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## OCCURRENCE OF HAEMOPARASITES AMONG SMALL RUMINANTS REARED UNDER TRADITIONAL HUSBANDRY SYSTEM IN OWERRI, SOUTHEAST NIGERIA

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### Abstract

This study investigated the prevalence of haemoparasites of small ruminants reared under traditional husbandry system in Owerri southeast Nigeria, from June to August, 2010. Blood samples were collected from 50 animals 26 goats and 24 sheep animals and examined for haemoparasites. The results showed that 16 (32.0%) of the animals were infected with blood parasites. Of these, 9 (34.6%) goats were infected, while 7 (29.2%) of the sheep were infected. *Plasmodium* sp recorded the highest prevalence rate of 56.3% in both sheep and goats. This was followed by *Anaplasma* sp and *Eperythrozoon* sp, each of which gave a prevalence rate of 18.8%. *Trypanosoma* sp had the least prevalence of 6.3%. Although not statistically tested; the prevalence of the infection was higher in female and males (81.3%) than male, which was (18.8%). Of the blood parasites, *Plasmodium* sp occurred in the female with a prevalence rate of 69.2%, but none was observed in the males. The prevalence of *Anaplasma* sp was 66.7% in the males and 7.7% in the females. It was therefore concluded that, with the high prevalence of blood parasites recorded in this study, the economic gains by the peasant farmers, could be limited, because of the accompanying morbidity, reduced production and growth and mortality. Consequently, this might further discourage the production of small ruminants in this region.

**Key words:** Haemoparasites, small ruminants, humid tropical rainforest, Owerri, Nigeria

## PRESENCE D'HEMOPARASITES CHEZ LES PETITS RUMINANTS ELEVES EN SYSTEME TRADITIONNEL A OWERRI DANS LE SUD-EST DU NIGERIA

### Résumé

La présente étude a examiné la prévalence d'hémoparasites chez des petits ruminants élevés en système traditionnel dans le Sud Est du Nigeria, de juin à août 2010. Des prélèvements de sang ont été effectués sur 50 animaux (26 chèvres et 24 moutons) et examinés pour la présence d'hémoparasites. Les résultats ont montré que 16 (32,0%) animaux étaient infectés avec des hémoparasites, dont 9 chèvres (34,6%) et 7 moutons (29,2%). *Plasmodium* sp a enregistré le taux de prévalence le plus élevé de 56,3% à la fois chez les moutons et les chèvres. Ce taux était suivi de celui de *Anaplasma* sp et *Eperythrozoon* sp, dont chacun avait un taux de prévalence de 18,8%. *Trypanosoma* sp avait le plus faible taux de prévalence de 6,3%. Bien qu'elle n'ait pas été statistiquement testée, la prévalence de l'infection était plus élevée chez les femelles (81,3%) que chez les mâles (18,8%). Parmi les parasites sanguins, *Plasmodium* sp était présent chez les femelles avec un taux de prévalence de 69,2%, mais il n'a pas été constaté chez les mâles. La prévalence de *Anaplasma* sp était de 66,7% chez les mâles et 7,7% chez les femelles. On a donc conclu que la forte prévalence de parasites sanguins notée dans cette étude constitue une entrave aux gains économiques des paysans, en raison de la morbidité, de la réduction de la production et de la croissance, et de la mortalité qui vont de pair avec ces parasites. Par conséquent, cela pourrait décourager davantage la production de petits ruminants dans cette région.

**Mots-clés :** Hémoparasites, petits ruminants, forêt dense tropicale humide, Owerri, Nigeria

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## Introduction

Small ruminant production is popular in Nigeria and a great number of them are sold annually (Aboul Naga *et al.*, 1992). These animals have high economic potential because of their high fertility and early maturity as well as their adaptability to humid environments (Ademosun, 1988). Apart from producing hides, skins and other products, meat production is the most important.

However, the benefits derived from these animals are well below the expected mainly due to low productivity. This low productivity is due to numerous factors of which the major one is disease (Akerejola, *et al.*, 1979).

For example mortality rates of 34.12% and 36.20% have been reported respectively for sheep and goats respectively in the old Bendel State (ILCA, 1987), one of the close neighbouring states of Owerri. Diseases caused by blood parasites are most prevalent.

Blood parasites are transmitted by insects especially ticks and tsetse flies. Such blood parasites include: *Erlchia ruminatum*, Eperythrozoon, Babesia, Theileria, Trypanosoma, Plasmodium and Anaplasma species.

The effects of blood parasites on the susceptible hosts vary from reduced productivity to death (Urquhart *et al.*, 1988). Rue (1974) in his study of haemoparasites of sheep observed Anaplasma, Babesia and Eperythrozoon species in local and exotic sheep, while Nicholls and Veale (1986), in a two year study of 22 shires in Australia reported Eperythrozoon infection in 10% and 51% of weaner and adult sheep, respectively. Locally, Dipeolu *et al.*, (1982) reported mixed infections of Babesia and Eperythrozoon species as blood parasites of local and exotic pigs in Ibadan.

Similar work involving small ruminants reared in Owerri, south eastern Nigeria has not been done. This work was therefore, conducted to investigate and document the prevalence of blood parasites of small ruminants in Owerri south eastern,

Nigeria.

## Materials and Methods:

Initial visits were made to the homes of the small ruminant owners for introduction and explanation of the purpose and procedures of the study. Thereafter, blood samples were collected through the jugular vein, using sterile syringes and needles into Ethylene Diamine Tetra-acetic Acid (EDTA) containers. The samples were gently mixed with the anticoagulant to prevent coagulation and kept in an ice box, until they were taken to the laboratory for parasitological examination. The examinations were done following standard procedures as described by Shah-Fischer and Say, (1989) and Foreyt (2001).

Data obtained were analyzed, using simple averages and percentages.

## Results

The overall prevalence of haemoparasites among small ruminants reared in Owerri, southeast Nigeria from June to August 2010 is shown in table 1. Out of 50 animals examined, 26 (52%) were goats, and 24 (48%) were sheep. Of these small ruminants, 16 (32%) were infected with various types of blood parasites. Goats had infection rate of 9 (34.6%) while the rate of infection in sheep was 7 (29.2%).

**Table 1:** Overall Prevalence of Haemoparasites among Small Ruminants reared in Owerri.

Animal Species	No. examined (%)	No infected	% infected
Goats	26 (52)	9	(34.6)
Sheep	24 (48)	7	(29.2)
<b>Total</b>	<b>50</b>	<b>16</b>	<b>(32.0)</b>



**Table 2:** Prevalence of Various Haemoparasites among Small Ruminants reared in Owerri.

Animal species	No. of animals (%) infected with <i>Plasmodium</i> sp (%)	No. of animals (%) infected with <i>Anaplasma</i> sp (%)	No. of animals (%) infected with <i>Eperythrozoon</i> sp (%)	No. of animals (%) infected with <i>Trypanosoma</i> sp (%)	Total of animals (%) involved in infection
Sheep	4 (57.1)	1 (14.3)	2 (28.6)	0 (0)	7 (43.8)
Goat	5 (55.6)	2 (22.2)	1 (11.1)	1 (11.1)	9 (56.3)
<b>Total</b>	<b>9 (56.3)</b>	<b>3 (18.8)</b>	<b>3 (18.8)</b>	<b>1 (6.3)</b>	<b>16 (32.0)</b>

**Table 3:** Occurrence of Haemoparasites by sex of Small Ruminants reared in Owerri

Sex of all Animals examined	<i>Plasmodium</i> sp (%) infection	<i>Anaplasma</i> sp (%) infection	<i>Eperythrozoon</i> sp (%) infection	<i>Trypanosoma</i> sp (%) infection	Total (%) infection
Female	9 (69.2)	1 (7.7)	2 (15.4)	1 (7.7)	13 (81.3)
Male	0 (0)	2 (66.7)	1 (33.3)	0 (0)	3 (18.8)
<b>Total</b>	<b>9 (56.3)</b>	<b>3 (18.8)</b>	<b>3 (18.8)</b>	<b>1 (6.3)</b>	<b>16 (32)</b>

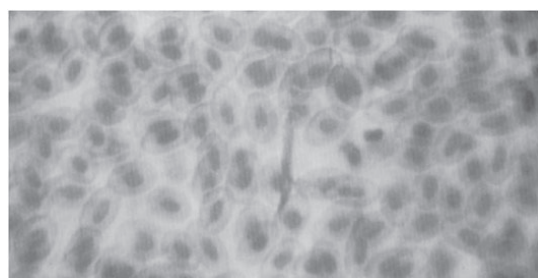
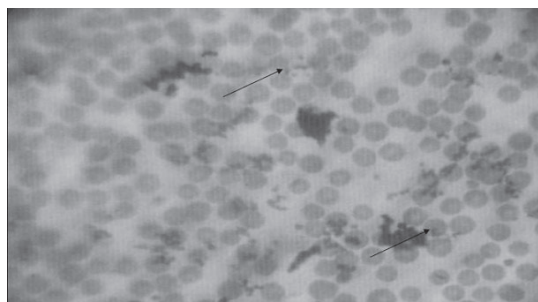
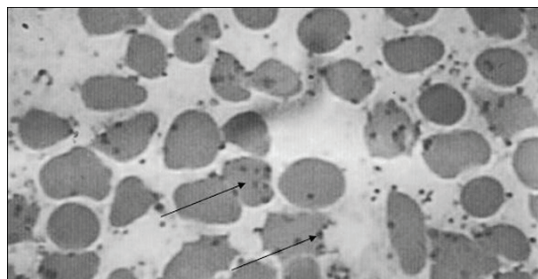
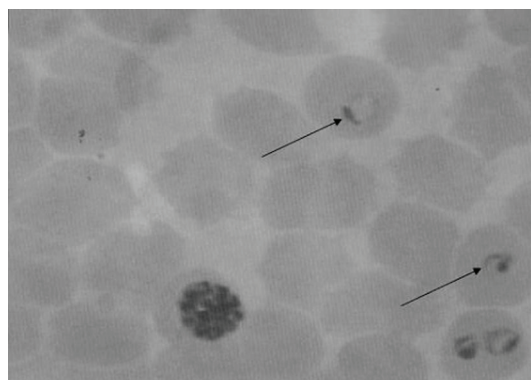
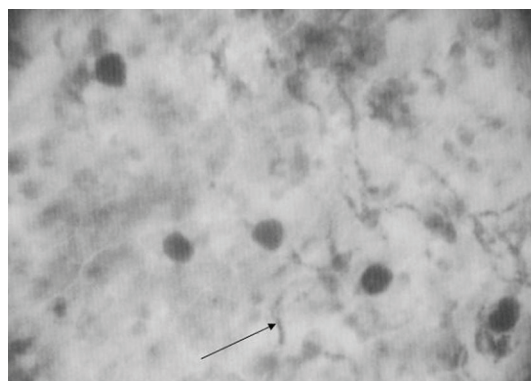
**Fig 1:** Leishman stain showing normal erythrocytes in small ruminants.**Fig. 2:** Showing *Anaplasma ovis* in the erythrocytes of infected sheep.**Fig.3:** Showing *Eperythrozoon* ovis on the erythrocytes of infected goat.**Fig. 4:** Showing the ring form of *Plasmodium* sp in the erythrocytes of an infected goat.**Fig. 5:** Showing *Trypanosoma* sp organisms in the blood of an infected goat.

Table 2 shows the prevalence of various haemoparasites among small ruminants reared in Owerri from June to August 2010. *Plasmodium* sp recorded the highest prevalence rate of 56.3% in both sheep and goats. This was followed by *Anaplasma* sp and *Eperythrozoon* sp, each of which gave a prevalence rate of 18.8%, while *Trypanosoma* sp had the least prevalence of 6.3%. Considering the prevalence among infected animals, sheep recorded 43.8%, while it was 56.3% among the goats. Haemoparasite infection within an animal species, shows that *Plasmodium* gave a prevalence of 4 (57.1%) in goats, while *Eperythrozoon* was 2 (28.6%). There was no *Trypanosoma* infection among the sheep examined (fig. 5). In the goats, again *Plasmodium* yielded a prevalence of 5 (55.6%) and 2 (22.2%) by *Anaplasma*, while *Eperythrozoon* and *Trypanosoma* gave a prevalence of 1 (11.1%) each. The blood film of an uninfected small ruminant, showing the normal erythrocytes is presented in figure 1, while figures 2, 3, 4 and 5 show blood cells of infected with *Anaplasma*, *Eperythrozoon*, *Plasmodium* and *Trypanosoma* organisms respectively.

The result of the occurrence of haemoparasites by sex of small ruminants reared in Owerri, southeast Nigeria is reported in table 3.

The prevalence of the infection was higher in female small ruminants (81.3%) than the male, which was (18.8%). Within the same sex, *Plasmodium* gave a prevalence of 9 (69.2%) among the female animals examined, followed by *Eperythrozoon*, which yielded a prevalence of 2 (15.4%). Among the male animals, *Anaplasma* had the highest prevalence of 2 (66.7%), while *Plasmodium* and *Trypanosoma* species were not observed.

## Discussion

This study was carried out in the months of June to August, which is the rainy season in Owerri, southeast Nigeria. This could be the reason for the relatively high prevalence rate of haemoparasites recorded among the small ruminants examined.

The favareble environmental conditions favareble for the survival and proliferation of the arthropod vectors responsible for the transmissions of the parasites which breed during the rainy season.

However, the overall prevalence (32%) of haemoparasites reported in this study, is similar to with the equally high prevalence (34.12%) and 36.20%) reported for sheep and goats respectively in the Old Bendel State (ILCA, 1987). This shows that for over two decades now, there has not been adequate veterinary attention given in the country to control blood parasitism in small ruminants. This might lead to little or no development in the national small ruminant production system and reduced growth of their population.

The result of the investigation revealed that sheep and goats reared in Owerri were predominantly infected with *Plasmodium* sp. Other parasites observed were *Anaplasma*, *Eperythrozoon* and *Trypanosoma* species. Ukaga et al., (2002) had earlier documented the prevalence of these haemoparasites in animals too. *Plasmodium*, the causative organism of malaria in man was commonly encountered in these animals, suggesting that they serve as reservoirs of infection in the study areas. Malaria parasitaemia in humans is wide-spread in the study area and had been reported by Akpa and Iwuala, (1983) and Egwunyenga et al., (1996).

*Anaplasma* parasites are also major constraints for livestock production. In this study, 18.8% of the small ruminants examined harboured *Anaplasma* in their blood. This partially agrees with Rue (1974) who reported that 11% of 80 local and exotic sheep sampled were positive for *Anaplasma*.

*Eperythrozoon* has also been incriminated as one of the major causative agents of economically important diseases of livestock (Seifert, 1996). This is the first report of this haemoparasite in small ruminants in Owerri. The prevalence of 18.8% could not be compared with any other result, since information on *Eperythrozoon* infection is scarce. Although, Dipeolu et al., (1982) reported a 49% prevalence in blood

parasites of 800 dogs they sampled in Ibadan, southwest Nigeria.

Results obtained from this study showed that there were mild cases of trypanosomiasis (6.3%) in the goats. This may be due to several factors including the low feeding success of tsetse flies on goats, related to the small size and anti-feeding behaviour such as leg kicks and stamping, tail and ear flicks, head movement and skin rippling (Snow *et al.*, 1996). Also biting flies prefer cattle to small ruminants on the account of larger size. In any communal grazing area, the flies are observed to attack cattle, leaving most of the small ruminants uninfested (Kniepert, 1981). Trypanosoma was the least common in occurrence, probably because the animals in the study areas were semi – intensively reared and might have been protected from the bites of tsetse flies found there. This partly agrees with Dipeolu *et al.*, (1982), who reported lower prevalence rate for Trypanosoma organism in exotic than local pigs and attributed it to the system of production engaged.

Goats are considered to be resistant to trypanosome infection, showing only mild or sub clinical manifestation of the disease under natural conditions (Oladele and Adenegan 1998). These workers also reported that the problem of diagnosis of trypanosomiasis in sheep and goats might be due to an existence of some degree of trypanotolerance.

The prevalence of the haemoparasite infection was higher in female than male small ruminants. This may be explained by the stress inducing activities (reproduction and lactation) that female animals undergo which reduce their immunity to infections (Blood and Radostits, 2000 and Opara *et al.*, 2005). Of the blood parasites encountered, *Plasmodium* *sp* occurred in the female small ruminants but not in the male. It is possible that the smell of the male sex hormone in small ruminants is capable of repelling mosquitoes. Other reasons for this difference call for further investigation in the future.

