983) specify that the species of fish used in toxicity analysis should be such that is easy to rear and widely available throughout the year. It should also be capable of being bred and culture either at fish farms or in the laboratory, under disease and parasite free condition. It must also be sensitive to a wide

range of possible pollutants. Clarias gariepinus is suitable for both indoor and outdoor cultivation and remain the most cultivated fish species in Nigeria (Akintunde, 2009).

The African catfish, Clarias gariepinus is an ecologically important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). These mudfish are frequently and widely cultured in ponds and they also occur freely in Nigerian natural fresh waters. The demand for this fish species by almost 75% of Nigerian population has necessitated the cropping of it in large number using piscicides.

Materials and Method

Test fish species

Two hundred and fifty healthy juvenile catfish (Clarias gariepinus) were sourced from kofo's hatchery with no history of disease outbreak. Ten catfish juvenile were placed in a 20litre capacity translucent rectangular plastic cover with transparent mosquito net to prevent fish from jumping out of the holding plastic: aeration of dechlorinated water was constantly maintained. The fish was acclimatised for 14 days, and fed twice daily with commercial floating feed. Unused feed and faeces were siphoned out to prevent water contamination even though water was change at regular interval. Feeding was stopped 24hours prior the experiment and was not fed throughout the experimental period. Their weight and length were sampled randomly by picking three (3) juvenile fish from each container and weighed on a sensitive scale while length was measured with tape rule.

Test plant: African Mesquite (Prosopis africana)

African Mesquite (Prosopis Africana) was obtained locally from Federal College of Wildlife Management field laboratory, New Bussa, Niger state. Collection of the plant seeds was done aseptically in sterile poly bags and two (2) kg of the seed was pounded with pestle and mortar to obtain powdered samples. Aqueous extract was obtained by adding 4 litre of distilled water to the powdered sample to simulate local procedures (Ofogba *et al.,* 1998) and left to ferment for 48 hours. Afterward, the plant extract was obtained by shaking before filtering using a dry Whatman no. 5 filter paper. This was immediately used to ensure its maximum effect and freshness.

Experimental set-up

Ten (10) catfish of Clarias gariepinus with an average weight of 8±1.56g each with no history of any disease outbreak were used for the experiment were placed in a 20litre capacity translucent rectangular plastic covered with transparent mosquito net to prevent fish from jumping out of the holding plastic during the experiment. The different test concentrations were used: 0ml, 10ml, 20ml, 40ml, 80ml, 160ml, 320ml and 640ml/litre of water. Fish mortality was observed and recorded for the first 3hours, every 3hour for the next 6, 12, 24hour and subsequently every 24hour until the time limit of 48hour from the commencement of the experiment (EIFAC, 1983). The inability of fish to respond to external stimuli was used as an index of death (Ayotunde and Ofem, 2008).

Lethal Concentration

The lethal concentration (LC50) of the test plant was determined by plotting logarithm concentration of the plant against fish mortality within 24hours and 48 hours after experiment to the treatment. Interpolation between two concentrations where the mortality occurred at less or greater than 50% was carried out.

The median lethal concentration (LC50) is the concentration at which 50% of the test fish survived and 50% died while LC100 is the lowest concentration at which 100% of the fish died. A fish was considered affected by the toxicant when it manifested erratic swimming behaviour, air gulping, loss of reflex, discolouration and pronounced ataxia coinciding with decreased capacity to respond to visual stimuli: A fish was considered dead when it does not respond to mechanical prodding. The dead fish were removed continuously and counted for determination of lethal concentration (LC50).

Statistical Analysis

Data collected was subjected to probit statistical test. The logarithm of concentration of Prosopis africana used was determined. Also, the percentage of the mortality response was established for each treatment, then their probit values. The linear relationship between the probit values and the logarithm concentration was established using regression analysis. The slope (b) of the regression linear relationship between the probit values and logarithm concentration was calculated as:

$y = bx + a$----------equation 1

$b = \sum (x - \bar{x}) (y - \bar{y})$ = slope) = slope----------equation 2

$\sum (x - \bar{x})2$

The equation for the intercept of the regression line, a is;

$a = \bar{y} - b\bar{x}$ Where y = intercept (constant by regression) ---------- equation 3

The equation of the relationship was obtained from the plotted graph. Then, from the relationship, the LC50 of the Prosopis africana was estimated for 24hour and 48hour. This was done by setting $\bar{y}$ to LC50 while a and b taking the values as:

$y = bx + a$

Where y = Concentration (LC50)
b = Slope
c = $\bar{y}$ intercept constant.

Results

Mean mortality observed in Clarias gariepinus exposed to different concentrations of Prosopis africana seed extract.

Table 1 show that the aqueous extract of Prosopsis africana seed was observed to be toxic causing Clarias gariepinus juvenile mortality at different exposure time and at different concentrations. No mortality was recorded for all concentration in the first three hour (3hours) while the highest mortality was recorded 640ml/litre at six hour (6hour). All fish were dead at 640ml at the termination of the experiment in forty-eight hour (48 hour).

Probit values obtained for different concentrations of Prosopis africana seed (24 hours)

Probit analysis show a probit of mortality value of 4.48 in 10ml/l and 4.16 in both 20ml/l and 40ml/l at 24h exposure time while 80ml/l, 160ml/l, 320ml/l and 640ml/l had probit of mortality values of 3.72, 5.00, 5.84 and 6.28 respectively at 24h exposure time. The logarithmic concentration of 2.20 was observed for probit of mortality values 5.0 (50% mortality) (Table 2 and 3). The corresponding probit value of percentage mortality and logarithmic value of each concentration used were shown in table 2.

Relationship between probit and log concentration (24 hours)

Table 3 revealed the relationship between probit and log concentration. Probit values (Y) representing mortality were recorded against log concentration (X) of each treatment.

Table 1: Mean mortality observed in Clarias gariepinus exposed to different concentrations of Prosopis africana seed extract.

Time (hour)	No. of fish/tank	Control	10ml	20ml	40ml	80ml	160ml	320ml	640ml
3	10	0	0	0	0	0	0	0	0
6	10	0	0	0	0	0	2	2	5
12	10	0	2	2	2	1	2	5	3
24	10	0	1	0	0	0	1	1	1
48	10	0	0	0	0	0	4	1	1

Table 2: Probit values obtained for different concentrations of Prosopis africana seed (24 hours)

Concentration (ml)	Mortality	% Mortality	Probit	Log. Concentration
10	3	30	4.48	1.00
20	2	20	4.16	1.30
40	2	20	4.16	1.60
80	1	10	3.72	1.90
160	5	50	5.00	2.20
320	8	80	5.84	2.50
640	9	90	6.28	2.81

Table 3: Relationship between probit and log concentration (24 hours)

Probit (Y)	Log. Concentration (X)
4.48	1.00
4.16	1.30
4.16	1.60
3.72	1.90
5.00	2.20
5.84	2.50
6.28	2.81

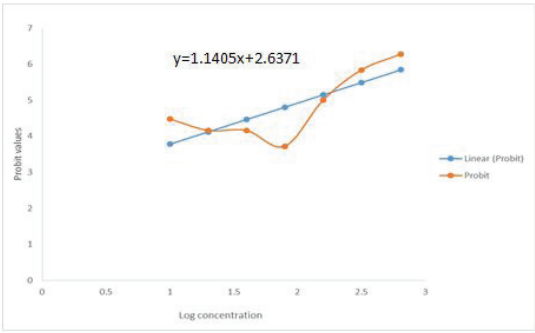

Figure 1: 24 hours LC50 of Prosopis africana on African catfish (Clarias gariepinus) juvenile

Figure 1 revealed regression equation that showed the relationship between the mortality and the concentration used when probit values were plotted against log concentration. However, the regression equation that gives the positive relationship between probit and log concentration in 48 hours was given as:

$$Y = 1.14X + 2.637$$

So, the LC50 of Prosopis africana at 24 hours can be calculated as Y is set at 5.0

$$5 = 1.14X + 2.637$$

$$X = 2.07$$

The antilogarithm of the value of X is found to be 118.25 ml/l. This value is taken as the LC50 of Prosopis africana at 24 hours.

LC50 at 48 hours of observation

Table 4 shown percentage (%) mortality of Clarias gariepinus at each concentration over the period of 48 hours, percentage (%) mortality obtained from mean mortality as well as the corresponding probit values of percentage mortality.

Relationship between probit and log concentration

Table 5 shows the relationship between probit and log concentration. Probit value (Y) representing mortality and logarithmic concentration (X) of each treatment.

Table 4: Probit values obtained for different concentrations of Prosopis africana seed extract (48 hours).

Concentration (ml)	Mortality	% Mortality	Probit	Log. Concentration
10	3	30	4.48	1.00
20	2	20	4.16	1.30
40	2	20	4.16	1.60
80	1	10	3.72	1.90
160	9	90	6.28	2.20
320	9	90	6.28	2.50
640	10	100	8.72	2.81

Table 5: Relationship between probit and log concentration (48 hours)

Probit (Y)	Log. Concentration (X)
4.48	1.00
4.16	1.30
4.16	1.60
3.72	1.90
6.28	2.20
6.28	2.50
8.72	2.81

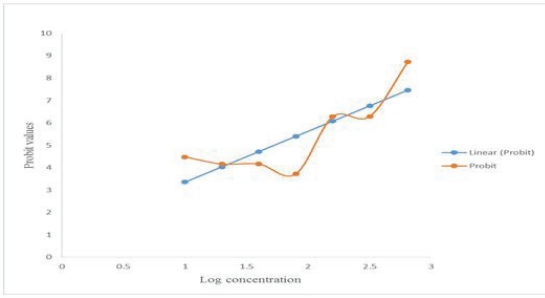

Figure 2: 48 hours LC50 of Prosopis africana on African catfish (Clarias gariepinus) juvenile

The regression equation shows the relationship between the mortality and the concentration used. However, the regression equation that gives the positive relationship between probit and log concentration in 48 hours was given as:

$$Y = 2.268X + 1.086 \text{ (Where } Y = 5.0)$$
$$\text{Therefore, } X = 1.73$$

Therefore, the antilogarithm of the value of X was found to be 53.70 ml/l. This value is taken as the LC50 of Prosopis africana at 48 hours.

Discussion

The Clarias gariepinus juvenile were exposed to seven different concentrations of liquid extract of Prosopis africana seed as treatments. An untreated experiment was set as control. The concentrations of the plant extract were in logarithmic ratios, i.e. 10ml, 20ml, 40ml, 80ml, 160ml, 320ml and 640ml (EIFAC, 1983). The bioassay was carried out for 48 hours. During this period, there was no mortality recorded in the control and at the first three hours of exposure in all the treatments, obvious signs of stress were observed.

The stressful behaviour of Clarias gariepinus juveniles showed feelings of respiratory impairment due to toxicity effect of Prosopis africana. Weaken respiration in fish have been reported to be toxic effect of botanical piscide (Fafioye *et al.,* 2002). It was also observed that the treatment of lower concentrations didn't feel the toxicity effect much as they (fish) hardly showed these responses but signs of discomfort increased with increasing concentrations. This is in

consonance with Ayotunde *et al.,* (2010) who reported similar observation using pawpaw seed extract on Clarias gariepinus. Mortalities recorded also increased as the concentration and time of exposure increases.

The LC50 of liquid extract of Prosopis africana seed at 24 hours of exposure on Clarias gariepinus juvenile was found to be 118.25ml/l. This concentration was smaller compared with 252mg/l, LC50 of aqueous extracts of *Moringa oleifera* seed on Oreochromis niloticus fingerlings, at 24 hour of exposure (Ayotunde, *et al.,* 2010). This showed that Prosopis africana seed is more toxic than *Moringa oleifera* seed. Cagauan *et al.,* (2004), also reported that LC50 of neem (Azadichrata indica) at 24 hour of exposure is 6.4ml/l, this also suggested that neem is more toxic than Prosopis africana.

The LC50 of liquid extract of Prosopis africana seed at 48 hours of exposure on Clarias gariepinus juvenile was found to be 53.70ml/l. This concentration seems smaller than the result recorded for LC50 earlier. It was also smaller when compared with 251mg/l, LC50 of aqueous extracts of *Moringa oleifera* seed on Oreochromis niloticus fingerlings, at 48 hour of exposure (Ayotunde, *et al.,* 2010). This implies that Prosopis africana seed is more toxic than *Moringa oleifera* seed. Cagauan et al. (2004), also reported that LC50 of neem at 48 hour of exposure is 3.22ml/l, this suggest that neem is more toxic than Prosopis Africana even after 48 hours of exposure.

Conclusion

Apparent toxicity effect of aqueous extract of Prosopis africana seed on juveniles of African catfish (Clarias gariepinus) was confirmed in this study. Therefore, the piscidal effect of aqueous extract of African Mesquite (Prosopis Africana) seed had been established in this study. It is safe to use plant origin piscides to harvest fish species with potential difficulty in the fish management and to eradicate unwanted fish from the ponds. This is because of residual effect of botanical is biodegradable while chemicals origin are detrimental to human health aside the fact that it is costlier and many times are not readily available at time of use. Further toxicological studies on this plant (Prosopis africana) using extracts from other solvents are strongly recommended.