## Conclusion

The prevalence rates of *Plasmodium*, *Anaplasma* and *Eperythrozoon* species were relatively high and so require control. This is necessary because of the fact that diseases of blood parasites generally devastate and drain animals' condition and population. These effects cannot be compromised in livestock production as they reduce the economic gains from these animals.

In Owerri in particular, ruminants are of great economic importance. These animals serve as sources of income for their owners who sell them for use in traditional ceremonies such as funerals, marriages and other social functions. They also serve as sources of animal protein and farm yard manure.

It is therefore, recommended that high level of control of these blood parasites be effectively implemented through control of the vectors. Also improvement in the level of education and standard of environmental hygiene, as well as social habits of the inhabitants of the study areas could help to bring the problem of blood parasite infections in both man and animals under control.

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## **PREVALENCE OF ANTIBODIES AGAINST THREE ACTIVE TYPES OF FOOT AND MOUTH DISEASE VIRUS IN CATTLE IN KHARTOUM STATE AT CENTRAL SUDAN: EPIDEMIOLOGICAL SIGNIFICANCE**

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This work was carried out at Central Veterinary Research Laboratories, Khartoum, Sudan.

### **Summary**

Prevalence of antibody against types "O", "A" and "SAT2" of foot-and-mouth disease virus (FMDV) was studied in cattle sera collected in the year 2005 from Khartoum State at central Sudan, using the liquid-phase blocking ELISA (LPBE). The test was optimized for the screening assay by selection of optimum antigen doses that produced parallel serum titration curves. Results showed high prevalence of type "A" antibody (85.65%) followed by that of type "O" (81%) then "SAT2" antibody (65.78%). Apart from an observed natural resistance in local breeds to type "O" infection, no epidemiological factor seemed to affect separately the prevalence's of each of the three serotypes; prevalence rates of the serotype-specific antibody increased or decreased simultaneously in different locations. Prevalence was higher in the west and south than in the east and north of the State, coinciding with the known direction of animal movements in Sudan, and higher near traffic lines than in milking farms, where sedentary type of management prevails.

The result indicated the maintained activity of three serotypes of FMDV at central Sudan. Prevalence of type "O" antibody was similar to that previously reported in Sudanese cattle and that of "SAT2" was coinciding with the history of its introduction in Sudan. The much higher prevalence of type "A" antibody than the earlier report was likely to be due to testing of sera at low dilution (1/32) in the present LPBE in comparison to high dilutions (1/100 to 1/200) in previous work. This is apparently more to be relevant since the present LPBE distinguished positivity to type "A" from that to type "O" and "SAT2" by lower number of strong positive sera and more sharp decline of their titration curves, consistent with the known antigenic diversity of this virus type.

**Keywords:** LPB ELISA-FMDV-Prevalence-Types "O", "A" and "SAT2"-Sudan

## **PREVALENCE D'ANTICORPS CONTRE TROIS TYPES ACTIFS DU VIRUS DE LA FIEVRE APHTEUSE CHEZ LES BOVINS DANS L'ETAT DU KHARTOUM DANS LE SOUDAN CENTRAL: IMPORTANCE EPIDEMIOLOGIQUE**

### **Résumé**

La prévalence des anticorps contre les types « O », « A » et « SAT2 » du virus de la fièvre aphteuse (VFA) a été étudiée sur la base de sérums de bovins prélevés en 2005 dans l'Etat du Khartoum dans le Soudan central, en utilisant l'épreuve ELISA avec blocage en phase liquide (LPBE). L'épreuve a été optimisée pour le test de dépistage par la sélection de doses optimales d'antigènes qui ont produit des courbes de titrage de sérums parallèles. Les résultats ont montré une forte prévalence de l'anticorps du type « A » (85,65%), suivie de celle du type « O » (81%) et de l'anticorps « SAT2 » (65,78%). A part une résistance naturelle à l'infection de type « O » observée chez les races locales, aucun facteur épidémiologique ne semblait affecter isolément la prévalence de chacun des trois sérotypes ; les taux

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de prévalence de l'anticorps spécifique au sérotype ont augmenté ou diminué simultanément dans différents endroits. La prévalence était plus élevée dans l'Ouest et le Sud par rapport à l'Est et au Nord de l'État, coïncidant avec la direction connue du mouvement des animaux au Soudan. Elle était plus élevée près des couloirs de circulation par rapport aux fermes laitières où prédomine l'élevage sédentaire.

Le résultat a révélé l'activité continue des trois sérotypes du VFA au centre du Soudan. La prévalence de l'anticorps de type « O » était similaire à celle signalée auparavant chez des troupeaux soudanais, et celle du type « SAT2 » coïncidait avec l'historique de son introduction au Soudan. La prévalence beaucoup plus élevée de l'anticorps du type « A » par rapport à celle signalée précédemment était probablement due au test de sérums à faible dilution (1/32) dans la LPBE actuelle par rapport aux fortes dilutions (1/100 à 1/200) dans les travaux antérieurs. Cela semble particulièrement pertinent puisque la LPBE actuelle a distingué la positivité au type « A » de celle au type « O » et au « SAT2 » par le faible nombre de sérums positifs forts et une chute abrupte de leurs courbes de titrage, ce qui est cohérent avec la diversité antigénique connue de ce type de virus.

**Mots-clés:** LPB ELISA-VFA-Prévalence-Types "O", "A" et "SAT2"-Soudan

### Introduction

Foot-and-mouth disease (FMD) is the most important animal viral disease that constrains international animal's trade (Doel, 2003; Rweyemamu and Leforban, 1999; Abu Elzein, 1983) and affects livestock productivity (Perry *et al.*, 2002). Indeed, Sudan massive livestock is far from being fully exploited largely because of the problem of FMD. The disease is long known to be existing in Sudan with serotypes "O", "A", "SAT1" and "SAT2". Clinical disease was reported in cattle only and serological evidence of infection was also obtained from sheep and goats. No general vaccination against FMD has been practiced in the country but some commercial vaccines have been introduced to cover trade cattle (Abu Elzein, 1983).

Of animal viral diseases FMD is the one with the most complex epidemiology and more complex situations are expected in developing countries of Africa and Asia (Fenner *et al.*, 1987). Prior to formulation and implementation of a control program, excessive passive and active disease surveillances are required to identify high and low risk areas and to gain through knowledge of its epidemiology. Otherwise, the last survey against FMD antibodies in Sudan was in 1980 and the available data were not sufficient to determine the geographical distribution of the virus.

The presented work is a study of prevalence of serotypes "O", "A" and "SAT2"

antibody in cattle sera, the main target species, which was carried out in Khartoum state at the centre of Sudan. Particularly, in Sudan and in sub-Saharan Africa, such studies could give more accurate index of prevalence's of FMD viruses than direct disease surveillance. In the area, mild and subclinical FMD (Kitching, 2002) is widely known and a considerable number of the disease outbreaks remain unrecorded (Vosloo *et al.*, 2002). The prevalence of "SAT1" antibody in cattle in Khartoum state was described recently in the first part of this work (Raouf *et al.*, 2009). Prevalence's of antibodies against serotypes "O", "A" and "SAT2" in Sudanese cattle in earlier reports were (75.6%), (18%) and (0.2%) respectively, (Abu Elzein *et al.*, 1987).

### Materials and Methods

#### *Study area and serum collection:*

Khartoum State is located in central Sudan and it is divided by the White Nile, Blue Nile and River Nile into three cities; Khartoum, Khartoum North and Omdurman. The state contains different breeds of cattle; exotic, cross breeds and local breeds that reared under different management systems; nomadic, semi-nomadic (intensive system that turns to nomadic in dry seasons) and sedentary (intensive livestock production). Estimated number of cattle in the State is a quarter of a million (Anon, 2006).

Six hundred and thirty seven (637) cattle sera were randomly collected in

August 2005 from 14 different sites in the state in proportion to estimated numbers of cattle at each site. Sera were obtained from apparently healthy cattle, at least one year old and with no history of vaccination. Sera were collected in plain vacutainers, separated by centrifugation, labeled and stored at  $-20^{\circ}\text{C}$  till use.

#### Sample size:

The most important factor in determining the sample size suitable for studying a variable, which is prevalence in this case, is the degree of variability itself (Putt *et al.*, 1987). According to previous information, prevalence of the three studied serotypes; "O", "A" and "SAT2" varied widely. In present preliminary trials, it was observed that prevalence of type "SAT2" was around 50% and that of types "O" and "A" was well above 50%. It was decided that at such expected high true prevalence's, relative accuracy of 10 would not affect our interpretation of results of estimated prevalence's. The sample size was computed applying the following equation (Putt *et al.*, 1987):

$$n = (100 - P) 10000 \div P \times SE^2 \text{ where}$$

$n$  = sample size

$P$  = prevalence (expected true prevalence)

$SE$  = standard error as relative accuracy

Compensating for  $P$  with 50 in case of "SAT2" and with 70 in case of types "O" and "A", and compensating for  $SE$  with 10, a sample size of 100 for "SAT2" and 43 in case of types "O" and "A".

The above sample size is suitable in case of random sampling (in the sense that every animal has an equal chance of being selected). When random sampling is not achievable, as in an area with the size of Khartoum State, the computed sample size should be multiplied with a factor of 4 (Putt *et al.*, 1987). It follows that the selected sample size would be 400 for type "SAT2" and around 200 for each of types "O" and "A".

#### Testing of sera:

The collected sera were divided into

groups of 30. The selected sample size was picked at random from these groups, in such a manner that the number of sera tested from each site would be proportional to the number of animals at this site.

Sera were tested using the liquid-phase blocking ELISA (LPBE) (Hamblin *et al.*, 1986). The test was modified (Raouf *et al.*, 2006 and 2007) to avoid false positive results reported for the LPBE (Mackay *et al.*, 2001; Alexandersen *et al.*, 2003; Mouchantat *et al.*, 2005; Chénard *et al.*, 2003; Anon, 2000).

#### ELISA reagents and methods:

All ELISA reagents were prepared and supplied by the World Reference Laboratory (WRL) of FMD (Pirbright UK).

Essentially the LPBE procedure (Hamblin *et al.*, 1986) was adopted with slight modification. The modification involved selection of an optimum antigen dose that produced parallel titration curves of sera (strong, medium and weak) (Raouf *et al.*, 2007). In brief, antigen titration was performed. The antigen dilution that gave OD value of 1.5 was determined e.g. 1/200. Starting with twice this dilution, as described in the original procedure (1/100 equivalent to 60  $\mu\text{l}$ /6 ml), a panel of test and reference sera were titrated. The antigen dose was increased gradually, e.g. to 75, 90 and 105  $\mu\text{l}$ /6 ml, and used to titrate the same panel of sera. The antigen dose which produced parallel titration curves of sera (Figure 1) was used for the screening assay. Usually, this dose represents less than two fold increase of the original antigen dose of 1.5, lies in the upper part of the antigen titration curve and gives OD values well below the predefined test limit of 1.9.

The assay application, data expression, calculation and interpretation were all done following the WRL kit information as described before (Raouf *et al.*, 2009).

#### Titration assay:

Similar to screening assay except that sera were used in duplicated two fold serial dilution starting from 1/16 to 1/128.

#### Precision and reproducibility of the assay:

To check the appropriateness of the described optimization, criteria specified for precision and reproducibility (Paiba *et al.*, 2004) for the recently developed solid-phase competition ELISA (SPCE) were used to evaluate type “O” assay. Reproducibility of the assay was evaluated at two levels. The first was reproducibility of results of duplicate wells of each sample within the same test run and the second was reproducibility of results from day to day using control wells and repeatedly tested samples. The duplicate wells of each test sample had to give the same result interpretation i.e. fall either side of the negative/weak positive cut-off point (COP). Secondly, when the duplicate wells fell either side of the negative/weak positive COP the allowable difference was 30 percent inhibition (PI) and when they fell either side of the weak/high positive COP the allowable difference was 14 PI.

## Results

### *Estimated prevalence rates:*

Number of cattle sera tested against each serotype and prevalence rates detected in the three cities of Khartoum State are shown in Table 1. Highest prevalence was detected for type “A” (85.65%) followed by that of type “O” (81%), both at relative accuracy of 10%. Antibodies to type “SAT2” were of a medium prevalence of 65.78% at similar relative accuracy. Omdurman showed the highest prevalence rates followed by Khartoum and at last Khartoum North.

Result presented in Table 2 shows that only a small fraction (25.4%) of type “A” positive sera had shown strong positivity (PI values between 90 and 100). In contrast, about 60% of type “O” and almost one half of type “SAT2” positive sera were strong positive. That seems to fit with the known antigenic diversity of serotype “A” in comparison to that of serotypes “O” and “SAT2” and reflect specificity of the screening assay.

### *Simultaneous increase and decrease of prevalence rates in different locations:*

In Figure 2 prevalence rates of

the most prevalent type “A” (series 1) in different locations are plotted from high to low. Associated prevalence rates of the other serotypes are shown in series 2 and 3. While in Figure 3 prevalence rates of the lowest prevalent type “SAT2” in different locations are plotted from low to high (series 1) and associated prevalence rates of the other serotypes are shown in series 2 and 3. It is observable that in the two figures, the three curves, particularly “A” and “SAT2”, almost have a similar direction from high to low (Fig. 2) or from low to high (Fig. 3) i.e. prevalence rates increased or decreased simultaneously indicating similarity of the epidemiological factors that affect prevalence of the three serotypes.

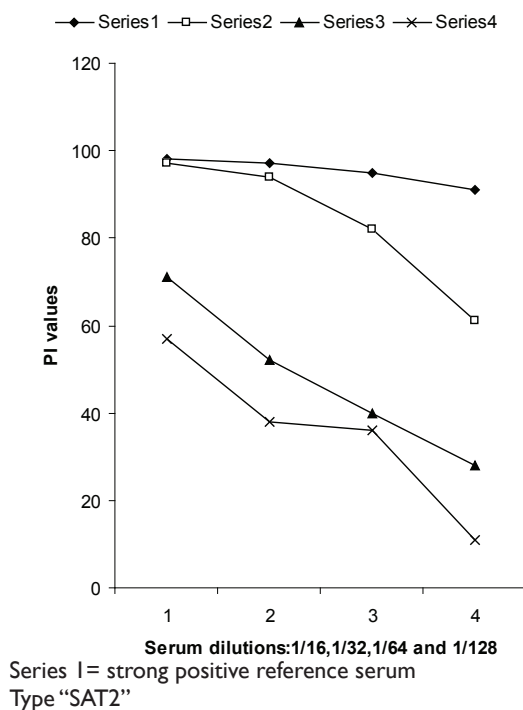
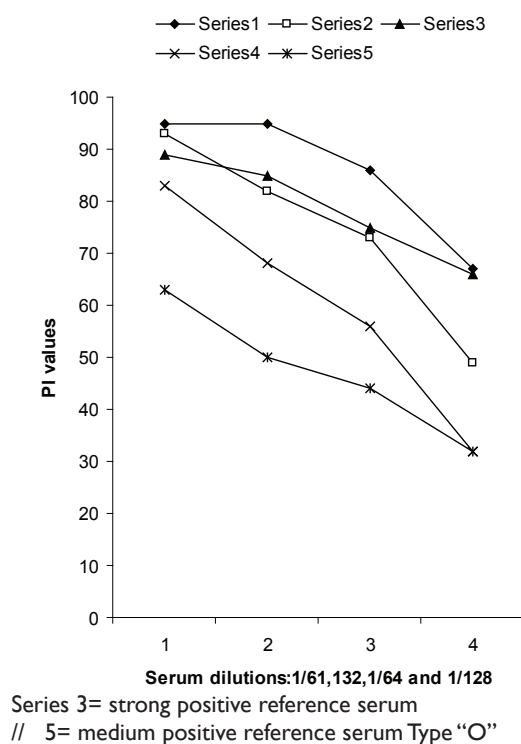
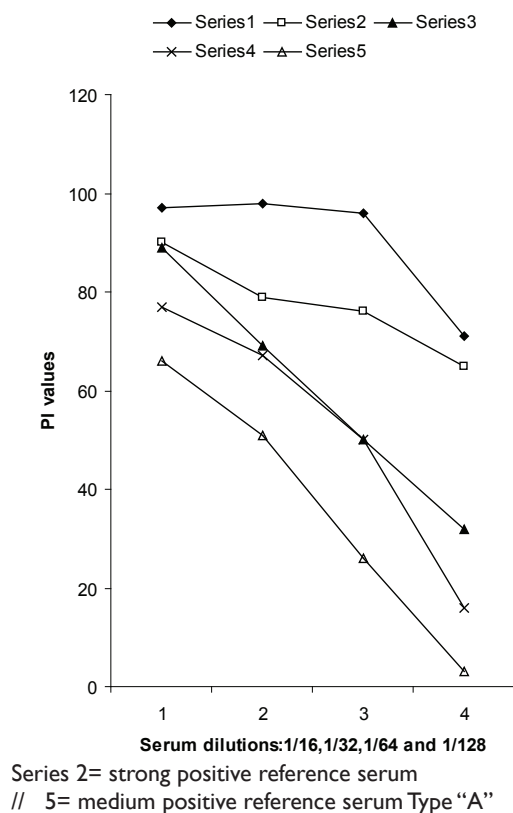
In Figure 2 the first 4 locations (Al Rdwan, Al Azharie, Al Srha and Al Sabiel) are either in the west or south. In Figure 3 the first 3 sites (Ed Babiker, Al Zakiab and Abu El Gasim) are in the east and north indicating a general direction of infection from west and south to east and north.