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PRINCIPALES ESPÈCES MÉDICINALES UTILISÉES EN MÉDECINE VÉTÉRINAIRE AU BÉNIN: DISPONIBILITÉ ET CARACTÉRISTIQUES DENDROMÉTRIQUES

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Résumé

L'utilisation des plantes pour soigner les maladies des hommes et des animaux tient une place importante dans la vie des populations en Afrique au sud du Sahara. La présente étude vise à inventorier les principales plantes médicinales utilisées pour traiter les pathologies des animaux, à recenser les différentes parties de ces plantes qui sont utilisées et à évaluer la disponibilité de *Zanthoxylum zanthoxyloïdes* et *Newbouldia laevis*, deux plantes aux propriétés anthelminthiques prouvées. A cet effet, une enquête ethnobotanique dans quatre localités du Bénin: Ouidah et Allada dans l'Atlantique, Abomey et Djidja dans le Zou et un relevé de végétation ont été réalisés. Au total, 18 espèces à usage vétérinaire ont été recensées dans l'Atlantique et 36 dans le Zou. Les principales plantes antiparasitaires sont *Zanthoxylum zanthoxyloïdes* dans l'Atlantique et *Moringa oleifera* et *Carica papaya* dans le Zou. La principale forme d'administration de ces plantes est le broutage des feuilles (61,11% dans l'Atlantique et 86,11% dans le Zou). Les autres formes de préparation que sont les décoctions, les macérations et les triturations sont faiblement utilisées. En ce qui concerne la disponibilité des deux plantes ciblées, il est noté une densité totale des peuplements des deux espèces plus significative dans l'Atlantique que dans le Zou (183,3 tiges/ha et 62,96 tiges/ha respectivement dans l'Atlantique et dans le Zou pour *Zanthoxylum zanthoxyloïdes* et 174,07 tiges/ha et 102,96 tiges/ha respectivement dans l'Atlantique et dans le Zou pour *Newbouldia laevis*). On retient une prédominance des jeunes individus (dbh<10cm) dans les deux départements et une absence totale des individus de dbh≥10cm dans le Zou en ce qui concerne *Zanthoxylum zanthoxyloïdes*. La mise en place d'une politique de conservation de ces espèces, soumises à une forte pression anthropique serait une alternative à l'érosion des ressources naturelles de nos pays.

Mots-clés : Ethnobotanique, disponibilité, Atlantique, Zou, *Zanthoxylum zanthoxyloïdes*, *Newbouldia laevis*, Bénin

Abstract

The use of plants to treat diseases of humans and animals is an important part of the lives of populations in sub-Saharan Africa. The present study aims to identify the main medicinal plants used to treat animal diseases, to identify the different parts of these plants that are used and to evaluate the availability of zanthoxylum zanthoxyloides and *Newbouldia laevis*, two plants with proven anthelmintic properties. To this end, an ethnobotanical survey was carried out in four localities of Benin: Ouidah and Allada in the Atlantic, Abomey and Djidja in the Zou and a survey of vegetation. A total of 18 veterinary species were recorded in the Atlantic and 36 in the Zou. The main antiparasitic plants are zanthoxylum zanthoxyloides in the Atlantic and *Moringa oleifera* and *Carica papaya* in the Zou. The main form of administration of these plants is leaf browsing (61.11% in the Atlantic and 86.11% in the Zou). Other forms of preparation such as decoctions, macerations and triturations are poorly used. As for the availability of the two targeted plants, total stand

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density of the two species is more significant in the Atlantic than in the Zou (183.3 stems / ha and 62.96 stems / ha, respectively, Atlantic and Zou for Zanthoxylum zanthoxyloides and 174.07 stems / ha and 102.96 stems / ha respectively in the Atlantic and Zou for *Newbouldia laevis*). A predominance of young individuals (dbh <10cm) in both departments and a total absence of individuals of dbh≥10cm in the Zou with respect to Zanthoxylum zanthoxyloides is retained. The implementation of a conservation policy for these species, subject to strong anthropogenic pressure, would be an alternative to the erosion of the natural resources of our countries.

Keywords: Ethnobotany, availability, Atlantic, Zou, *Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, Benin

Introduction

Le milieu tropical auquel appartient l'Afrique en grande partie, dispose d'une diversité biologique très élevée, à tel point que l'avenir de notre planète dépend de sa survie. Mais cette survie se voit menacée par des attaques catastrophiques dues aux variations climatiques provoquées ou non et aux interventions humaines contrôlées ou non (Adjanohoun *et al.,* 1999 ; Delvaux *et al.,* 2010). La perte de couverture forestière a atteint dans la décennie 1990-2000, 14,2 millions ha/an et l'Afrique, avec seulement 16,8% du couvert mondial, a contribué pour 56% à cette réduction du couvert forestier (Djègo, 2006). C'est le cas en Afrique au Sud du Sahara et particulièrement au Bénin où les ressources génétiques, précisément les espèces médicinales s'amenuisent progressivement à cause de leur utilisation abusive. Les menaces qui pèsent sur ces formations végétales comprennent les pratiques culturales, l'élevage, l'exploitation du bois et des Produits Forestiers Non Ligneux (Sokpon et Agbo, 2001 ; Sinsin *et al.,* 2009), la croissance démographique et l'urbanisation (Yessoufou, 2005). L'Afrique de l'Ouest perd chaque année 4% de forêt dense (Harrison, 1991). Quant au Bénin, c'est 60 000 ha de forêt par an qui sont concernés soit un taux annuel de déforestation évalué à 1,2%. Cette déforestation n'est pas sans conséquences sur la conservation des ressources biologiques et notamment sur les plantes médicinales qui demeurent encore une source de soins médicaux dans les pays en voie de développement, en l'absence d'un système médical moderne (Tabuti *et al.,* 2003 ; Deleke *et al.,* 2009). En effet, la destruction des forêts tropicales est la cause essentielle de réduction de la diversité biologique (Djègo et Sinsin, 2006) et présente des conséquences économiques et écologiques graves. Aussi, de nombreuses espèces végétales utiles sont-elles vulnérables ou menacées d'extinction de nos jours. C'est pourquoi la présente étude réalisée au Sud et au Centre du Bénin a été initiée. Elle a pour objectif l'évaluation des principales espèces médicinales utilisées en médecine vétérinaire traditionnelle, des différentes parties exploitées pour ces plantes, de leur mode de préparation et de l'étude de la disponibilité dans le milieu de deux d'entre elles (*Zanthoxylum zanthoxyloides* et *Newbouldia laevis*) dont les propriétés anthelminthiques ont été étudiées et confirmées (Azando, 2011 ; Olounladé, 2011 ; Hounzangbé-Adoté, 2004).