### *Factors affecting prevalence rates at different locations:*

Figures 2 and 3 are a trial to simulate the Levey-Jennings chart. When prevalence at a particular site rises above or fell below the estimated general prevalence, it might indicate a significant epidemiological factor specific to this site. In Fig. 2, prevalence of type “SAT2” rose above the estimated general prevalence at Al Rdwan, Kuku and Jabra, while prevalence of type “A” Fig. 3 rose above the estimated general prevalence at Al Rdwan, Al Srha and Al Azharie. Al Rdwan, Al Srha and Al Azharie are in the west or south of the State, while Kuku and Jabra are at the centre of the cities near to traffic lines. Prevalence of types “SAT2” and “A” fell below the estimated general prevalence at Ed Babiker, Abu El Gasim and Al Zakiab Fig. 2 and 3 and at milking farms (type “A” in Fig. 3). Ed Babiker, Abu El Gasim and Al Zakiab are in the east and north of the State and milking farms are with sedentary type of management. The curve for type “O” rose above the estimated general prevalence also at Al Rdwan, Al Srha, Al Sabiel (points in the



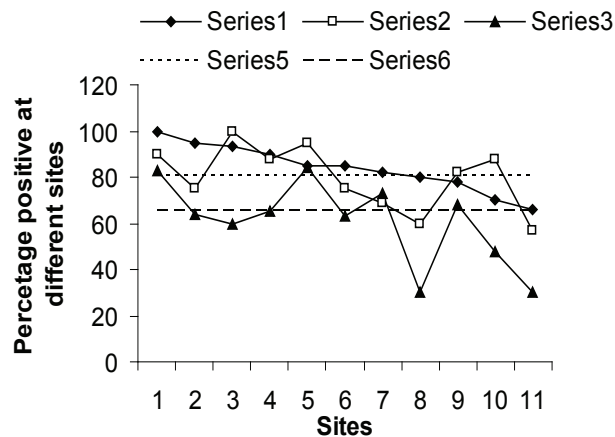
**Figure 1:** Titration curves of positive sera at the optimum antigen doses.



west and south) and Kuku (near traffic lines) (Fig. 3). However, it shows some differences; in addition to Ed Babiker and Al Zakiab (points in the east and north) prevalence rates fell below the estimated general prevalence at Al Azharie, Jabel Toria and Jabra (Figures 2 and 3). Unlike the other locations, Al Azharie, Jabel Toria and particularly Jabra were the only sites where cross and local breeds were reared together. The prevalence of type "O" at these sites was considerably lower than that of type "A" and got nearer to that of type "SAT2". At Jabra it was even lower than that of the moderately prevalent type "SAT2". Table 3 shows prevalence rates in cross and local breeds and Table 4 shows the situation at Jabra. These results could be taken as an indication of development of natural resistance in the local breeds to type "O" infection.

#### Precision and reproducibility of type "O" assay:

Table 5 shows variations between duplicate wells of tested sera. At strong positivity, the duplicate results (n=83) were almost identical. Maximum difference was 5 degrees and the mean difference was below

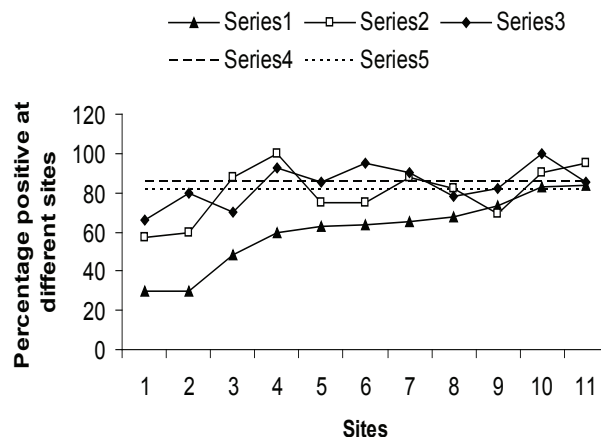
**Figure 2:** A chart showing prevalence rates at different sites in Khartoum State arranged according to the prevalence of type “A” antibody (from high to low)

Series 1 = type A

// 2 = // O (series 5=general estimated prevalence of type O)

// 3 = // SAT2 (series 6= // // of type SAT2)

Locations= 1-Al Rdwan 2-Al Azharie 3-Al Srha 4-Al Sabiel 5- Kuku 6- Jabel Toria 7-Jabra 8-Al Zakiab 9-Milking farms 10-Abu El Gasim 11-Ed Babiker.

**Figure 3:** A chart showing prevalence rates at different sites in Khartoum State arranged according to the prevalence of type “SAT2” antibody (from low to high)

Series 1 = type SAT2

// 2 = // O (series 5= general estimated prevalence of type O)

// 3 = // A (series 4= // // of type A)

Locations=1- Ed Babiker 2- Al Zakiab 3- Abu El Gasim 4- Al Srha 5- Jabel Toria 6- Al Azharie 7- Al Sabiel 8- Milking farms 9- Jabra 10- Al Rdwan 11- Kuku.

**Table 1:** Prevalence of antibodies against the three FMD virus types in the three cities of Khartoum State

	Type A		Type O		Type SAT2	
	No. tested	prevalence	No. tested	prevalence	No. tested	prevalence
Khartoum	70	88.6%	59	76.3%	147	68.7%
Khartoum North	98	79.6%	109	79.8%	175	61.1%
Omdurman	48	93.8%	48	89.6%	92	70%
Overall results	216	85.65%	216	81%	415	65.78%

**Table 2:** Comparison between strong positivity in positive sera of the three virus types (O, SAT2 and A)

Serotype	O	SAT2**	A
No. of +ve sera	175	101	185
No. of very strong +ve sera*	108	48	47
% of very strong +ve sera in the +ve group	61.7%	47.5%	25.4%

\* Pls between 90% and 100%.

\*\* No. of positive sera at Khartoum city only.

**Table 3:** Prevalence of types “O”, “A” and “SAT2” antibodies in cross and local breeds

	Type O			Type A			Type SAT2		
	Total	Cross	Local	Total	Cross	Local	Total	Cross	Local
No. tested	216	187	29	216	184	32	414	354	60
No. positive	175	154	21	185	159	26	273	228	45
% positive	81%	82.4%	72.4%	85.6%	86.4%	81.3%	65.8%	64.4%	75%

one degree. These figures increased to 12 and 4.4, respectively, at medium positivity and to 21 and 7.5 when duplicates were negative. Only 13 duplicate wells out of 200 analyzed did not fall either side of the negative/weak positive cut off point (6.5%). Reproducibility from day to day is shown in Table 6. It is evident that variation of results of repeatedly tested control and test samples was small.

### Discussion

It has long been known that FMD infection in Sudanese cattle was predominantly of the serotype “O” followed, at much lower extent, by types “A”, “SAT1” and “SAT2”, respectively. That gained support from results of serosurveillance (Abu Elzein *et al.*, 1987) conducted in the early 1980s, and frequency of isolation of FMD virus in samples from Sudanese cattle at The WRL of FMD (Pirbright-UK) (Abu Elzein, 1983). Since that time, little was done to control of FMD in Sudan. Recently, Raouf *et al.*, (2009), it was found that “SAT1” type of FMD virus has maintained its low activity and showed low antibody prevalence similar to what previously reported by (Abu Elzein *et al.*, 1987). Similarly, in this work seroprevalence detected showed that type “O” has also maintained its high activity (81%), but revealed higher activity for type “A” (85.65%) and “SAT2” (65.78%) as compared with 18%

and 0.2% respectively in 1980 (Abu Elzein *et al.*, 1987). Serosurvey conducted, thereafter, in other parts of the country also showed similar higher prevalence rates for types “A” (78.13%) and “SAT2” (44%) antibody (Habiela *et al.*, 2010). The detected rise in type “SAT2” antibody seroprevalence is consequent with the relatively recent introduction of this infection in Sudan in 1977 (Abu Elzein and Crowther, 1979) about the time of the first mentioned survey. It is, also, consequent with the high rate of reporting the condition throughout the African continent (Vosloo *et al.*, 2002) and from recent FMD events in Sudan (Habiela *et al.*, 2010; Raouf *et al.*, 2010).

A different state of affairs appears to be the case for type “A” seroprevalence and infection. It showed a high prevalence of 85.65% at relative accuracy of 10%. In other words, true prevalence might reach as high as 94.2%. It even surpassed the prevalence of type “O” which has long been known in the country as the most frequent and wide spread. Seroprevalence, s detected, using the LPBE, in cattle species in other parts of the country were also higher for type “A” (78.13%) than type “O” (69.39%) (Habiela *et al.*, 2010). On the other hand, earlier report (Abu Elzein *et al.*, 1987) of type “A” seroprevalence in Sudanese cattle was only 18% though the infection was recorded in Sudan since 1957 (Abu Elzein, 1983). Within all reported

**Table 4:** Differences between the cross and local breed in prevalence to types “A” and “O” antibodies at Jabra

	Type “A”			Type “O”		
	Total	Local	Cross	Total	Local	Cross
No. examined	28	18	10	29	19	10
No. positive	23	16	7	20	12	8
No. normal	2	-	2	9	7	2
No. retest	3	2	1	-	-	-
% positive	82.14	88.88	70	69	63.2	80

**Table 5:** Variations between pairs of sample wells (test sera) in type “O” serosurvey (reproducibility within the same test run)

% of duplicates that did not fall either side of the COP	6.5*
Differences between duplicate PI values of strong positive sera (PI between 85 and 100)	
No of pairs	83
Minimum difference	0
Maximum difference	5
Mean of difference	0.94
Differences between duplicate PI values of medium positive sera (PI between 50 and 84)	
No of pairs	48
Minimum difference	0
Maximum difference	12
Mean of difference	4.4
Differences between duplicate PI values of negative sera (PI below 50)	
No of pairs	57
Minimum difference	0
Maximum difference	21
Mean of difference	7.5

n=200

**Table 6:** Variations in percent inhibition between control wells (reference antisera and control samples) in type “O” serosurvey (reproducibility from day to day)

Serum group	Serum No.	No of pairs	Mean	S.D.	Minimum	Maximum
Strong	10	2	96	0.81	96	97
positive	C++	10	90	3.56	82	93
Medium	229	3	74	5.5	64	79
positive	388	6	71	4.59	66	80
	98*	4	70	6.24	64	78
Negative	C-	10	6	8.7	-9	18
sera	140	3	7	10	-6	23

\* Replaced C+

periods, type "A" has been isolated at lower frequencies than type "O" (Abu Elzein, 1983; Vosloo et al, 2002; Habiela et al, 2010; Raouf et al., 2010). Type "A" is known for its antigenic diversity. It was obvious, in this work, unlike types "O" and "SAT2", most of type "A" positive sera (75%) were medium positive (Table 2). Medium positive sera were likely to show negative results if tested at higher serum dilutions e.g. 1/100 or 1/200 (observe positivity in Fig. 1 of type "A" sera at the third and fourth dilutions which are equivalent to final dilutions of 1/128 and 1/256). In the present and previous work (Abu Elzein et al., 1987) the test antigen used was similar. It was A22 mahmatli. But, Abu Elzein and coworkers (1987) tested sera at dilutions 1/100 to 1/200 while in the LPBE, used presently, sera were tested at dilution 1/32. This could largely explain the wide divergence between results of the two surveys in regard to prevalence of type "A" antibody. However, the high prevalence of type "A" antibodies should not be unexpected. The infection is long known to be circulating in Sudan unopposed. Frequency of typing the condition increased considerably from 20% during the period between 1957 and 1981 (Abu Elzein, 1983) to 40% during the period between 1982 and 2007 (Vosloo et al., 2002) though during the latter period sending samples to the WRL was suspended for 10 years. It corresponds with the known endemicity of the virus in North Africa and the high frequency of reporting the infection in western and eastern Africa (Vosloo et al., 2002).

Throughout Africa, complex epidemiology of FMD is expected. Moreover, in Sudan different rates of prevalence in different ruminant species led Abu Elzein et al., (1987) to expect different cycles of infection in these species. It is encouraging to observe that in the present results, apart from the described natural resistance in local breeds to type "O" infection (Figures 2 and 3; Tables 3 and 4); no epidemiological factor, which is particular to either type (such as involvement of an other species), seemed to affect separately their seroprevalence's. Results presented in Figures 2 and 3

show that, the three curves of prevalence, particularly those of type "A" and "SAT2", have a general similar direction from high to low or from low to high. It was also evident that prevalence rates of types "A" and "SAT2" increased or decreased simultaneously, above or below their detected general prevalence's. They increased at points in west and south or near traffic lines while decreased at points in the east and north, and at milking farms (Fig. 2 and 3). On the other hand, the observed natural resistance to type "O" was consistent with the earliest recording of this serotype in Sudan in 1938 (Abu Elzein, 1983).

In Sudan animal movement was noted as the vital factor in the spread of FMD. Following animal movement, FMD infection is expected to move from west and south to east and north of the country (Abu Elzein, 1983). Foot-and-mouth disease infections in the study area follow this direction. Prevalence rates detected were highest in Omdurman in the west followed by Khartoum in the south and at last Khartoum North in the east and north of Khartoum state (Table 1). Similarly, at particular locations in the state, prevalence rates were higher at points in the west and south (Al Rdwan, Al Azharie, Al Srha and Al Sabiel), or at points near to traffic lines in the centre of the cities (Kuku and Jabra) which also might be indicative of the importance of transmission of infection by inanimate objects.

The test used throughout this work was the LPBE (Hamblin et al., 1986). It is the first time to be used in Sudan. Considerable care was taken optimization of the test in its screening form (Raouf et al., 2006 and 2007). The test detected, as previously known, similar low (9.5%) and similar high (81%) prevalence's for "SAT1" (Raouf et al., 2009) and "O" antibody, respectively. On the other hand, it detected a rise, from what previously known, in prevalence's of "SAT2" and "A" antibody. Moreover, the test distinguished positivity to type "A" from that to types "O" and "SAT2" by lower number of strong positive sera and more sharp decline of titration curves of type "A" positive sera (compare titration

curves of types “A” and “O” in Fig 1) what is consistent with the known antigenic diversity of this serotype. Also among the significant epidemiological findings in this work was that, apart from the observed natural resistance of the local breeds to type “O” infection, no epidemiological factor seemed to affect separately prevalence of each of the three serotypes; contrasting previous predictions. Through 14 different locations, prevalence rates increased or decreased simultaneously. Appropriateness of the serological test is necessary for validation of these findings. One measure of validation of serological tests is precision and reproducibility; within the same test run and from day to day. Reproducibility of the assay was examined when the test produced negative results (“SAT1”) (Raouf et al, 2009) or, in this work, when most of the tested sera were positive (type “O”). In type “O” survey, only 6.5% of the examined wells (n=200) did not fall either side of negative/weak positive COP Table 5. Moreover, figures reported for the S.D. PI of control wells (table 6) were similar or lower than those reported for the SPCE ( $94.8 \pm 5.1$ ,  $69.0 \pm 7.1$  and  $11.6 \pm 11.9$ ) (Paiba et al., 2004), the more recent approach in serotype specific serology of FMD.

### Impact

In sub-Saharan Africa, as FMD is known for its mild nature and many outbreaks remain unrecorded, serosurveillance could give a more accurate index of the prevalence of a particular FMDV type. The work detected high prevalence for the 3 surveyed serotypes; “A”, “O” and “SAT2” in cattle at central Sudan. Though, complex epidemiology of FMD always raises concern, however, in the study area no epidemiological factor seemed to affect separately the prevalence of each type, apart from detected natural resistance to type “O”. The hitherto unprecedented high prevalence of type “A” in this area was attributed to the known sensitivity of the LPBE and a demonstrated high specificity of the applied screening assay in comparison to former methods.