Matériel et méthodes

Milieu d'étude

La présente étude a été réalisée dans les départements de l'Atlantique (Communes d'Allada et de Ouidah) et du Zou (Communes d'Abomey et de Djidja). L'ensemble de ces quatre Communes est marquée par un climat du type sub-équatorial caractérisé par deux saisons sèches (mi-novembre à mi-mars, mi-juillet à mi-septembre) et deux saisons pluvieuses (mi-mars à mi-juillet et mi-septembre à mi-novembre). La pluviométrie moyenne annuelle est de 1200 mm environ, dont 700 à 800 mm pour la première saison pluvieuse et 400 à 500 mm pour la seconde saison pluvieuse. Les températures ambiantes moyennes mensuelles varient entre 25,79 et 33,98°C.

La région est caractérisée globalement par une diversité de sols : des vertisols (montmorillonite), des sols ferrugineux ; des

sols ferralitiques avec ou sans concrétions sur roches sédimentaires et des sols sableux ferralitiques.

La végétation de la zone est de type savane arborée où prédominent les palmiers à huile naturels qui occupent une place importante dans la physionomie du paysage. Dans la plupart des communes, les formations marécageuses et les mosaïques de cultures et de jachères sont très fréquentes. On rencontre par ailleurs quelques plantations et ilots forestiers (forêts sacrées).

Enquêtes ethnobotaniques

Des enquêtes ethnobotaniques ont été faites pour recenser les espèces médicinales utilisées traditionnellement dans les départements de l'Atlantique et du Zou contre diverses pathologies en médecine vétérinaire.

Au cours de ces enquêtes, les données socio-économiques recueillies ont concerné le profil de chaque enquêté (âge, niveau d'études, situation familiale, ethnie et activité principale). La technique d'entretiens structurés basés sur des questionnaires a permis d'avoir les informations sur :

- La liste des plantes qui servent pour le traitement des maladies animales (free listing). La majorité des espèces ont été mentionnées par les enquêtés par leur nom commun vernaculaire. L'identification taxonomique des espèces a été réalisée ultérieurement grâce au catalogue de de Souza (2008).
- Les organes ou parties de la plante qui sont utilisés (racines, tige, feuilles, fleurs, fruits, graines, sève ou autre) ;

Certaines informations ont été enrichies par des visites et des observations dans les paysages agraires pour reconnaissance des espèces rapportées.

Relevé de végétation

Les relevés de végétation ont été réalisés pour obtenir des données quantitatives sur deux espèces dont les propriétés antiparasitaires ont été largement prouvées parmi celles rapportées par les populations au cours des enquêtes ethnobotaniques. Il s'agit de : *Zanthoxylum zanthoxyloïdes* et *Newbouldia laevis* (Azando, 2011 ; Olounladé, 2011 ; Hounzangbé-Adoté, 2004). Les relevés ont été effectués dans les placeaux de 30 m x 30 m installées dans le terroir de chaque localité où les enquêtes ont été conduites. Au total, 60 placeaux dont 30 pour *Zanthoxylum zanthoxyloïdes* et 30 pour *Newbouldia laevis* ont été installés.

Ces relevés ont permis de mieux définir l'importance de ces deux espèces et leur disponibilité dans les différentes localités. La disponibilité de chacune des espèces ligneuses a été déterminée sur la base de leur effectif et la répartition des individus de chaque espèce selon leur diamètre à hauteur d'homme, dbh. Ainsi deux classes de diamètre ont été définies (dbh≥ 10cm et dbh< 10cm). Les individus de chaque espèce sont repartis dans ces différentes classes. La densité des individus adultes (dbh≥ 10cm) et des régénérations (dbh< 10cm) ont été déterminés.

Analyse des données

Des techniques d'analyse descriptive simple ont été utilisées. La méthode Tramyl IV a permis de dégager les espèces les plus significatives pour traiter chaque maladie. La fréquence d'utilisation des différents organes a été calculée afin d'évaluer les conséquences des prélèvements sur la survie des espèces cibles.

Les densités de peuplement calculées ont été comparées pour les deux départements avec le logiciel R2 et le seuil de comparaison est de 5%.

Résultats

Profils des enquêtés

Les personnes enquêtés dans les deux départements étaient en majorité Fons: Atlantique (96%) et Zou (91,11%). Les autres groupes ethniques rencontrés sont constitués de Houéda et de Yoruba dans le département de l'Atlantique, de Mahi, de Hla et de Nago dans le département du Zou.

Dans les deux départements, 61,05% des enquêtés se situent dans la tranche d'âge de

35 à 60 ans. Le ratio Homme/Femme enquêté est largement favorable pour le sexe masculin : 88% dans l'Atlantique et 93,33% dans le Zou.

Dans la plupart des cas, les enquêtés mènent des activités doubles (Agriculteur-Eleveur) ou multiples, les deux activités Agriculteur-Eleveur étant souvent liés. Ainsi, dans l'Atlantique, 64% des enquêtés sont des agriculteurs et/ou éleveurs, 2% des tradipraticiens et le reste est constitué de commerçants, taximan et conducteurs de zemidjan, d'instituteurs en activité ou retraités. Dans le Zou, 40% de l'échantillon est constitué des agriculteurs et/ou éleveurs, 16,67% d'instituteurs en activité ou non, 9,52% de commerçants, les autres étant des élèves, des artisans et des fonctionnaires (Figure 1).

Dans la zone d'enquête, le niveau d'instruction le plus élevé est le supérieur. Dans l'Atlantique, 66% des enquêtés sont instruits et 44% sans instruction. Dans le Zou par contre, 42,23% n'ont pas été à l'école contre 57,77% d'instruits.

Par ailleurs, 46% des personnes enquêtés estiment pouvoir bien reconnaitre les plantes et apprécier leur vertus dans l'Atlantique alors que dans le Zou, 44,45 % pensent le faire et 46,67% le faire passablement.

Inventaire des espèces à usage vétérinaire et maladies traitées

Au total, 18 espèces à usage vétérinaire réparties dans 16 familles ont été recensées dans l'Atlantique et 36 réparties dans 20 familles dans le Zou. Neuf (09) espèces à usage vétérinaire se retrouvent dans les deux départements soit 50% des espèces recensées dans l'Atlantique et 25% de celles rencontrées dans le Zou. Il s'agit notamment de : *Cesalpinia bonduc*, *Citrus limon*, *Elaeis guineensis*, *Mangifera indica*, *Mitracarpus hirtus*, *Morinda lucida*, *Moringa oleifera*, *Spondia monbin*, *Zanthoxylum zanthoxyloides*.

Les principales pathologies traitées par les espèces rapportées dans les deux départements sont : les parasitoses internes et externes, les troubles digestifs, l'agalactie, la maladie de Newcasttle (Tableau 1). D'autres maladies sont inconnues aux enquêtés. Les infections et la malnutrition ont été citées en plus dans le Zou tandis que dans l'Atlantique ce sont les problèmes de rumination, les fièvres et les plaies qui ont été rapportés.

Principales plantes antiparasitaires utilisées

Plusieurs plantes antiparasitaires ont été recensées dans les deux départements (Tableau 2). La principale plante utilisée contre les parasitoses gastro-intestinales dans l'Atlantique est le *Zanthoxylum zanthoxyloïdes* (98%). Dans le Zou par contre, les principales plantes utilisées sont : *Moringa oleifera* (33,33%), *Carica papaya* (31,11%), *Zanthoxylum zanthoxyloïdes* n'ayant été cité que par 6,67% des enquêtés.

Différentes parties des plantes utilisées, modes de préparation et voies d'administration

Les populations de la zone d'étude ont signalé cinq parties des plantes qui sont utilisées en médecine vétérinaire traditionnelle (Figure 3). Il s'agit des feuilles, des racines, des écorces, des fruits et des graines. Dans les deux départements, la plupart des espèces sont utilisées pour leur feuilles : 77,77% et 91,66% respectivement dans l'Atlantique et dans le Zou. Les espèces utilisées pour leur écorce dans le Zou (16,66%) sont : *Mangifera indica*, *Khaya senegalensis*, *Bauhinia purpurea* et *Anacardium occidentale*. Dans l'Atlantique, seul *Mangifera indica* est concerné (5,55%). L'utilisation des racines a été signalée seulement dans le Zou (2,77%) et ne concerne que Cesaplinia bonduc. Quant au graines, celles de *Carica papaya* et de Azadiracta indica ont été citées dans

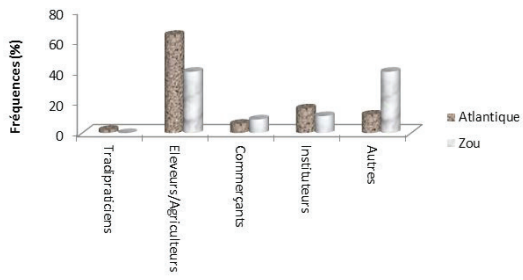

Figure 1 : Répartition de la population d'étude suivant les catégories socio-professionnelles

Tableau 1 : Principales pantes à usage vétérinaire et maladies traitées

Départements	Plantes	Taux de citation (%)	Maladies traitées
Atlantique	*Zanthoxylum zanthoxyloïdes*	98	Parasitoses internes Troubles digestifs (diarrhées, vomissements) Agalactie
	Spondia monbin	94	Agalactie
	Elaeis guineensis	74	Parasitoses externes, Plaies Maladies inconnues
	Moringa oleifera	42	Agalactie Fièvre Maladie de Newcasttle
	Funtumia elastica	32	Fièvre Problèmes de rumination
	Mangifera indica	22	Maladies inconnues
Zou	*Spondia monbin*	35,55	Agalactie, diarrhées, malnutrition du nouveau-né
	Carica papaya	33,33	Parasitoses internes Troubles digestifs (diarrhées, vomissements) Agalactie
	Moringa oleifera	31,11	Maladie de Newcasttle, Parasitoses internes Troubles digestifs Infections
	Azadiracta indica	22,22	Parasitoses externes

Tableau 2 : Principales plantes antiparasitaires recensées dans les deux départements

Départements	Espèces	Taux de citation
Atlantique	*Zanthoxylum zanthoxyloïdes*	98%
	Cajanus cajan	12%
	Cesalpinia bonduc	4%
Zou	*Moringa oleifera*	33,33%
	Carica papaya	31,11%
	Zanthoxylum zanthoxyloïdes	6,67%
	Cajanus cajan	6,67%
	Azadiracta indica	4,44%
	Ocimum graticimum	4,44%
	Cesalpinia bonduc	4,44%

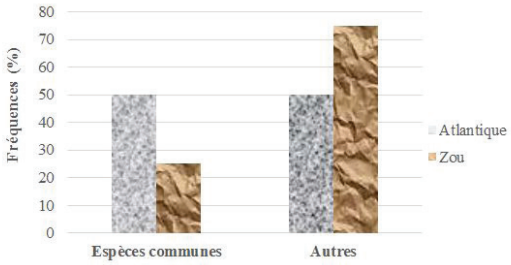

Figure 2 : Répartition des espèces à usage vétérinaire recensées dans les deux Départements

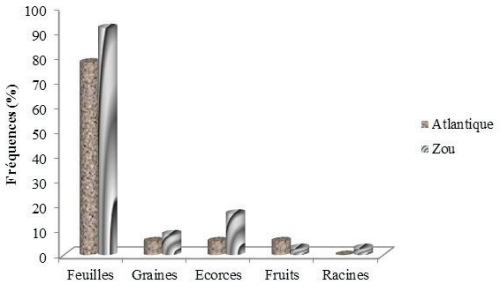

Figure 3 : Fréquences d'utilisation des différentes parties des plantes

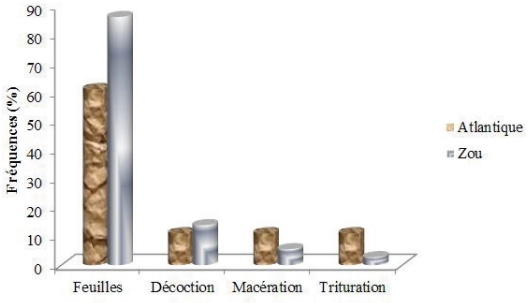

Figure 4 : Mode de préparation des différentes parties de plantes utilisées

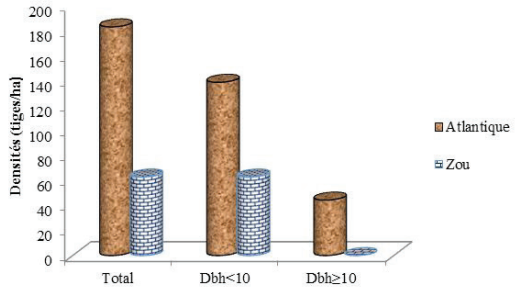

Figure 5 : Distribution par classe de diamètre du peuplement à *Zanthoxylum zanthoxyloïdes*

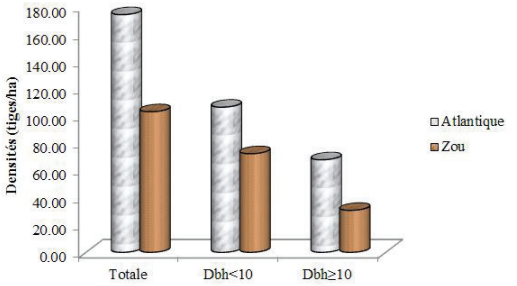

Figure 6 : Distribution par classe de diamètre du peuplement à *Newbouldia laevis*

le Zou (8,33%) tandis que celles de Citrus limon sont utilisées dans l'Atlantique (5,55%). Enfin, l'utilisation des fruits pour soigner les animaux concerne *Citrus limon* dans les deux départements.