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## SERO-PREVALENCE OF AVIAN INFLUENZA, NEWCASTLE AND GUMBORO DISEASE IN CHICKENS IN KOGI STATE, NIGERIA

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### Abstract

A survey was carried out in chickens from backyard farms, rural flocks and live bird markets in six local government areas of Kogi state to study the epidemiology of three viral diseases of high economic importance in Nigeria. A total of 750 sera were collected and screened for evidence of antibodies to Avian Influenza, Newcastle and Gumboro disease viruses. An overall prevalence of 22.4% and specific prevalence of 26.0% were obtained for avian influenza in the live bird market chickens using the agar gel immunodiffusion test. Antibodies to Newcastle disease using haemagglutination inhibition test gave an overall prevalence of 25.6% with a mean titre of  $1.39 \pm 0.088$  and a unit specific prevalence of 36.7% ( $2.07 \pm 0.233$ ) for rural chickens with 74.5% of the chickens surveyed having antibody titre  $<4 \log_2$ . Overall seroprevalence for Gumboro using agar gel immunodiffusion test was 16.3% with highest unit specific seroprevalence of 21.3% among chickens from live bird market. The study reveals that Avian Influenza, Newcastle and Gumboro disease viruses are circulating among chickens in Kogi state and the negative impacts of these diseases may explain the low level of commercial poultry production in the state.

**Key words:** Avian Influenza, apparently healthy, rural chickens, live bird market, Kogi state.

## SEROPREVALENCE DE L'INFLUENZA AVIAIRE, DE LA MALADIE DE NEWCASTLE ET DE LA MALADIE DE GUMBORO CHEZ LES POULETS DE L'ETAT KOGI AU NIGERIA

### Resume

Une enquête a été réalisée sur des poulets de basse-cour, du milieu rural et des marchés d'oiseaux vivants dans six zones de l'Etat de Kogi, afin d'étudier l'épidémiologie de trois maladies virales de grande importance économique au Nigeria. Au total, 750 sérums ont été prélevés et examinés pour rechercher des preuves de présence d'anticorps contre les virus de la grippe aviaire, de la maladie de Newcastle et de la maladie de Gumboro. L'épreuve d'immunodiffusion sur gélose a révélé une prévalence globale de 22,4% et une prévalence spécifique de 26,0% pour la grippe aviaire chez les poulets du marché d'oiseaux vivants. Pour les anticorps contre la maladie de Newcastle, le test d'inhibition de l'hémagglutination a révélé une prévalence globale de 25,6% avec un titre moyen de  $1,39 \pm 0,088$  et un taux de prévalence spécifique de 36,7% ( $2,07 \pm 0,233$ ) pour les poulets du milieu rural, 74,5% des poulets étudiés ayant un titre d'anticorps  $<4 \log_2$ . Le test d'immunodiffusion sur gélose a révélé que la séroprévalence globale de la maladie de Gumboro était de 16,3%, la plus forte séroprévalence spécifique de 21,3% étant notée chez les poulets du marché d'oiseaux vivants. L'étude révèle que les virus de la grippe aviaire, de la maladie de Newcastle et de la maladie de Gumboro circulent parmi les poulets de l'Etat de Kogi ; et les impacts négatifs de ces maladies peuvent expliquer le faible niveau de production du commerce de poulets dans cet Etat.

## Introduction

Until recently, the most important viral diseases of poultry in Nigeria were Newcastle disease (ND), Gumboro disease (GD), avian pox, Marek's disease (MD), duck viral enteritis and hepatitis (Abdu, 2007). These diseases were considered most important because of their enormous negative effects on productivity and economic impacts on human livelihood (Abdu, 2007).

The advent of highly pathogenic avian influenza (HPAI) on the African continent in an outbreak first confirmed in Nigeria on the 8th February, 2006, has widened the earlier views of the extent of viral diseases in poultry on the continent (Adene *et al.*, 2006). The estimated poultry population of Kogi state is above 3 million with 80% being rural and 20% commercial poultry (Adene and Oguntade, 2006). During the 2006-2008 period of HPAI outbreaks in Nigeria, Kogi state was reported free of HPAI (AICP, 2008) in spite of the fact that the state is surrounded by eight states including the Federal Capital Territory (FCT) where HPAI was reported.

However, apart from the periodic surveillance activity that started in the country in the wake of the HPAI outbreaks, in depth research on poultry diseases in Kogi state is minimal. As a result of this short fall, there are no research-based reports on poultry diseases in the state. Hence, inferences on the interplay of factors on poultry diseases were vague and not factual.

This study was designed to determine the epidemiology of avian influenza (AI), Newcastle disease and Gumboro disease and their impacts on poultry production in Kogi state, Nigeria. This was done by examining serum samples from chickens in backyard farms, rural poultry and live bird markets for evidence of antibodies to any of the three viral diseases.

## Materials and Methods

### Study Area

The study was carried out in chickens from backyard farms, rural poultry and live

bird markets in six out of the twenty-one local government areas (LGAs) of Kogi state. Kogi state lies between latitude 6° 44'N - 7°36'N and longitude 7° 49'E - 8° 27'E, situated at a height of about 789m above sea level.

### Sample Size

The sample size for the study was determined using the formula described by Mahajan, (1997):

$$N = Z^2Pq/L^2$$

Where  $Z = 1.96$ ;

$q = 1-P$ ;

$L^2 =$  allowable error (5%)

$P =$  Prevalence rate = 13.4% (Wakawa, 2007)

$N = 177.5 =$  for outbreak sample size.

For non – outbreak sample size,  $N^* =$  outbreak sample size multiply by  $Z^2$

$$= 177.5 \times (1.96^2) = 177.5 \times 4 = 710.$$

However, the sample size was increased to 750 to allow for lost samples.

### Sample Collection

Approval to undertake the study was obtained from the leaders in the live bird markets, farm owners and village (rural) heads. All the chickens sampled had no history of vaccination against AI. Simple random sampling without replacement was used to sample the chickens from each cluster. About 2–3 ml of blood was collected from the brachial vein of chickens using 21G needle and 5 ml syringe, observing asepsis after proper restraint by an assistant. A total of 750 serum samples were collected from apparently healthy chickens between the months of October, 2009 – January, 2010. The blood collected was allowed to clot between 2-4 hours after which the serum was decanted into serum bottles and stored at – 20°C until analyzed.

### Detection of Avian Influenza Antibodies

The test antigen used was an inactivated H5N2AI virus while the antiserum was an H5N3 serum both prepared by OIE/FAO laboratory for AI and NDV, delle Venezie, Italy. The antigen and antiserum were first

titrated against each other using a two-fold dilution and incubated at 37°C for 24 hours. The highest dilution that gave clear precipitin line was 1:16 but 1:8 dilution of antigen was used for the final experiment. The agar gel immunodiffusion (AGID) test was then carried out using 25 µl each of antigen, antiserum and test serum as prescribed by OIE (2008). The AGID test plates were then incubated at 37°C for 24 hours after which they were examined and read in a dark room with the chamber illuminated from behind.

**Detection of Newcastle Disease Antibodies**  
The antigen used was La Sota strain of ND Vaccine batch 07/2009 and antiserum obtained from the Veterinary Research Institute (NVRI), Vom, Nigeria. A 1% suspension of chicken red blood cells (RBC) was prepared and used as indicator in the haemagglutination (HA) and haemagglutination inhibition (HI) tests. The HA titre of the La Sota antigen was determined as prescribed by OIE (2008) and diluted to contain 4 HA units. This concentration of antigen was used for the HI test. The HI antibody titre for each test serum was determined and expressed in log<sub>2</sub> and the mean titre was also calculated.

#### *Detection of Gumboro Disease Antibodies*

The GD antigen used was prepared from infected bursae as described by Harai et al. (1972). The AGID test was carried out according to Harai et al. (1972) using 25 µl of antigen, known positive serum and test serum. The plates were incubated in a humidified chamber at room temperature for 48 hours after which they were read in a dark room with the plates illuminated from behind. Positive test samples gave precipitin lines that were identical and continuous with bursae antigen and the known positive serum.

## **Results**

A total of 750 serum samples made of 300 sera each from backyard farms and live bird markets with 150 sera from rural chickens were tested for antibodies to AI, ND and GD. Overall and sampling unit specific

antibody prevalence rates against individual virus antigen with mean antibody titre for ND were AI, 22.4%; ND, 25.6% ( $1.39 \pm 0.088$ ); and GD, 16.3% as indicated in Tables 1 and 3. A total of 13 (1.73%) sera were positive for AI, ND and GD antibodies while the antibody prevalence in other combinations was AI and ND, 58 (7.73%); AI and GD, 24 (3.20%); and GD and ND, 19 (2.52%) (Table 2). Based on LGAs, AI antibody prevalence was highest in Adavi LGA with 42.3%, lowest for Kabba/Bunu LGA with 21.9% while prevalence in Ankpa and Dekina LGAs was zero (Table 4). In addition, ND prevalence was highest in Adavi LGA with 58% ( $3.47 \pm 0.26$ ) and lowest in Dekina LGA with 6.1% ( $0.25 \pm 0.096$ ) (Table 4). However, GD antibody prevalence was highest in Okene LGA with 24.8% and lowest in Dekina LGA with 4.4% (Table 4).

## **Discussion**

Kogi state was thought to be free of AI infection during the 2006–2008 outbreaks period despite being surrounded by eight states and the Federal Capital Territory that reported HPAI infection (AICP, 2008). The result of this study shows the presence of AI antibodies among apparently healthy chickens in the state, which is an indication of natural infection because vaccination against AI is not routinely done in Nigeria. This implies that the AI virus of probable low virulence is circulating in the state with the likelihood of mutation to a virus of high virulence or the exacerbation of clinical disease and deaths in concurrent infection with ND (Easterday et al., 1997).

The congregation of various types of chickens and other poultry from different places in a common point of sale compromises biosecurity measures within LBMs thereby increasing the risk for the transmission and spread of HPAI (Aye, 2010). This may be the reason for the high level of AI antibody prevalence among chickens in LBMs because chickens are brought from different places even across state borders including states where HPAI had occurred without movement restriction. This is a risky practice

**Table 1:** Prevalence of avian influenza, Newcastle disease and Gumboro disease in the sera tested.

Disease	Test result		Total no. tested
	Positive (%)	Negative (%)	
Avian influenza	168 (22.4)	582 (77.6)	750
Newcastle disease	192 (25.6)	558 (74.4)	75
Gumboro disease	122 (16.3)	628 (83.7)	750

**Table 2:** Distribution of sera with combined antibodies to AI, ND and GD in the sera of chickens tested.

No. tested	Prevalence of AI, ND and GD	Prevalence of AI and ND	Prevalence of AI and GD	Prevalence of ND and GD
750(100%)	13 (1.73%)	58 (7.73%)	24 (3.20%)	19 (2.52%)

**Table 3:** Distribution of avian influenza, Newcastle and Gumboro disease antibodies in chickens by sample units.

Sample unit	Prevalence of avian influenza antibodies (%)	Prevalence and mean titre of Newcastle disease antibodies {%}	Prevalence of Gumboro disease antibodies (%)	No. of samples tested
Backyard chickens	62 (20.7)	97{32.3(1.66±0.145)}	41(13.7)	300
Rural chickens	28(18.7)	55{36.7(2.07±0.233)}	17(11.3)	150
Live bird market chickens	78 (26.0)	40{13.3(0.78±0.107)}	64(21.3)	300
Overall prevalence	168(22.4)	192{25.6(1.39±0.088)}	122(16.3)	750

**Table 4:** Distribution of avian influenza, Newcastle and Gumboro disease antibodies in chickens by local government areas.

Local government area	Avian influenza %	Newcastle disease % Mean±SEM}	Gumboro disease %	No. of samples tested
Adavi	60 (42.3)	83(58.5)3.47± 0.26	28(19.7)	142
Ankpa	0 (0.0)	26(18.7)0.91±0.167	19 (13.7)	139
Dekina	0 (0.0)	7(6.1)0.25±0.096	5(4.4)	114
Kabba/Bunu	27 (21.9)	14(11.4)0.65±0.143	24(19.5)	123
Lokoja	38 (33.0)	44(38.3)1.85±0.227	18(15.6)	115
Okene	43 (37.0)	18(15.4)0.84±0.186	29(24.8)	117
Overall prevalence	168(22.4)	192 (25.6) 1.39±0.088	122(16.3)	750

for the introduction, spread of AI and other contagious poultry diseases to new areas. This finding indicates the role LBM in the epidemiology of AI.

Ducatez *et al.*, (2006) reported wild birds and trade in poultry to be the likely sources of introduction of AI into Africa. The presence of AI antibodies in rural chickens and those of live bird markets confirmed the report. There are fears that the free entry of birds into the LBMs will enhance maintenance

and spread of the virus for a long time once introduced (Ducatez *et al.*, 2006).

This study reveals AI antibody prevalence in apparently healthy rural chickens similar to the report by Durosiniorun (2008) in Kaduna state. The presence of AI antibodies in rural chickens that roam freely and scavenge for food underscores the danger of this type of chickens to commercial poultry in the transmission and spread of the disease. This is also an indication that the country may not

be completely free of AI infection because rural chickens are kept for a long period of time, which may provide the time lag for the mutation of LPAI to HPAI (Alexander, 2000).

There is a relationship in the occurrence of AI antibodies in the four local government areas (LGAs) as seen in this study. These LGAs are contiguous to one another with common biophysical features such as close proximity to one another, location along major routes from the northern part of the country to south-west, south-south and south-east as well as free access to the live bird markets where the same live bird marketers move live poultry from one location to the other. These common features present the platform for the likely introduction and spread of AI virus in these areas. Based on proximity, Chen *et al.*, (2004) explained how the A/Guangdong/goose/1/96 HPAI H5N1 precursor virus jumped from waterfowls to domestic chickens and spread from Mainland China to Hong Kong SAR in 1997.

The various favorable conditions for the contact and airborne transmission of HPAI to chickens had been described (Tsukamoto *et al.*, 2007). The fact that AI antibodies were not detected among chickens in Ankpa and Dekina LGAs might be due to the absence of large water bodies and other favorable risk factors for AI infection in these areas. The LBMs in these two LGAs are well organized with built up stalls and strong market union that restricts traders from other areas to sell live poultry in their markets. The backyard poultry farms in these areas though few in number were also more organized and located away from households with improved biosecurity. In addition, the location of these two LGAs away from major routes as well as the long distance between them and the four LGAs where AI antibodies were detected could also be responsible for the absence of AI antibodies in chickens' sera in these areas.

The concurrent infection of birds with AI and ND viruses can lead to exacerbated clinical signs as well as misdiagnosis of either disease (Easterday *et al.*, 1997). This study shows a substantial percentage of rural

chickens with antibodies to both AI and ND antigens. This may be due to scavenging activity of rural chickens that may serve as interface for transmitting AI from migratory wild birds to commercial chickens. The rural chickens may in turn contract ND virus from the commercial chickens that are routinely vaccinated with attenuated live vaccines (Nwanta *et al.*, 2006) or contact with poultry manure in refuse dump. The live bird market system, which serves as a pool for the various types of birds offered for sale as well, may account for the combined sero-prevalence of antibody to both AI and ND. The evidence of the presence of concurrent infections of AI and ND has serious consequences on clinical diagnosis of both diseases and control activities. The absence of prior report of AI in the state may be due to misdiagnosis of AI for ND since they have similar clinical presentations (Wakawa *et al.*, 2009).

Mixed infection of HPAI and ND was reported among rural chickens in Jigawa state, Nigeria with severe clinical disease and deaths (Wakawa *et al.*, 2009). This study showed mixed infections of AI and ND but with no observable clinical signs and deaths similar to the report of the evidence of LPAI (H5N2) in apparently healthy rural chickens in Kaduna state (Durosinlorun, 2008). The presence of the mixed infection of AI of low virulence with ND may account for production losses and high seasonal mortality in rural chickens during outbreaks of ND virus in cold months (Wakawa *et al.*, 2009).

Poor antibody response to disease challenge has been associated with birds infected with GD early in life (Abdu, 2007). The combined effects of AI, ND and GD as seen in this study might explain the low antibody production in response to diseases and vaccinations among chickens especially in backyard poultry farms. This will result in birds so affected to become more susceptible to AI, ND and other diseases due to the immunosuppressive effect of GD. Equally, it shows that the antibodies sero prevalence of AI and ND would have been relatively higher especially in Okene LGA and in the LBMs if not for the immunosuppressive effect of GD.