Les feuilles à brouter directement par l'animal constituent la principale forme d'administration des plantes (61,11% et 86,11% respectivement dans l'Atlantique et dans le Zou). Les autres formes de préparation des plantes sont faiblement utilisées dans les deux départements : décoctions (11,11% et 13,88% respectivement dans l'Atlantique et dans le Zou), macérations (11,11% et 5,55% respectivement dans l'Atlantique et dans le Zou), et triturations ou baumes (11,11% et 2,77% respectivement dans l'Atlantique et dans le Zou), (Figure 4).

La principale voie d'administration est la voie orale dans les deux départements (93,05%), la voie cutanée (6,94%) étant utilisée pour les parasitoses externes et les plaies. Les espèces recensées dans les deux départements et leurs modes d'administration sont résumés dans le tableau 3.

Caractéristiques de la population de Zanthoxylum zanthoxyloïdes

La densité totale des individus au sein de la population de *Zanthoxylum zanthoxyloïdes* est plus importante dans l'Atlantique (183,3 tiges/ha) que dans le Zou (62,96 tiges/ha). La distribution par classe de diamètre montre que les individus de la classe de diamètre inférieur à 10 cm est plus importante dans les deux zones, 138,88 tiges/ha et 62,96 tiges/ha respectivement

Tableau 3 : Mode de préparation des différentes espèces recensées dans les deux départements

Modes de préparation des différentes parties de plantes utilisées	Atlantique	Zou
Feuilles à brouter	*Icacina trichantha, Zanthoxylum zanthoxyloides, Cesalpinia bonduc,Funtumia elastica, Olax subscorpioidea, Moringa oleifera, Dissotis fruticosa, Spondia monbin, Morinda lucida, Adansonia digitata, Margaritaria discoidea, Newbouldia laevis, Ficus spp.*	*Ocimum graticimum, Hyptis suaveolensis,Elaeis guineensis,Carica papaya, Moringa oleifera, Zanthoxylum zanthoxyloides, Ocimum basilicum, Parkia bocolor, Khaya senegalensis, Morinda lucida, Abrus precatorius, Cajanus cajan, Citrus reticulate, Ipomoea eriocarpa, Ricinus communis, Talinum triangulare, Boerhavia diffusa, Spondia monbin, Chenopodium ambrosoides, Ocimum basilicum, Bauhinia purpurea, Mitracarpus hirtus, Anacardium occidentale, Cesalpinia bonduc, Elaeophorbia grandifolia, Caesalpinia pulcherima, Manihot esculenta*
Décoction	*Garcinia cola*	*Clausena anisata, Carica papaya, Mangifera indica, Annona senegalensis, Vernonia amygdalina, Bryophyllum pinnatum, Nicotiana tabacum,*
Macération	*Mangifera indica*	*Khaya senegalensis*
Trituration	*Mitracarpus hirtus, Elaeis guineensis,*	*Azadiracta indica*

dans l'Atlantique et dans le Zou (Figure 5). Les individus de la classe de diamètre supérieur ou égal à 10 cm n'ont pas été retrouvés dans le Zou.

La comparaison des différentes densités entre les deux zones montre que les différences observées sont significatives ($p<0,05$) tant pour les individus de dbh<10 que pour ceux de dbh≥10.

Caractéristiques du peuplement à Newbouldia laevis

La population de *Newbouldia laevis* présente une densité totale des individus plus élevée dans l'Atlantique (174,07 tiges/ha) que dans le Zou (102,96 tiges/ha). Par ailleurs, la distribution par classe de diamètre montre que les individus de classe de diamètre inférieur à 10 cm est importante dans les deux zones, 106,30 tiges/ha et 72,22 tiges/ha respectivement dans l'Atlantique et dans le Zou (Figure 6). Quant aux individus de la classe de diamètre supérieur ou égal à 10 cm, ils sont plus importants dans l'Atlantique (67,78 tiges/ha) que dans le Zou (30,7 tiges/ha).

L'analyse de variance des densités entre les deux zones montre que les différences observées sont significatives ($p<0,05$) tant pour les individus de dbh<10 que pour ceux de dbh≥10.

Discussion

La méthode rétroactive d'enquête utilisée pour évaluer l'importance d'utilisation et la pression sur les espèces végétales porte en elle selon les travaux de Dossou *et al.,* (2014), des biais relatifs à la mémoire des personnes interrogées et pourrait entacher l'appréciation personnelle de l'enquêté. L'importance

accordée à l'utilisation des espèces est donnée par les individus qui tiennent implicitement compte d'une appréciation personnelle; laquelle fait souvent référence à leur préférence. Selon Dossou *et al.,* (2012), en dépit de ces quelques biais, cette méthode est largement utilisée en ethnobotanique par bon nombre d'auteurs et a le privilège de faire ressortir des résultats assez concluants.

Les personnes enquêtées étaient en majorité Fon tant dans l'Atlantique que dans le Zou. Cela s'explique par le fait que la population de la zone enquêtée (échantillon) est à dominance Fon (90% dans l'Atlantique et 91,11% dans le Zou). Les autres ethnies n'étant que faiblement représentées et constituées de Houéda, Yoruba (Atlantique) et de Mahi, Hla et Nago (Zou).