It was reported that ND is endemic and the most devastating disease of rural chickens (Adene, 1996). The high sero-prevalence of ND antibodies among rural chickens in this study, confirms the endemic nature of ND similar to the report in Kaduna state. The presence of ND antibodies in rural chickens might be due to natural infection, as this type of chickens are not routinely vaccinated or the ingestion of vaccinal virus from the faeces of vaccinated commercial poultry during scavenging (Nwanta *et al.*, 2006). In spite of the unit specific high sero-prevalence of ND among rural chickens, the mean antibody titre is below the protective level of  $4 \log_2$ . This indicates the high susceptibility of most chickens to virulent ND virus with extensive disease impacts (Boven *et al.*, 2008). The scavenging rural chickens that often roam freely pose serious danger to chickens in backyard farms and may hinder effective ND control especially, when the strain of the field virus is different from the vaccinal virus.

The endemic nature of ND among rural chickens may be the reason why chickens from backyard farms, which are routinely vaccinated, showed low antibody titre to ND virus. It may be inferred that the low ND antibody titre among chickens from backyard farms was as a result of challenge by the endemic field strain of the virus, which was different from the vaccine strain. It may also be due to recovery from recent disease challenge after vaccination or due to errors in vaccination (Sa'idu *et al.*, 2004). The mean antibody titre for ND is below the protective level of  $4 \log_2$  for chickens in all the sample units surveyed including the backyard farms (Boven *et al.*, 2008). This calls for the review of the current vaccination program for ND among commercial chickens and the institution of vaccination against ND in rural chickens.

The ND sero-prevalence was highest in Adavi LGA but with low level of mean antibody titre for protection. The high sero-prevalence of ND antibody may be due to the increased activity of the endemic field virus among rural chickens as higher number

of sera was obtained from rural chickens in this area. It may also be due to a response of recent vaccination by chickens in backyard poultry farms in this area. This has serious implications on the clinical diagnosis of AI considering the fact that its sero-prevalence was equally high in this area. The low prevalence of ND virus antibodies in Dekina LGA shows that chickens in this area will be highly susceptible to ND virus infection during outbreaks (Adene and Oguntade, 2006).

After ND, GD is ranked as the second endemic disease of poultry that limits rural poultry production in Nigeria (Adene and Oguntade, 2006). Mbuko *et al.* (2010) reported that broilers, and cockerels kept with layers have the highest odds of contracting GD. The sero-prevalence of GD is highest among chickens in LBM owing to the fact that chickens from backyard farms that are routinely vaccinated against GD and susceptible rural chickens that are not vaccinated are often kept together for sale. Sharing or cross-infection also occurs among birds within this period thereby, resulting in high antibody response. This study reveals that chickens from the LBMs are potential sources of spread of infection of GD and other diseases to the areas they may be taken to after purchase.

Okene LGA has the highest GD antibody prevalence probably due to anamnestic response of chickens from the backyard poultry farms. The immunosuppressive effect of GD (Abdu, 2007) is apparent in this area as antibody response to other diseases especially ND is low as seen in this study. This leaves chickens in this area more susceptible to other contagious poultry diseases resulting in high economic losses to farmers.

It can be inferred from this study that the impacts of these three important viral diseases might be the cause of the low level of poultry production in Kogi state. Therefore, there is need to urgently institute preventive and control measures to mitigate their effects on poultry production.

Furthermore, it is recommended that AI surveillance in the state should be sustained and AI virological investigation carried out in LBMs and in rural poultry to ascertain the presence of the virus and its serotype. Equally, rural poultry farmers should be encouraged to improve their production systems as well as being incorporated into the AI control and compensation plan by government.

The findings of this study reveal the evidence of AI (H5) antibodies in Kogi state, an area earlier reported to be free of the disease. Equally, the study confirms the endemicity of ND and GD, which calls to question the effectiveness of the vaccination options used in their prevention.

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## STUDY ON GASTROINTESTINAL HELMINTHES OF BACKYARD LOCAL CHICKENS IN EAST SHOA ZONE OF OROMIA REGIONAL STATE, ETHIOPIA

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### Abstract

A study was conducted to determine the prevalence of gastrointestinal helminthes in chickens raised under traditional management system in three selected agro ecological areas of East Shoa namely Akaki, Ada'a and Adama. The study showed that 517 (86.17%) of the examined chickens (n= 600) were found to be infected with various helminthes. Out of 600 chickens, 370 (61.65%) carried nematodes including *Ascaridia galli* (45.2%), *Hetrakis gallinarium* (18.2%), *Capillaria* spp (0.66%), *Trichostrongylus tennis* (1%) and *Subulura* spp (9%). The predominant cestodes recovered included *Ralletina tetragona* (21.8%), *Ralletina echinobothria* (32.9%), *Ralletina cesticulus* (5.7%), *Davina proglottina* (1.8%), *Amoebotaenia sphrnoides* (2%), *Choanotania infundibulum* (2.2%) and *Hymenelopsis* spp (1.6%). The over all mean (+ SD) nematode burden per chicken was 9.5+ 8.6. The highest mean ((± SD) nematode count per chicken obtained was 12.1± 10.8 from Ada'a (Mid altitude) where as the over all mean (± SD) cestode burden per chicken was 10.0± 9.7. The highest mean cestode count per chicken was 10.6±10.4 from Ada'a. Mixed infection of both nematode and cestode was encountered in 195 (32.5%) of the cases.

**Key words:** Akaki, Ada'a, Adama, Helminthes, Prevalence, chickens.

## ETUDE SUR LES HELMINTHES GASTROINTESTINAUX DES POULETS DE BASSE-COUR LOCAUX DANS LA ZONE SHOA EST DE L'ETAT REGIONAL D'OROMIA EN ETHIOPIE

### Résumé

Une étude a été menée afin de déterminer la prévalence des helminthes gastro-intestinaux chez les poulets élevés en système traditionnel dans trois zones agro écologiques en Ethiopie. L'étude a révélé que 517 (86,17%) des poulets examinés (n = 600) étaient infectés par des helminthes différents. Des 600 poulets, 370 (61,65%) portent des nématodes, notamment *Ascaris galli* (45,2%), *Hetrakis gallinarium* (18,2%), *Capillaria* spp (0,66%), *Trichostrongylus tennis* (1%) et *Subulura* spp (9%). Les cestodes prédominants identifiés comprennent : *Ralletina tetragona* (21,8%), *Ralletina echinobothria* (32,9%), *Ralletina cesticulus* (5,7%), *Davina proglottina* (1,8%), *Amoebotaenia sphrnoides* (2%), *Choanotania infundibulum* (2,2%) et *Hymenelopsis* spp (1,6%). Globalement, le nombre moyen de nématodes (+ SD) par poulet était de 9,5 + 8,6. La numération moyenne la plus élevée de nématodes (+ SD) obtenue par poulet était de 12,1 + 10,8 à Ada'a (moyenne altitude), tandis que le nombre global moyen (+ SD) de cestodes par poulet était de 10,0 + 9,7. La numération moyenne la plus élevée de cestodes par poulet était de 10,6 10,4 à Ada'a. Une infection mixte à la fois par des nématodes et des cestodes a été identifiée dans 195 (32,5%) des cas.

**Mots-clés:** Akaki, Ada'a, Adama, Helminthes, Prévalence

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## Introduction

Poultry production is an important and widespread agricultural industry in the tropics. There have been more changes in the methods of keeping poultry than any other sectors of livestock agriculture production system (Sainsbury, 1992). The poultry population in Ethiopia is estimated to be 56.5 million (ILCA, 1993). Indigenous birds raised under traditional or backyard condition without any input comprised over 99%, while 1% are exotic breeds maintained under intensive system. Raising poultry has a long line tradition in Ethiopia and the production systems showed a clear distinctions among traditional subsistence, low input systems, and small scale and large-scale commercial system using relatively advanced technology (Ziyad, 2007).

The free ranging system is one of the methods of managing birds extensively. The birds are allowed to move freely over a large area. The movement is usually limited by fences around the farmers' own boundary, roads and neighboring house holds. Farmer ensure that the birds have adequate feed and water available near the house (Teshome, 1993).

Despite the importance of village poultry in developing countries, little research and development work has been carried out to characterize, understand and develop the system (Harst, 1989).

Although poultry is important for economic development and food security in Ethiopia, systematic studies have not been conducted to assess the rate and intensity of adoption of exotic poultry breeds and farmers' response to improved poultry technologies. Information regarding use of exotic poultry breeds and associated improved management practices (feeding, housing, health, etc) are limited (Hailemariam *et al.*, 2006).

The gastrointestinal helminth parasites of poultry are classified as round worms (nematodes), tape worms (cestodes) and fluke worms (trematodes). in terms of number of species affecting poultry and in

the extent of damage done, it is difficult to give an accurate estimate of the economic importance of parasitic disease, because it varies greatly between areas (Teshome, 1993).

There little is Recorded information on the occurrence of gastrointestinal helminthes parasites of Ethiopian indigenous chicken is very little. Hence investigation of helminthes parasites in relation with the management systems is important. Therefore, the objectives of this study were:

1. To determine the prevalence of gastrointestinal helminthes of backyard chickens in Eastern Shoa (Akaki, Ada'a and Adama districts)
2. To identify gastrointestinal helminthes species in the study areas
3. To assess the effect of sex and agro-ecology on the prevalence of gastrointestinal helminthes

## Materials and Methods

### Study areas

#### Akaki

Akaki is located 80 9'N latitude and 380 8' E longitude, 27 km South of Addis Ababa at 2720 meter above sea level with an annual rainfall of 1200 mm and annual average maximum and minimum temperatures of 20°C and 18°C respectively. Poultry population kept under back yard system is 31,000 (NMSA 2000) and AKAO (2006).

#### Ada'a

Ada'a is located in Oromia national regional state in East Shoa zone at about 47 km South East of the capital city, Addis Ababa. The town is found at latitude of 8.7°N and a longitude of 39°E with an altitude of 1850 meter above sea level. Its mean annual rainfall is 866 mm. The mean annual maximum and minimum temperatures are 26°C and 14°C, respectively with a relative humidity of 61.3% (10). Poultry population of small holder farmers in Ada'a area is 71,000 and almost all poultry are raised under backyard or traditional low input production system

(AADO, 2008).

### Adama

Adama is located at about 95km south east of Addis Ababa (39.17°E and 8.33°N), with an altitude of 1622 meters above sea level in the rift valley. Its annual rainfall ranging from 400 mm to 800 mm and has a temperature of 13.9°C to 27.7°C NMSA (2006). Adama is one of the most populous towns of the Oromia region and an important multidirectional trade route. The number of livestock by species is 70,662 bovine; 36,142 ovine; 42,968 caprine; 31,933 equines and 193,155 poultry (ADAO, 2003).

### Study Population

The target populations were local backyard and non-vaccinated chickens in the study areas. The sample size was determined based on the formula recommended by Thrusfield (2005).

$$N = \frac{1.962 * p_{exp} * q}{d^2}$$

Where,

N = sample size required

P<sub>exp</sub> = expected prevalence (Any prevalence report in the study area)

q = 1 - p<sub>exp</sub>

d = desired absolute precision

Since there was no study conducted in the areas, expected prevalence of 50% was considered at 5 % absolute precision. Accordingly, a total 384 chicks were required for this study. However, a total of 600 chickens were purchased from local markets in the three agro-ecological zones (200 chickens from each agro-ecology) to increase the precision of the study.

### Study Design

Across-sectional study design was used to determine the prevalence of helminthosis in non-vaccinated backyard local chickens from November 2007 to April 2008.

### GIT Parasite identification

The birds were killed by cervical dislocation and the parasites were identified and counted according to Hofstad et al., (1978).

### Data Analysis

The data collected were stored and analyzed using STATA (Version 7) Software. Chi-Square test, percentages were used for the data analysis. P-values < 0.05 were considered significant.

## Results

### Prevalence and parasitic burden of helminthes of chickens

Out of the 600 local chickens examined, 370 (61.6%) were found harboring different species of nematodes. The prevalence of nematode infection in mid-land (Ada'a) (67%) was relatively higher than high-land Akaki, (58.3%) and low-land (Adama) (59.2%). However, the difference was not statistically significant (P > 0.05) among the three study sites.

Five major nematode species, *Ascaridia galli* (45.2%), *Heterakis gallinarum* (18.2%), *Capillaria spp* (0.7%), *Trichostrongylus tenuis* (1%) and *Subulura brumpti* (9%) were encountered in the three agro-ecological sites. The most prevalent nematode encountered was *A. galli* (45.2%), where as *Capillaria Spp.* was the least prevailing nematode parasite (0.7%).

The overall mean (±SD) nematode burden of chickens was 9.5 ± 8.6. The highest mean (±SD) nematode count obtained was 10.6 ± 10.8 from Ada'a (mid-altitude) chickens. The mean (±SD) nematode burden in Akaki (high-land) and Adama (low-land) chickens were 8.7 ± 6.5 and 8.1 ± 7.2, respectively (Table 3).

Out of the total 600 local chickens examined, 342 (57%) were found harboring at least one species of cestode infections. The observed cestode infection prevalence rate (65.5%) was significantly higher (P < 0.05) in chickens from Akaki (highland) than the other two sites (Ada'a-52% and Adama-53.5%).

Moreover, the cestode infection rate (69.3%) in male chickens was significantly higher ( $P < 0.05$ ) than in female chickens (44.6%) (Table 1). The highest prevalence rate of cestode infection was recorded for *R. echinobothridia* (32.5%), followed by *R. tetragona* (21.8%), whereas *Hymenolepis* spp (1.6%) had the least prevalence rate (Table 4).

The overall mean ( $\pm$ SD) cestode burden of chickens was  $10.03 \pm 9.66$ . The highest mean ( $\pm$ SD) cestode count recorded was from Ada'a (mid-altitude) ( $12.1 \pm 10.8$ ) chickens. The mean ( $\pm$ SD) cestode burden from Akaki (highland) and Adama (lowland) chickens were  $8.1 \pm 9.6$  and  $9.4 \pm 7.5$ , respectively (Table 5).

Mixed infection of cestode and nematode was found in all study sites. The prevalence rate of mixed infection in Akaki (38.6%) was significantly ( $P < 0.05$ ) higher than that of Ada'a (32%) and Adama (27%). Moreover, male chickens had significantly higher ( $P < 0.05$ ) mixed infection rate (39%) than female chickens (26%) (Table 1).

A total of 12 species of gastrointestinal helminthes were encountered from this study; of these a mixed infection of gastrointestinal tract helminthes ranging up to 8 species from the Ada'a, six species from the Adama and five species from Akaki were recorded.

## Discussion

The present study indicated an overall high prevalence of gastro-intestinal helminthes (86.2%) in backyard chickens in three selected districts of Oromia Regional State. This study further revealed the occurrence of various species of helminthes in poultry production particularly in the low input backyard system of production. The high prevalence of cestode and nematode infections in backyard chickens may be attributed to the prevailing husbandry practice of rural farmers with low input management system as well as conducive environmental factors favoring the propagation and life cycle progression of the diverse parasites in the studied sites.

In Ethiopia, the reported prevalence rates of GIT helminthes in local chicken

were variable: 100% from Southern Ethiopia (Tegene 1992); (92.5%) from Bahir Dar (Awoke 1987); 97.9% in and around Addis Ababa (Abebe et al., 1997); 91% from North East Amhara Regional State (Eshetu et al., 2001); 90.8% from Soddo (Teshome 1993); 86.3% Central Ethiopia (Hagos 2000). Reports from overseas are also variable. For instance, Eisa (1976) reported 89% prevalence from Sudan; 100% from Zimbabwe (Tensen and Pandey 1989), Morocco Dakka and Houadfi (1992) and Tanzania (Permin et al., 1995).

Generally, most reports from the backyard or scavenging chicken production indicated that there have been diverse parasitic species with high prevalence rates in different parts of Ethiopia and from other tropical countries.

The most prevalent nematode in this study was *A. galli* (45.2%). This result is relatively similar to the report of (25) (47.3%) and lower than the reports of Abebe et al., (1997) (71.6%), Awoke (1987) (61.2%), Teshome (1993) (64.3%). However, this result is relatively greater than the reports of Eshetu et al., (2001 from different corners of Ethiopia.

*Heterakis gallinarum* was detected in the caecal tubes of 18.2% of chickens. Secondary infections are associated with nodule formation in the mucosa and sub mucosa of the caceum. Experimental infection has caused reductions in body weight gain and feed efficiency (Abdu-Hamed 1984). *Subulura brumpti* was most frequently found in combination with *H. gallinarum*, but had a less prevalence rate (9%) than *H. gallinarum*, although the association was not correlated with morbidity.

In this study, 12.6% of the local chickens were carrying between two and four different species of nematode and almost all of the chickens were infected by one or more helminth parasites, the impact of these helminthes on local poultry should not be underestimated.