La majorité des enquêtés est mature (35-60 ans) et de sexe masculin. Ceci indique que les connaissances sur les espèces et leurs utilisations sont inégalement réparties suivants l'âge et le sexe. Ces résultats corroborent ceux obtenus par Deguenonvo (2011) sur M. whitei et Akouehou *et al.,* (2014) sur l'espèce *Artocarpus altilis*. Par ailleurs, même si les hommes possèdent plus de connaissances sur les espèces que les femmes, les valeurs de densité pour *Newbouldia laevis* (174,07 tiges/ha dans l'Atlantique et 102,96 tiges/ha dans le Zou) et pour *Zanthoxylum zanthoxyloïdes* (183,3 tiges/ha dans l'Atlantique et 62,96 tiges/ha dans le Zou) en relation avec les utilisations des organes des espèces, pour les catégories d'âge n'indiquent pas une forte discrimination entre celles-ci au sein de ces différents groupes. Ceci montre que, contrairement aux conclusions rapportées par Hanazaki *et al.,* (2000) ; Matavele et Habib (2000) et Amorozo (2004), le niveau de valorisation et par conséquent de connaissance des espèces augmente très peu avec l'âge. Cela pourrait traduire une transmission des connaissances au fil des générations de façon à assurer une homogénéité des connaissances dans le milieu. Ces résultats écartent la possibilité d'un risque d'érosion qui planerait sur les connaissances endogènes relatives aux usages des espèces. Notons que la grande majorité des enquêtés est constitué d'agriculteurs-éleveurs et que très peu de tradipraticiens ont été enquêtés.

Les connaissances ethnobotaniques des populations du Sud et du Centre Bénin sont traditionnellement riches à cause de la diversité des groupes ethniques, des coutumes et des traditions mais aussi de la diversité des espèces végétales et de leur habitat. L'enquête ethnobotanique révèle que la zone d'étude est une source importante de matières premières végétales pour la pharmacopée. Pour satisfaire leurs besoins vitaux, les populations n'hésitent pas, à exploiter les espèces végétales à usages traditionnels multiples dans la zone d'étude. Ces espèces sont utilisées pour traiter de nombreuses affections comme : les parasitoses internes et externes, les troubles digestifs, l'agalactie, la maladie de Newcasttle, la gale et d'autres maladies inconnues aux enquêtés. Ces résultats montrent le rôle important de la flore dans la vie des populations rurales et plus particulièrement dans le traitement des pathologies animales. On dénote l'utilité diversifiée de ces plantes à travers les diverses affections traitées. Ces données sont en accord avec les résultats des travaux de Ogni *et al.,* en 2014 qui ont montré qu'au Bénin les utilisateurs les plantes avaient une connaissance plus large respectivement sur les espèces végétales efficaces dans le traitement des helminthiases, des ectoparasitoses, de la gale, de la trypanosomiase, de la coccidiose et de la piroplasmose.. De toutes les plantes à usage vétérinaire recensées dans notre étude, *Moringa oleifera*, *Cajanus cajan*, *Zanthoxylum zanthoxyloides* et *Carica papaya* sont les plus utilisées dans le traitement des parasitoses internes et des troubles digestifs. Ces résultats s'accordent avec ceux de Hounzangbé-Adoté (2004) qui ont fait ressortir Zanthoxylum zanthoxyloides et *Carica papaya* comme des plantes utilisées dans le Sud du Bénin par les éleveurs dans le traitement endogène de la diarrhée et des affections gastro-intestinales.

Toutes les parties ou organes des plantes sont exploitables et peuvent être prélevés selon les pathologies. La nature de la partie ou de l'organe récolté semble varier avec le domaine d'usage. En effet, en pharmacopée,

plusieurs auteurs ont signalé que les organes les plus utilisés sont les feuilles et les écorces (Aké-Assi et Guinko, 1991 ; N'guessan, 1996 ; Tra-bi, 1997 ; Houessou, 2010). Notre étude a montré que les feuilles sont les plus utilisées du fait qu'elles se prêtent mieux aux manipulations et du fait aussi de leur facile accessibilité. Les formes d'utilisation diffèrent et parmi celles rencontrées, l'utilisation directe des feuilles est la forme la plus utilisée. Les feuilles sont effet données aux animaux comme fourrage dans le traitement des parasitoses aussi bien internes qu'externe. Bien qu'elles soient utilisées, les autres formes de préparation comme la décoction, la macération et la trituration sont plus difficile à appliquer.

Les aires de distribution des espèces végétales et plus particulièrement celles ayant des vertus thérapeutiques s'amenuisent. Les menaces qui pèsent sur ces formations végétales comprennent les pratiques culturales, l'élevage, l'exploitation du bois et des Produits Forestiers Non Ligneux (Sokpon et Agbo, 2001 ; Sinsin *et al.,* 2009), la croissance démographique et l'urbanisation (Yessoufou, 2005).

Les espèces comme *Zanthoxylum zanthoxyloïdes*, Spondia monbin, Funtumia elastica, Dissotis fruticosa ont été citées comme plantes menacées de disparition dans la zone de l'Atlantique du fait de leur exploitation incontrôlée. Le mode de prélèvement des organes très sensibles (racines, fruits, graines, écorce) est aussi l'une des causes de vulnérabilité des espèces. De pareils résultats sont en accord avec ceux obtenus par Sinsin *et al.,* (2009) qui démontrent que les ressources végétales sont soumises à de fortes pressions humaines (agriculture, transhumance, émondage, feux de brousse, collecte des Produits Forestiers Non Ligneux (PFNL) et occupation aux fins d'habitation qui à divers degrés influencent négativement la conservation durable des forêts. Ces mêmes facteurs pourraient être la cause de la rareté d'espèces comme Olax subscorpioidea et Margaritaria discoidea. Cette menace de disparition est due aux prélèvements intenses, aux mutilations, à la déforestation incontrôlée ou mal gérée, aux pratiques agricoles dévastatrices (Adomou *et al.,* 2007 ; Deleke *et al.,* 2009 ; Delvaux *et al.,* 2010).

Aucune espèce cependant parmi les plantes recensées n'a été citée comme en danger ou rares dans la localité du Zou. Cela s'expliquerait aisément par le phénomène de l'urbanisation assez poussée dans le Sud-Bénin (Atlantique). L'urbanisation est d'ailleurs la principale raison évoquée par les tradithérapeutes, les vendeuses ou les agriculteurs. Cette urbanisation étant perçue comme l'occupation des espaces par les infrastructures (routes, bâtiments, etc.), réduisant du coup les espaces occupés par les espèces.