There was a significant difference in the prevalence rate of cestode infections between male and female chickens as well as among the three study sites. As Tizard

**Table 1:** The prevalence of gastrointestinal helminthes by site and sex

Factors		No. of chickens examined	Prevalence rate (%)					
			Nematode		Cestode		Both Nematode & Cestode	
			No. +Ve	%	No. +Ve	%	No. +Ve	%
Site	Akaki	200	117	58.3	131	65.5	77	38.5
	Debre Zeit	200	134	67	104	52	64	32
	Nazareth	200	119	59.5	107	53.5	54	27
Chi-square results			X <sup>2</sup> =3.65, P=0.16		X <sup>2</sup> =8.9, P=0.011		X <sup>2</sup> =6.06, P=0.048	
Sex	Male	300	188	62.6	208	69.3	117	39
	Female	300	182	60.6	134	44.6	78	26
Chi-square results			X <sup>2</sup> =0.25, P=0.61		X <sup>2</sup> =37.2, P=0.00		X <sup>2</sup> =11.4, P=0.001	
Overall		600	370	61.6	342	57	195	32.5

**Table 2:** Gastrointestinal nematode identified from backyard local chickens examined in Akaki, Ada'a and Adama

Nematode Species	No. positive (%)
<i>Ascaridia galli</i>	271 (45.2)
<i>Hetrakis gallinarum</i>	109 (18.2)
<i>Capillaria spp</i>	4 (0.7)
<i>Trichostrongylus tenuis</i>	6 (1)
<i>Subulura brumpti</i>	54 (9)
Mixed infection (more than one species)	76 (12.6)

**Table 3:** The parasitic burden of nematode infection in backyard local chickens in Akaki, Ada'a and Adama.

Nematode Spp.	Mean burden $\pm$ SD and range			
	Akaki	Ada'a	Adama	Total
<i>A. galli</i>	9 + 6.0 (2-22)	13.4 + 12.7 (1-63)	8.5 + 8.1 (2-31)	10.6 + 9.8 (2-63)
<i>H. gallinarum</i>	8.4 + 6.0 (2-25)	7.4 + 4.7 (2-25)	8.7 + 6.8 (4-23)	8.3 + 6.1 (2-25)
<i>S. brumpti</i>	5.5 + 2.6 (2-8)	7.4 + 4.6 (2-21)	6 + 3.4 (5-20)	8.3 + 7.8 (2-20)
<i>T. tenuis</i>	4.5 + 3.5 (2-7)	3.5 + 2.1 (2-5)	4.5 + 0.7 (4-5)	4.2 + 1.9 (2-7)
<i>Capillaria spp.</i>	17.5 + 17.7 (5-30)	4.5 + 3.8 (2-12)	8 + 5.6 (4-12)	5.5 + 3.6 (2-30)
<b>Total</b>	<b>8.7 + 6.5 (2-30)</b>	<b>10.6 + 10.8 (1-63)</b>	<b>8.1 + 7.1 (2-31)</b>	<b>9.5 + 8.6 (2-63)</b>

**Table 4:** Cestodes identified and their respective prevalence

Cestode Species	No. of Chickens infected	Prevalence rate (%)
<i>Davina Proglotina</i>	11	1.83
<i>Raillietina cesticillus</i>	34	5.66
<i>R. tetragona</i>	131	21.8
<i>R. echinobothridia</i>	195	32.5
<i>Amoebotaenia Sphenoides</i>	12	2
<i>Choanotaenia infundibulum</i>	13	2.16
<i>Hymenolepis spp</i>	10	1.6
Mixed (more than one species)	57	9.5

**Table 5:** The parasitic burden of cestode infection in backyard local chickens originated from Akaki, Ada'a and Adama

Cestode spp	Mean cestode burden + SE and range			
	Akaki	Ada'a	Adama	Total
<i>D. Proglotina</i>	2.5 + 0.7 (2-30)	19 + 9.9 (12-26)	4.5 + 2.1 (3-6)	11 + 10.7 (3-26)
<i>R. cesticillus</i>	4.8 + 2.7 (2-10)	7.5 + 4.3 (2-14)	5.3 + 5.8 (2-12)	5.4 + 4.0 (2-14)
<i>R. tetragona</i>	13 + 18.4 (2-60)	12.1 + 10.3 (2-44)	8.7 + 6.5 (2-23)	10.7 + 11.2 (3-60)
<i>R. echinobothridia</i>	9.4 + 7.7 (4-32)	15.3 + 12.0 (2-51)	12.0 + 8.6 (3-40)	12.1 + 10.0 (2-51)
<i>A. sphenoides</i>	6.5 + 4.94 (3-10)	4.5 + 0.70 (4-8)	14.5 + 9.2 (8-21)	9.2 + 7.2 (3-21)
<i>Ch. Infundibulum</i>	2.3 + 0.6 (2-3)	3.8 + 2.9 (2-8)	3.3 + 1.5 (2-5)	3.3 + 2.0 (2-8)
<i>Hymenolpis spp</i>	4.3 + 2.6 (2-8)	4.7 + 3.1 (2-8)	4.0 + 2.8 (2-6)	4.4 + 2.6 (2-8)
<b>Total</b>	<b>8.1 + 9.6 (2-60)</b>	<b>12.1 + 10.8 (2-51)</b>	<b>9.4 + 7.5 (2-40)</b>	<b>10.0 + 9.6 (2-60)</b>

(1992) described, the effect of host factors, such as sex, on the prevalence of helminthes appears to be largely hormonal. On the other hand, the difference in the prevalence rate of cestode infection among the three study sites might be due to the presence of favorable bionomic phenomenon, which is conducive for the survival and multiplication of intermediate hosts and eggs of parasite in highland areas than in the other climatic zones.

The most prevalent cestode recorded in the area was *R. echinobothridia* (32.5%) which is lower than the previous report (48.9%) in Arsi Zone Eshetu and Tilahun (2000) but higher than in Amhara

Regional State (25.8%) Eshetu *et al.*, (2001). These differences in the prevalence rate of cestode could be partly attributed to the difference in the prevailing environmental conditions.

The prevalence rate of *R. tetragona* was less than that reported from different parts of the country, 26.3% in and around Addis Ababa (Abebe *et al.*, 1997), 34% in and around Bahir Dar (Awoke 1987) and 30.9% in and around Dire Dawa (Gedion 1991)) but higher than in Arsi zone (22.3%) (Eshetu and Tilahun 2000).

In this study, mixed infections have been recognized. Mixed infections of up to 11 species by Bersabeh (1999) and Helina



(2000), 10 species from Bahir Dare (Awoke 1987), 7 species from Dire Dawa (Gedion 1991) and in Soddo by (Teshome 1993) were also reported. A mixed infection up to 5 and 10 species of helminthes were recorded from Sudan (Eisa 1976) and Zambia (Shamsul 1985). This could be due to the availability of intermediate hosts and undoubtedly contribute to the low productivity of local chickens.

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## SEROLOGICAL EVIDENCE OF BLUETONGUE VIRUS ANTIBODIES IN SHEEP AND GOATS AT TWO REARING REGIONS OF NORTH SOMALIA

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### Abstract

To determine the presence and prevalence of bluetongue virus infection in sheep and goats at different geographical regions of North Somalia, a competitive enzyme-linked immune-sorbent assay (cELISA) for the detection of serum antibody against BTV in clinically healthy sheep and goats was carried out in Northern Somalia in two main districts of sheep and goats-rearing regions namely, Togdheer, and Waqoyi Galbed in the period between July 2008 to April 2009. Results for bluetongue infection, herd size, and herd location, mixing with other animal species with various other associations were detected among demographic, husbandry and disease variables. All animals tested were apparently normal without showing clinical signs and without history of any specific clinical signs for BTV infection. Out of 24 (601) sheep/herds investigated, 3 (12.5%) herds were seronegative and 21 (87.5%) were seropositive by cELISA with seroprevalence on herd level ranged from 4.2 % - 42.9 % with a total seroprevalence of both districts 21.8% (n=131). Out of 24 (466) goat/herds investigated, 8 (33.3%) herds were seronegative and 16 (66.7%) were seropositive by cELISA to BT virus infection with seroprevalence ranged from 12.5 % - 38.5 % on herd level with a total district seroprevalence of 16.0 % (n=77). The results of the present investigation indicate that the bluetongue virus exists within the sheep and goat herds. The findings suggest that the disease is widely distributed in most investigated parts of the North Somalia where possible insect vectors may prevail and may suggest disease endemicity which is probably subclinical or in-apparent in sheep and goats of North Somalia. The prevalence differed significantly between herd types but did not show a geographical trend. The results presented here may record the first confirmation of bluetongue virus (BTV) antibody in sheep and goats in North Somalia.

**Keywords:** Bluetongue, Seroprevalence, North Somalia, ELISA

## PREUVE SÉROLOGIQUE DES ANTICORPS DU VIRUS DE LA FIÈVRE CATARRHALE DU MOUTON CHEZ LES OVINS ET CAPRINS DE DEUX RÉGIONS D'ÉLEVAGE DANS LE NORD DE LA SOMALIE

### Resume

Dans la perspective de déterminer la présence et la prévalence de l'infection par le virus de la fièvre catarrhale du mouton chez les ovins et caprins dans différentes régions géographiques du Nord de la Somalie, on a utilisé une épreuve immuno-enzymatique par compétition (cELISA) pour la détection d'anticorps sériques contre le virus de la FC chez des ovins et caprins cliniquement sains. Les résultats pour l'infection de la fièvre catarrhale du mouton, la taille du troupeau, et l'emplacement des troupeaux, le contact avec d'autres espèces animales avec diverses autres associations ont été détectés parmi les variables de la démographie, de l'élevage et de la maladie. Des 24 (601) moutons / troupeaux étudiés, trois (12,5%) étaient séronégatifs et 21 (87,5%) étaient séropositifs par cELISA, avec une séroprévalence variant de 4,2% à 42,9% au niveau du troupeau, et une séroprévalence totale des deux districts de 21,8% (n = 131). Des 24 (466) chèvres / troupeaux étudiés, huit (33,3%) étaient séronégatifs et 16 (66,7%) étaient séropositifs par cELISA pour l'infection du virus de la fièvre catarrhale, avec une séroprévalence variant entre 12,5% et 38,5% au niveau du troupeau et une séroprévalence totale des

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districts de 16,0% ( $n = 77$ ). Les résultats de la présente enquête révèlent que le virus de la fièvre catarrhale existe parmi les troupeaux d'ovins et de caprins dans le Nord de la Somalie.

## Introduction

Bluetongue (BT) is a viral, non-contagious, arthropod-borne, infectious disease of domestic and wild ruminants caused by the BT virus (BTV) of the family Reoviridae, genus Orbivirus (Erasmus, (1975). To date, twenty four virus serotypes have been identified worldwide (Gibbs and Greiner, (1994). The clinical form of the disease is usually only seen in sheep. Infection in cattle and goats is generally not noticeable, although clinical BT has been reported in both species (Luedke and Anakwenze, (1972); and Hourrigan and Klingsporn, (1975). For bluetongue, clinical symptoms vary from being subclinical to mild and acute, and can terminate in the death of the animal depending on the pathogenicity of the viral strain and the sensitivity of the breed. In cattle and goats, the disease usually assumes a subclinical course without severe symptoms (Erasmus, 1975); and Buxton and Frazer, 1977). Ruminants naturally infected with one serotype of BTV have a solid, lifelong immunity to the homologous serotype but only partial or no protection against other (heterologous) serotypes (Verwoerd and Erasmus, 2004), thus recovered animals pose no threat for transmission of infection if they are confirmed to be virus-free prior to their movement. However, BTV infection of ruminants is prolonged but not persistent (MacLachlan, (2004); Melville *et al.*, (2004); White and Mecham, (2004); and Lunt *et al.*, (2006). A true persistent BTV carrier state occurred in some animals (Barratt-Boyes and MacLachlan, (1995); MacLachlan, (2004); and Lunt *et al.*, (2006). BT is found only in regions where competent vectors occur. Between 1998 and 2004, an epizootic of BT occurred in the Mediterranean Basin, also affecting countries where BT had never previously been recorded. The biological vectors of BTV, play the principal role in disease spread. *Culicoides imicola*, subgenus *Avaritia*, which is the most widely spread species of bloodsucking *Culicoides*, acting

as vectors of BT and African horse sickness, occurs in Africa and most countries of the Mediterranean Basin (Luedke and Anakwenze, (1972); and Hourrigan and Klingsporn, (1975). In Africa at least 10 species of *Culicoides* breed exclusively in the dung of indigenous herbivores (Meiswinkel *et al.*, 2004b). The involvement of novel insect vectors (Mellor and Wittmann, 2002), together with an effective overwintering mechanism, could dramatically increase the threat posed by BTV in areas that were previously free of the disease (Takamatsu *et al.*, 2003). It is evident that a reservoir of the virus must exist through the winter but its location and the mechanism by which it re-establishes overt infection of either the vector insect or the mammalian host has not been satisfactorily explained or demonstrated. There are to date, 24 distinct serotypes of BTV have been described that all share common group antigens but which are distinguished on the basis of serotype-specific virus neutralization assays (Bonneau *et al.*, (1999); Pritchard *et al.*, (2004) and Bréard *et al.*, (2007b). Prevalence of sheep and goats bluetongue in North Somalia has not been discussed. Therefore, the aim of the present study was to explore the serologic-prevalence and potential risk factors of BTV in North Somalia using a commercially available competitive enzyme-linked immune-sorbent assay (c-ELISA).

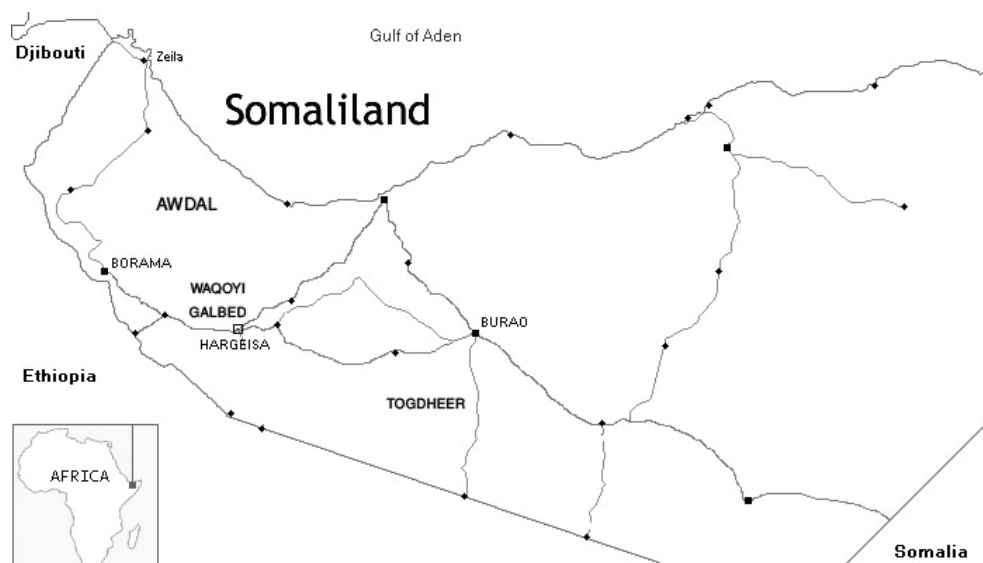
## Materials and Methods:

### Study Area

The present study was conducted during the period from July 2008 to April 2009 in the northern part of Somalia (North Somalia). Two main districts were covered in this study namely, Waqoyi Galbed, and Togdheer.

### Study Animals

A total of 601 unvaccinated sheep blood sera were examined in this study and involved 24 herd/flocks (12, and 12 herds from



**Fig. 1:** A map of North Somalia showing locations of sample collection from sheep and goats for serosurvey of Bluetongue viral antibodies

Togdheer and Waqoyi Galbed respectively. The number of sheep heads per herd/flock ranged from 16-32. Information of each sheep sampled were obtained including, location, herd size, sex, age, health status, history of disease, whether reared individually, with other species or in a sheep herd. Sheep's sera were collected as 289 samples from Togdheer (132 from males and 157 from females) and 312 samples from Waqoyi Galbed (141 from males and 171 from females).

A total of 466 unvaccinated goats blood sera were examined in this study and involved 24 herd/flocks (12, and 12 herds from Togdheer and Waqoyi Galbed respectively. The number of goat heads per herd/flock ranged from 14 - 27. Information of each goat sampled were obtained including, location, herd size, sex, age, health status, history of disease, whether reared individually, with other species or in a goat herd. Goat's sera were collected as 213 samples from Togdheer (86 from males and 127 from females) and 253 samples from Waqoyi Galbed (112 from males and 141 from females).

#### *Sample collecting*

Samples were collected from each herd from unvaccinated sheep and goats of both sexes, with a 100% of the total number

of each herd. Ten ml blood samples were collected and transferred to the laboratory of the Gulf International Veterinary Quarantine in Berbera city of North Somalia. The sera were separated and stored at -20°C until testing.

#### *Serological test*

Bluetongue virus antibody kit, (VMRD, Inc.) which detects all 24 known serotypes of Bluetongue Virus (BTV) was used. The test procedure was as directed by the manufacturer; and test results were read on a plate reader (MR-96A Microplate Reader (Shenzhen mindary biomedical electronics CO., LTD) at 620 nm. Test sera are positive if they produce an optical density less than 50% of the mean of the Negative Controls. Test sera that produce an optical density greater than or equal to 50 % of the mean of the Negative Controls are negative.

## **Results**

#### *Sheep:*

*Overall Seroprevalence results of the two districts*  
Out of 24 (601) sheep/herds

investigated, 3 (12.5%) herds were seronegative and 21 (87.5%) were found to be seropositive by cELISA to BT virus infection with seroprevalence ranged from 4.2 % - 42.9 % as herd prevalence with a total district seroprevalence of 21.8% (n=131). For 273 males included in the 24 herds, 5 (20.8%) herds were found negative and 19 (79.2%) were positive with seroprevalence ranged from 15.4 % - 50.0 % on herd level with a total district seroprevalence of 21.8% (n=60). For 328 females included in the 24 herds, 3 (12.5%) herds were seronegative and 21 (87.5%) herds were found positive with seroprevalence ranged from 6.7 % - 38.5 % on herd level with a total district seroprevalence of 21.7% (n=71).

#### *Results for Togdheer*

Out of 12 (289) sheep/herds investigated, 2 (16.7%) herds were seronegative and 10 (83.3%) were found to be seropositive by Competitive Enzyme Linked Immunosorbent Assay (cELISA) to Bluetongue virus infection with seroprevalence ranged from 13.3 % - 30.8 % on herd level with a total prevalence of 18.2% (n=55). For 132 males included in the 12 herds, 2 (16.7%) herds were found negative and 10 (83.3%) were positive with seroprevalence ranged from 15.4 % - 40.0 % on herd level with a total prevalence of 17.64% (n=27). For 157 females included in the 12 herds, 2 (16.7%) herds were seronegative and 10 (83.3%) herds were found positive with seroprevalence ranged from 11.8 % - 36.4 % on herd level with a total prevalence of 19.8% (n=31). All sheep were apparently normal without showing clinical signs and without history of any specific clinical signs for BT viral infection.

#### *Results for Waqoyi Galbed*

Out of 12 (312) sheep/herds investigated, 1 (8.3%) herds were seronegative and 11 (91.7%) were found to be seropositive by Competitive Enzyme Linked Immunosorbent Assay (cELISA) to Bluetongue virus infection with seroprevalence ranged from 4.2 % - 42.9 % on herd level with a total prevalence

of 24.4% (n=76). For 141 males included in the 12 herds, 3 (25.0%) herds were found negative and 11 (75.0%) were positive with seroprevalence ranged from 15.4 % - 50.0 % on herd level with a total prevalence of 25.5% (n=36). For 171 females included in the 12 herds, 1 (8.3%) herds were found negative and 11 (91.7%) herds were found positive with seroprevalence ranged from 6.7 % - 38.5 % on herd level with a total prevalence of 23.4% (n=40). All sheep were apparently normal without showing clinical signs and without history of any specific clinical signs for BT viral infection.

#### *Goats:*

#### *Overall Seroprevalence results of the two districts*

Out of 24 (466) goat/herds investigated, 8 (33.3%) herds were seronegative and 16 (66.7%) were found to be seropositive by cELISA to BT virus infection with seroprevalence ranged from 12.5 % - 38.5 % as herd prevalence with a total district seroprevalence of 16.0 % (n=77). For 198 males included in the 24 herds, 8 (33.3%) herds were found negative and 16 (66.7%) were positive with seroprevalence ranged from 12.5 % - 40.0 % on herd level with a total district seroprevalence of 15.7% (n=31). For 268 females included in the 24 herds, 8 (33.3%) herds were seronegative and 16 (66.7%) herds were found positive with seroprevalence ranged from 11.1 % - 38.5 % on herd level with a total district seroprevalence of 17.5% (n=47).

#### *Results for Togdheer*

Out of 12 (213) goat/herds investigated, 4 (33.3%) herds were seronegative and 8 (66.7%) were found to be seropositive by Competitive Enzyme Linked Immunosorbent Assay (cELISA) to Bluetongue virus infection with seroprevalence ranged from 13.3 % - 30.8 % on herd level with a total prevalence of 18.2% (n=55). For 86 males included in the 12 herds, 4 (33.3%) herds were found negative and 8 (66.7%) were positive with seroprevalence ranged from 12.5 % - 40.0 % on herd level with a total prevalence of 15.1%

**Table 1:** Seroprevalence of cELISA of Bluetongue virus in two district of northern Somalia in North Somalia of sheep

District	Herd No.	Herd size	Male				Female				Total	
			ELISA+	%	-	+	%	-	+	%	-	
Togdheer	1	30	2	15.4	11	2	11.8	15	4	13.3	26	
	2	29	3	23.1	10	4	25.0	12	7	24.1	22	
	3	32	0	0.0	14	0	0.0	18	0	0.0	32	
	4	23	2	18.2	9	3	25.0	9	5	21.7	18	
	5	22	2	18.2	9	4	36.4	7	6	30.0	16	
	6	21	2	20.0	8	2	18.2	9	4	19.1	17	
	7	27	4	30.8	9	5	35.7	9	9	33.3	18	
	8	20	2	22.2	7	3	27.3	9	5	25.0	15	
	9	24	0	0.0	11	0	0.0	13	0	0.0	24	
	10	28	3	25.0	9	4	25.0	12	7	25.0	21	
	11	17	2	25.0	6	2	22.2	7	4	23.5	13	
	12	16	2	28.6	5	2	22.2	7	4	25.0	12	
Waqoy Galbed	1	32	5	35.7	9	5	27.8	13	10	31.3	22	
	2	24	0	0.0	9	1	6.1	14	1	4.2	23	
	3	25	2	16.7	10	3	23.1	10	5	20.0	20	
	4	28	4	30.8	9	4	26.7	11	8	28.6	20	
	5	31	5	35.7	9	4	23.5	13	9	29.0	22	
	6	27	2	15.4	11	2	14.3	12	4	14.8	23	
	7	31	5	33.3	10	5	31.3	11	10	32.3	21	
	8	30	4	28.6	10	5	31.3	11	9	30.3	21	
	9	20	0	0.0	9	2	18.2	9	2	10.0	18	
	10	23	5	50.0	5	4	30.8	9	9	42.9	14	
	11	18	0	0.0	8	0	0.0	10	0	0.0	18	
	12	23	4	40.0	6	5	38.5	8	9	39.1	14	

(n=13). For 127 females included in the 12 herds, 4 (33.3%) herds were seronegative and 8 (66.7%) herds were found positive with seroprevalence ranged from 11.1 % - 37.5 % on herd level with a total prevalence of 16.5% (n=21). All goats were apparently normal without showing clinical signs and without history of any specific clinical signs for BT viral infection.

#### Results for Waqoyi Galbed

Out of 12 (253) goat/herds investigated, 4 (33.3%) herds were seronegative and 8 (66.7%) were found to be seropositive by Competitive Enzyme Linked Immunosorbent Assay (cELISA) to Bluetongue virus infection

with seroprevalence ranged from 21.1 % - 32.0 % on herd level with a total prevalence of 17.4% (n=44). For 112 males included in the 12 herds, 4 (33.3%) herds were found negative and 8 (66.7%) were positive with seroprevalence ranged from 12.5 % - 28.6 % on herd level with a total prevalence of 16.1% (n=18). For 141 females included in the 12 herds, 4 (33.3%) herds were found negative and 8 (66.7%) herds were found positive with seroprevalence ranged from 16.7 % - 38.5 % on herd level with a total prevalence of 18.4% (n=26). All goats were apparently normal without showing clinical signs and without history of any specific clinical signs for BT viral infection.

**Table 2:** Seroprevalence of cELISA of Bluetongue virus in two districts of northern Somalia in North Somalia in goats

District	Herd No.	Herd size	Male			Female			Total		
			ELISA +	%	-	+	%	-	+	%	-
Togdheer	1	18	1	12.5	7	2	20.0	8	3	16.7	15
	2	21	2	22.2	7	4	33.3	8	6	26.6	15
	3	16	0	0.0	7	0	0.0	9	0	0.0	16
	4	14	1	16.7	5	1	12.5	7	2	14.3	22
	5	22	3	37.5	5	4	30.8	9	7	31.8	15
	6	15	1	16.1	5	2	22.2	7	3	20.0	12
	7	16	1	14.3	6	1	11.1	8	2	12.5	14
	8	19	0	0.0	7	0	0.0	12	0	0.0	19
	9	13	2	40.0	3	3	37.5	5	5	38.5	8
	10	18	0	0.0	7	0	0.0	11	0	0.0	18
	11	21	2	22.2	7	4	33.3	8	6	28.6	15
	12	20	0	0.0	7	0	0.0	13	0	0.0	20
Waqoyi Galbed	1	24	2	18.2	9	3	23.1	10	5	20.8	19
	2	27	3	25.0	9	4	26.7	11	7	25.9	20
	3	19	0	0.0	8	0	0.0	11	0	0.0	19
	4	18	2	28.6	5	2	18.2	9	4	22.2	14
	5	22	2	20.0	8	3	25.0	9	5	22.7	17
	6	18	0	0.0	8	0	0.0	10	0	0.0	18
	7	25	3	25.0	9	4	30.8	9	7	28.0	18
	8	18	1	12.5	7	3	30.0	7	4	22.2	14
	9	25	3	25.0	9	5	38.5	8	8	32.0	17
	10	21	0	0.0	9	0	0.0	12	0	0.0	21
	11	19	2	28.6	5	2	16.7	10	4	21.1	15
	12	17	0	0.0	8	0	0.0	9	0	0.0	17

### Discussion

To supply information on the occurrence of bluetongue virus in sheep and goats, a serological survey was initiated in the northern regions of Somalia (North Somalia). To our knowledge, no available literature on the prevalence of sheep and goats BTV infection published over the last decades to determine the presence or distribution of the disease. BTV have been and continue to be a significant impediment to international livestock trade. Although severe disease is restricted to certain breeds of sheep and some species of deer (Taylor, 1986), it has been estimated that BTV causes losses of

US \$3 billion a year to trade in animals and animal products. Thus BT is classified as a List-A disease by the Office International des Epizooties (OIE). North Somalia had never previously recorded the presence of BTV in sheep and goats and the present study revealed an overall serologic-prevalence of 24 (601) sheep/herds investigated, 3 (12.5%) herds were seronegative and 21 (87.5%) were found to be seropositive by cELISA to BT virus infection with seroprevalence ranged from 4.2 % - 42.9 % as herd prevalence with a total district seroprevalence of 21.8% (n=131). Out of 24 (466) goat/herds investigated, 8 (33.3%) herds were seronegative and 16 (66.7%) were found to be seropositive by cELISA to BT



virus infection with seroprevalence ranged from 12.5 % - 38.5 % as herd prevalence with a total district seroprevalence of 16.0 % (n=77). The differences observed between geographic areas were not significant (Table I). Climatic factors may play an important role in the occurrence of BTV infection in animals and also influence the size of vector populations and periods of their seasonal activity. The presence of BTV antibodies in these sera had previously been demonstrated by the c-ELISA. In endemic areas, serologic-prevalence's of 46–52% in sheep, 44% in goats and 33–95% in cattle have been reported (Formenty *et al.*, 1994 and Thevasagayam *et al.*, 1996). The seroprevalence for BT virus was 23%, and seropositive animals were widespread suggesting endemicity, despite the disease not having been previously reported (Lundervold *et al.*, 2004). Competitive ELISA was applied to detect antibodies against bluetongue virus in sheep sera collected from different agro-climatic areas in Ethiopia. A total of 90 serum samples were tested and 42 (46.67%) were positive for bluetongue virus antibodies. A prevalence rate ranging from 9.67% for sheep sampled in the highland to 92.85% for sheep sampled in the lowland was recorded. The prevalence correlated with the probable distribution of the *Culicoides* vector. This is the first report indicating the presence of bluetongue virus infection in animals from Ethiopia (Woldemeskel *et al.*, 2000). Our findings of serologic-prevalence are relatively low, however, they suggest that BTV is widespread and endemic in the country. This result may be the first confirmation of BTV antibody in sheep and goats from North Somalia. Our interviews with farmers and officials highlighted the fact that vaccination against BTV diseases as a major economic and public health importance is not performed in North Somalia. Epidemic disease may constitute a serious problem for North Somalia's rural economy in future, and the situation is likely to worsen in the next few years. Failure to detect serologic-reactors in these serologic-negative herds may be due to the relatively low number of animals screened. In addition, mild disease may go unnoticed

and/or unreported. This may be attributed to the nearness of these districts to the states of Awdal, where BTV is considered endemic in Ethiopia as a border country, and to the unrestricted movement of relatively large numbers of camels, cattle, sheep and goats from these 'endemic' regions into the North Somalia districts. BTV, therefore, it is essential to develop methods for accurate prediction of BT risk in space and time. BTV can only survive under such constraints by continually moving to new locations occupied by naïve vertebrate hosts. These movements are via the agency of viraemic hosts or infected vectors. BTV is therefore a peripatetic virus and even within its enzootic zones its activity may be envisaged as a pattern of endlessly shifting viral "hot spots". Where annual bouts of BT occur, they may represent new introductions (from adjacent infected areas) or may be the visible evidence of low-level persistence from year to year. In conclusion, BTV antibodies do exist in studied animals. Based on our results, recommendations for further studies on prevalence of BTV should be carried-out to avoid the epidemic for this disease in North Somalia. Serotyping of the existence and persistence of BTV in North Somalia is extremely urgent. Moreover, epidemiological studies need to be done to explore the current status of the disease in other ruminants and other animals to enable the public veterinary authorities to construct a concrete program for prevention of the disease within animal herds in North Somalia or transmission of the disease via animal trading to the other countries.

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## EFFECT OF INSULIN ON FEVER IN ENDOTOXIC SHEEP

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### Abstract

A study was conducted to determine the effect of intravenous (i.v.) administration of insulin on fever in sheep challenged with bacterial endotoxin, lipopolysaccharide (LPS). Six castrated male Suffolk-cross wethers were randomly assigned to receive one of the following treatment combinations i.v: Saline control (SAL+SAL); SAL + LPS (0.06 µg kg<sup>-1</sup> BW) or various doses of insulin (I) (2, 6, 12 or 20 mU kg<sup>-1</sup> BW) + LPS (0.06 µg kg<sup>-1</sup> BW). Serial blood samples were collected at hourly intervals for 10 h after the start of i.v injections. Glucose concentrations in the plasma were measured. Rectal temperature was monitored at the same time as for serial blood sampling. Temperatures for the saline control sheep (SAL+SAL) remained relatively constant throughout the study period ranging from 38.9 ± 0.1 to 39.1 ± 0.1 °C. The SAL+LPS treated sheep had significantly (P<0.05) elevated temperatures compared to the saline controls from 1 to 8 h post LPS injection. The sheep injected with 12mUI +LPS had significantly (P<0.05) lower body temperature compared to the SAL +LPS treated sheep from 3 to 6 h post LPS injection. Within the insulin + LPS treatment combinations the 12mUI +LPS combination was found to significantly reduce (P<0.05) body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS injection. Sheep on the SAL+LPS or I+LPS treatments had reduced (P<0.05) glucose levels than the saline control sheep from 5 to 8 h post LPS injection. This study demonstrates the ability of insulin to reduce fever in LPS challenged sheep.