Les caractéristiques des peuplements de *Zanthoxylum zanthoxyloïdes* et *Newbouldia laevis*, deux plantes réputées antiparasitaires ont été évaluées. Il ressort des valeurs observées pour les deux espèces, une dominance des individus jeunes. Pour *Zanthoxylum zanthoxyloïdes* la densité des individus dans l'Atlantique (183,3 tiges/ha) est significativement supérieure à celle des individus présents dans le Zou (62,96 tiges/ha). De plus, les individus de diamètre supérieur à 10cm sont inexistants dans ce dernier département. Cette tendance est en accord avec celle observée par Orou (2009) qui explique cela par le fait que le Zou ne constitue pas l'habitat naturel de l'espèce. Néanmoins, les densités qu'il a relevées étaient nettement supérieures à celle trouvées par la présente étude : 357,57 tiges/ha et 244,81 tiges/ha respectivement dans l'Atlantique et dans le Zou. On note aussi une différence significative entre la densité des individus de *Newbouldia laevis* dans l'Atlantique (174,07 tiges/ha) et celle des individus dans le Zou (102,96 tiges/ha). Cette tendance est aussi celle obtenue par Orou (2009) qui avait trouvé une densité des individus de *Newbouldia laevis* de 301,57 tiges/ha dans l'Atlantique et de 200 tiges/ha dans le Zou. Cette diminution des peuplements de ces deux espèces pourrait s'expliquer par la forte pression anthropique dont elles font l'objet. En effet, ces deux plantes sont utilisées tant en pharmacopée vétérinaire qu'en médecine traditionnelle humaine. L'activité de *Zanthoxylum zanthoxyloïdes* a été reconnue dans

le traitement de plusieurs affections : parasiticide polyvalent interne et externe (Arbonier, 2004), fongicide, antiparasitaire, anti-inflammatoire (de Souza, 2008), anti-odontalgique et pour traiter les stomatites, les gingivites et les caries (Rotimi et al., 1988 Arbonnier, 2004) et antidrépanocytaire (Chaaib, 2004). Quant à *Newbouldia laevis*, il est considéré comme un arbre fétiche (Kokou et Sopkon, 2006) au Bénin et au Togo et intervient souvent dans les cérémonies traditionnelles sous forme de bain purificateur (Houzangbé-Adoté, 2004). Il est utilisé comme un véritable marqueur territorial et par les guérisseurs traditionnels en médecine humaine pour traiter: la diarrhée, l'ictère (Tra-Bi, 1997), les affections gastro-intestinales , les infections uro-génitales, les helminthiases, les hémorroïdes, les maladies cardiaques les maladies sexuellement transmissibles (Abbiw,1990 ; Ayensu, 1978; Eyong et al., 2005), la stimulation de la contraction utérine, lors de la parturition et l'expulsion du placenta après la délivrance (Bafor et Sanni, 2009).

La classe d'individus de *Zanthoxylum zanthoxyloïdes* à dbh ≥10 cm est absente dans le Zou. Cela s'explique par les menaces qui pèsent sur l'espèce à savoir les pratiques culturales, l'élevage, l'exploitation du bois et des Produits Forestiers Non Ligneux, la croissance démographique et l'urbanisation (Yessoufou, 2005). Cette raréfaction peut aussi s'expliquer par la présence de jachères et de savanes qui hébergent plus les jeunes peuplements dans le Zou alors que les peuplements plus âgés sont rencontrés dans les forêts sacrées dans l'Atlantique. Par contre, on rencontre, à faible densité, une population d'individus de *Newbouldia laevis* à dbh ≥10. Ces individus se trouvant un peu éparpillés dans la zone. Cette faible densité de la classe des espèces à dbh ≥10 peut s'explique par le fait de la mise en place dans le Centre du Bénin des Jardins de proximité ou de jardins de case. Selon les travaux de Djego et al. (2011), ce phénomène de jardin permet d'aménager un petit lopin de terre pour cultiver certaines espèces rares ou couramment utilisées. C'est cela donc qui expliquerait la faible densité des espèces adultes à grand diamètre (dbh>10). Ces jardins assurent la disponibilité des espèces en voie de disparition dans cette zone et c'est cela même qui expliquerait le fait que les enquêtés n'aient pas cité ces deux espèces comme rares ou menacées de disparition dans le Zou. Cette pratique doit donc être encouragée aussi dans l'Atlantique pour permettre la régénération des plantes et la conservation des potentiels génétique de nos espèces.

La grande majorité des espèces de la flore utilisée en médecine traditionnelle est prélevée dans la nature, et, vu la réduction des peuplements de ces deux espèces, on pourrait supposer qu'au fur et à mesure que la population s'accroîtra, les prélèvements s'intensifieront et l'impact des hommes sur les écosystèmes naturels s'aggraveront. Cette situation pose la problématique de la durabilité de l'exploitation des ressources et par conséquent de la conservation de la biodiversité car on risque d'assister dans très peu d'années à la perte de certaines espèces, en l'occurrence celles qui sont intensément exploitées et qui ne font jusqu'à présent, objet de domestication de la part des populations. C'est le cas de *Zanthoxylum zanthoxyloïdes* qui est classé parmi les espèces menacées de disparition (Sinsin *et al.,* 2009).

Conclusion

L'utilisation des plantes est une nécessité vitale pour les pays en voie de développement. Les résultats de la présente étude ont permis de noter 18 espèces dans l'Atlantique et 36 dans le Zou utilisées pour traiter les affections des animaux. L'enquête ethnobotanique révèle que la zone d'étude est une source importante de matières premières végétales pour la pharmacopée. L'évaluation des caractéristiques des peuplements de *Zanthoxylum zanthoxyloïdes* et *Newbouldia laevis*, deux plantes réputées antiparasitaires montre que ces deux espèces sont à des niveaux différents disponibles dans la zone d'étude. Il faut cependant prévoir des stratégies de conservation de ces espèces à travers par exemple la mise en place des jardins de case ou des campagnes de lutte contre la déforestation

qui semblent être une alternative à l'érosion des ressources naturelles de nos pays.

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2013

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

- Veterinary microbiology, epidemiology
- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
- wildlife management
- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
- Emerging and re-emerging issues in animal resources
- Biosecurity
- Animal resources trade and value chain
- Socio economics and economics of animal resources development

Language

The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

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Authors are invited to submit electronically their manuscripts via attachment only at bahpa@au-ibar.org in a secured PDF and word format. Manuscript can be sent by post in case of unavailability of internet services (authors should be aware that in this case it will take longer time to be published).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance.

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- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and hold readers' attention.
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- Well written to ensure clear and effective presentation of the work and key findings. The BAHPA editorial staff does not copyedit the text of accepted manuscripts, it is therefore important for the work, as presented, to be intelligible. Perfect, stylish language is not essential but it must be clear and unambiguous. If the language of a paper is not clear, Academic Editors should recommend that authors seek independent editorial help before submission of a revision. Poor presentation and language is a justifiable reason for rejection.
- Experiments, statistics, and other analyses performed are described in sufficient detail. The research must have been performed to a technical standard to allow robust conclusions to be drawn from the data. Methods and reagents must also be described in sufficient detail so that another researcher is able to reproduce the experiments described.
- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
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Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

Short Communications: are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

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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.

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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.

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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.
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9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by '*et al.,*'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.,*. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.,* 2001)

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Examples of References

- *Journal Articles:* Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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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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