**Key words:** Endotoxin, fever, glucose, insulin, sheep

## EFFET DE L'INSULINE SUR LA FIÈVRE DANS LES MOUTONS INFECTÉS ENDOTOXINE BACTÉRIENNE

### Résumé

Une étude a été menée afin de déterminer l'effet de l'administration par voie intraveineuse (iv) de l'insuline sur la fièvre des moutons infectés d'endotoxine bactérienne lipopolysaccharide (LPS). Six mâles croisés Suffolk castrés ont été randomisés pour recevoir l'une des combinaisons suivantes de traitement IV: Saline de contrôle (SAL + SAL); SAL + LPS (0,06 mg kg<sup>-1</sup> pc) ou différentes doses d'insuline (I) (2, 6, 12 ou 20 kg<sup>-1</sup> mU BW) + LPS (0,06 mg kg<sup>-1</sup> pc). Des échantillons de sang ont été collectés en série à une cadence horaire de 10 h après le début des injections. Les concentrations de glucose dans le plasma ont été mesurées. La température rectale a été contrôlée au même moment que le prélèvement sanguin. Les températures pour les moutons témoins (SAL + SAL) sont demeurées relativement constante pendant toute la période d'étude allant de 38,9 ± 0,1 à 39,1 ± 0,1 °C. Chez les moutons traités SAL + LPS elles ont significativement (P < 0,05) augmentées par rapport aux contrôles salines entre 1 à 8 heures après l'injection. Le mouton injecté avec 12mUI + LPS avaient une température corporelle significativement (P < 0,05) plus basse que celle des animaux SAL + LPS 3 à 6 h après l'injection de LPS. Au sein des groupes traités à différentes concentration d'insuline ceux ayant reçu LPS l'12mUI ont eu une température corporelle considérablement (P < 0,05) abaissée pour atteindre celle du groupe 5 à 8 h après l'injection. Cette étude démontre la capacité de l'insuline à réduire la fièvre chez les moutons infectés d'endotoxines bactérienne lipopolysaccharide

**Mots clés:** endotoxine, la fièvre, de glucose, d'insuline, les moutons

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## Introduction

Fever, anorexia, and reduced physical activity in ruminants are characteristic features of the coordinated host response to lipopolysaccharide (LPS) challenge, microbial infection or chronic inflammatory diseases which are generally thought to be mediated by host-derived proinflammatory cytokines acting on the central nervous system (Baile *et al.*, 1988; Plata-Salaman, 1991). Experimental studies in humans, mice and rats indicate that insulin can act as an anti-inflammatory agent by decreasing the proinflammatory response and increasing the anti-inflammatory cascade (Jeschke *et al.*, 2002; Dandona *et al.*, 2009). Insulin treatment was reported to dampen inflammatory and acute phase responses by decreasing interleukin-6 (IL-6) and acute phase proteins in humans (Jeschke *et al.*, 2010). Also the administration of insulin subcutaneously was reported to decrease proinflammatory cytokine expression in the liver and serum levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 in endotoxic rats (Jeschke *et al.*, 2004). Differences exist among species with regard to their endotoxin sensitivity (McKusky *et al.*, 1984). Moreover, reports on the effects of insulin on the inflammatory response and metabolic changes in endotoxic sheep is limited.

The purpose of this study was therefore to determine the ability of insulin to suppress fever in sheep challenged with LPS. The effect of LPS on carbohydrate metabolism was also characterized by determining the levels of glucose in the plasma.

## Materials and Methods

### Experimental procedures

Six castrated male Suffolk-cross wethers (weight range of 58.6 to 65.9 kg) were housed indoors under 12:12-h light dark cycle. They were randomly assigned to receive one of the following treatment combinations; 0.9 % saline vehicle (SAL+SAL) as control, SAL + LPS (0.06  $\mu$ g kg<sup>-1</sup> body weight (BW), *Escherichia coli* 055:B5; Sigma-Aldrich, Co. St

Louis, MO) or insulin (2, 6, 12 or 20 mU kg<sup>-1</sup> BW; Human recombinant; Sigma-Aldrich Co. St. Louis MO, USA) + LPS (0.06  $\mu$ g kg<sup>-1</sup> BW) injected intravenously (i.v.). The treatments were designed such that the first i.v injection in each treatment combination was given 2 h prior to the second. The sheep were fed 3 % of BW on a concentrate diet made up of 12% crude protein on the morning of an experiment and had free access to water. Treatment was administered to each sheep. The order of treatments was randomized. Jugular blood was sampled at 1 h before and then every 1 h thereafter for 10 h after the insulin bolus. Body temperature was also monitored at the same time as for blood sampling using a rectal digital thermometer.

### Blood Metabolite Analysis

Blood samples were placed into vacutainer tubes each containing 100  $\mu$ L of 7.5 mg Ethylenediaminetetraacetic acid (EDTA) anticoagulant and centrifuged (1600 x g for 15 min at 4°C) immediately within 10 min of collection. Plasma samples were then stored on ice until transported to the lab. Once in the lab, aliquots of plasma were stored at -20°C until assayed for glucose. Glucose concentrations in the plasma were determined using a commercially available test kit (Autokit Glucose C II kit; Wako Chemicals Inc, Richmond, VA, USA).

### Statistical Analysis

The effect of treatment and time on temperature and glucose concentrations were determined using the Generalized Linear Models procedures of SAS (1999) for repeated measures. Mean separation was performed by using the LS Means/PDIFF statements of SAS. Values of  $P < 0.05$  were considered significant.

## Results

The effect of intravenous administration of saline (SAL+SAL), SAL+LPS or various doses of insulin plus LPS on body temperature in sheep is shown in Table 1. Temperatures for the Saline control sheep

**Table 1:** Effect of intravenous administration of saline (SAL), lipopolysaccharide (LPS) or insulin and lipopolysaccharide (I+LPS) on body temperature (°C) in sheep (LS Mean  $\pm$  SEM).

Time (h)	Treatments					
	SAL+SAL	SAL+LPS	2mUI+LPS	6mUI+LPS	12mUI+LPS	38.9 $\pm$ 0.1
1	38.9 $\pm$ 0.1	38.8 $\pm$ 0.1	38.9 $\pm$ 0.1	39.0 $\pm$ 0.1	38.8 $\pm$ 0.1	38.9 $\pm$ 0.1
*0	39.1 $\pm$ 0.1	39.0 $\pm$ 0.1	39.0 $\pm$ 0.1	39.1 $\pm$ 0.1	39.0 $\pm$ 0.1	38.9 $\pm$ 0.1
1	38.9 $\pm$ 0.2	39.0 $\pm$ 0.1	39.1 $\pm$ 0.1	39.1 $\pm$ 0.1	39.0 $\pm$ 0.1	39.0 $\pm$ 0.1
#2	39.1 $\pm$ 0.1	39.3 $\pm$ 0.1	39.2 $\pm$ 0.1	39.1 $\pm$ 0.2	39.4 $\pm$ 0.2	39.2 $\pm$ 0.1
3	39.1 $\pm$ 0.2 <sup>a</sup>	40.6 $\pm$ 0.2 <sup>b</sup>	40.5 $\pm$ 0.2 <sup>b</sup>	40.5 $\pm$ 0.2 <sup>b</sup>	40.4 $\pm$ 0.2 <sup>b</sup>	40.6 $\pm$ 0.2 <sup>b</sup>
4	39.1 $\pm$ 0.2 <sup>a</sup>	41.2 $\pm$ 0.1 <sup>b</sup>	40.9 $\pm$ 0.2 <sup>b</sup>	40.8 $\pm$ 0.2 <sup>b</sup>	40.7 $\pm$ 0.2 <sup>b</sup>	41.0 $\pm$ 0.2 <sup>b</sup>
5	39.0 $\pm$ 0.3 <sup>a</sup>	41.6 $\pm$ 0.3 <sup>b</sup>	40.9 $\pm$ 0.3 <sup>bc</sup>	41.0 $\pm$ 0.3 <sup>bc</sup>	40.7 $\pm$ 0.3 <sup>c</sup>	41.0 $\pm$ 0.3 <sup>bc</sup>
6	39.0 $\pm$ 0.3 <sup>a</sup>	41.2 $\pm$ 0.3 <sup>b</sup>	40.6 $\pm$ 0.3 <sup>bc</sup>	40.5 $\pm$ 0.3 <sup>bc</sup>	40.0 $\pm$ 0.3 <sup>c</sup>	40.7 $\pm$ 0.3 <sup>bc</sup>
7	39.1 $\pm$ 0.3 <sup>a</sup>	40.5 $\pm$ 0.3 <sup>b</sup>	40.2 $\pm$ 0.3 <sup>b</sup>	40.2 $\pm$ 0.3 <sup>b</sup>	39.6 $\pm$ 0.3 <sup>a</sup>	40.2 $\pm$ 0.3 <sup>b</sup>
8	39.1 $\pm$ 0.2 <sup>a</sup>	40.1 $\pm$ 0.2 <sup>b</sup>	39.8 $\pm$ 0.2 <sup>ab</sup>	39.8 $\pm$ 0.2 <sup>ab</sup>	39.5 $\pm$ 0.2 <sup>a</sup>	39.8 $\pm$ 0.2 <sup>ab</sup>
9	39.0 $\pm$ 0.2 <sup>a</sup>	39.9 $\pm$ 0.2 <sup>b</sup>	39.7 $\pm$ 0.2 <sup>b</sup>	39.7 $\pm$ 0.2 <sup>b</sup>	39.4 $\pm$ 0.2 <sup>ab</sup>	39.8 $\pm$ 0.2 <sup>b</sup>
10	39.0 $\pm$ 0.2 <sup>a</sup>	39.6 $\pm$ 0.2 <sup>b</sup>	39.5 $\pm$ 0.2 <sup>b</sup>	39.7 $\pm$ 0.2 <sup>b</sup>	39.3 $\pm$ 0.2 <sup>ab</sup>	39.5 $\pm$ 0.2 <sup>b</sup>

Means within the same row with different superscripts (a,b,c) are significantly different ( $P < 0.05$ ).

-1 = initial body temperature 1 h before start of intravenous injections

\*0 = Time of intravenous administration of insulin

#2 = Time of intravenous administration of LPS.

**Table 2:** Effect of intravenous administration of saline (SAL), lipopolysaccharide (LPS) or insulin and lipopolysaccharide (I+LPS) on plasma glucose concentrations (mg dL<sup>-1</sup>) in sheep (LS Mean  $\pm$  SEM).

Time (h)	Treatments					
	SAL+SAL	SAL+LPS	2mUI+LPS	6mUI+LPS	12mUI+LPS	20mUI+LPS
-1	72.5 $\pm$ 3.1	71.6 $\pm$ 3.1	75.0 $\pm$ 3.1	68.2 $\pm$ 3.1	73.2 $\pm$ 3.1	70.3 $\pm$ 3.1
*0	73.0 $\pm$ 4.1	70.5 $\pm$ 4.1	68.4 $\pm$ 4.1	69.0 $\pm$ 4.1	64.2 $\pm$ 4.1	63.9 $\pm$ 4.1
1	71.7 $\pm$ 4.5	71.9 $\pm$ 4.5	69.2 $\pm$ 4.5	63.3 $\pm$ 4.5	67.9 $\pm$ 4.5	62.1 $\pm$ 4.5
#2	70.3 $\pm$ 3.5	69.1 $\pm$ 3.5	69.2 $\pm$ 3.5	63.0 $\pm$ 3.5	70.9 $\pm$ 3.5	69.0 $\pm$ 3.5
3	73.0 $\pm$ 5.0	77.2 $\pm$ 5.0	70.2 $\pm$ 5.0	76.8 $\pm$ 5.0	74.6 $\pm$ 5.0	72.1 $\pm$ 5.0
4	74.2 $\pm$ 5.3	77.1 $\pm$ 5.3	73.9 $\pm$ 5.3	76.9 $\pm$ 5.3	79.9 $\pm$ 5.3	80.0 $\pm$ 5.3
5	76.9 $\pm$ 3.9	73.3 $\pm$ 3.9	73.7 $\pm$ 3.9	75.0 $\pm$ 3.9	78.8 $\pm$ 3.9	75.7 $\pm$ 3.9
6	73.8 $\pm$ 5.1	69.3 $\pm$ 5.1	75.7 $\pm$ 5.1	67.2 $\pm$ 5.1	69.6 $\pm$ 5.1	64.0 $\pm$ 5.1
7	73.6 $\pm$ 3.5 <sup>a</sup>	58.3 $\pm$ 3.5 <sup>b</sup>	60.9 $\pm$ 3.5 <sup>ab</sup>	55.4 $\pm$ 3.5 <sup>b</sup>	55.8 $\pm$ 3.5 <sup>b</sup>	55.6 $\pm$ 3.5 <sup>b</sup>
8	76.3 $\pm$ 4.7 <sup>a</sup>	55.5 $\pm$ 4.7 <sup>ab</sup>	56.4 $\pm$ 4.7 <sup>ab</sup>	52.2 $\pm$ 4.7 <sup>b</sup>	48.3 $\pm$ 4.7 <sup>b</sup>	46.5 $\pm$ 4.7 <sup>b</sup>
9	75.5 $\pm$ 5.4 <sup>a</sup>	52.8 $\pm$ 5.4 <sup>ab</sup>	59.5 $\pm$ 5.4 <sup>ab</sup>	47.9 $\pm$ 5.4 <sup>b</sup>	48.0 $\pm$ 5.4 <sup>b</sup>	46.6 $\pm$ 5.4 <sup>b</sup>
10	80.0 $\pm$ 6.4 <sup>a</sup>	52.3 $\pm$ 6.4 <sup>b</sup>	57.8 $\pm$ 6.4 <sup>ab</sup>	51.8 $\pm$ 6.4 <sup>b</sup>	56.6 $\pm$ 6.4 <sup>ab</sup>	51.9 $\pm$ 6.4 <sup>b</sup>

Means in the same row with different superscripts (a,b,c) are significantly different ( $P < 0.05$ ).

-1 = initial body temperature 1 h before start of intravenous injections

\*0 = Time of intravenous administration of insulin

#2 = Time of intravenous administration of LPS.

(SAL+SAL) remained relatively constant throughout the study period ranging from  $38.9 \pm 0.1$  to  $39.1 \pm 0.1^\circ\text{C}$ . The administration of LPS to sheep in this study resulted in instant fever. Sheep administered with SAL + LPS had significantly ( $P < 0.05$ ) elevated temperature compared to the saline controls from 1 h to 8 h after LPS administration. Temperatures in the SAL+LPS treated sheep reached a maximum of  $41.6 \pm 0.1^\circ\text{C}$  3 h after LPS administration. This was followed by a gradual decline towards initial normal levels by 8 h post LPS administration. Temperatures in sheep administered with a combination of various doses of insulin (2, 6, 12 and 20 mU) and LPS ( $0.06 \mu\text{g kg}^{-1}$  BW) were also elevated from 1 h to 3 h after LPS administration. These were followed by steady declines towards normal levels by 8 h after LPS injection. The sheep injected with 12mUI +LPS had significantly ( $P < 0.05$ ) lower body temperature than the SAL +LPS treated sheep from 3 to 6 h post LPS injection. Within the insulin + LPS treatment group the 12mUI +LPS combination was found to significantly reduce ( $P < 0.05$ ) body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS administration.

Sheep injected with SAL+LPS or various doses of insulin+LPS had reduced ( $P < 0.05$ ) glucose levels than the saline control sheep from 5 to 8 h post LPS injection.

## Discussion

The dose of  $0.06 \mu\text{g kg}^{-1}$  BW of LPS used in this study effectively induced typical acute inflammatory symptoms similar to the findings of other studies in sheep (Coleman *et al.*, 1993; Soliman *et al.*, 2001). A fever in response to LPS is presumably due to the prostaglandin-mediated action of cytokines, in particular IL-1 on the hypothalamus (Lohuis *et al.*, 1988; Kluger, 1991). A large body of evidence shows prostaglandin E2 is a principal downstream mediator of fevers produced by LPS and most pyrogenic cytokines, including IL-1 $\beta$  and IL-6 (Tavares *et al.*, 2006).

The ability of 12mUI+LPS combination within the insulin + LPS treatment group to

significantly reduce body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS administration (Table 1) suggests the effectiveness of this treatment combination in alleviating febrile condition in sheep.

The significantly reduced glucose levels in sheep injected with SAL+LPS or various doses of insulin+LPS compared to the saline control sheep from 5 to 8 h post LPS injection (Table 2) could be attributed to the metabolic responses to endotoxin. This includes disruptions in glucose homeostasis (Holzman *et al.*, 1974; Hinshaw, 1976). A transient early hyperglycemia is generally followed by a progression to profound hypoglycemia and a depletion of body carbohydrate reserves (Hinshaw, 1976; Knowles *et al.*, 1986). The hypoglycemia is due primarily to hypersecretion of insulin by the endotoxic pancreas (Yelich, 2001; Lang, 2001) consequently increasing glucose utilization.

The present study demonstrates the ability of insulin at a dose of 12 mU  $\text{kg}^{-1}$  BW to reduce fever in sheep challenged with LPS. Understanding the role of insulin in the inflammatory response to disease in ruminants could help in the development of feeding strategies and novel treatment interventions to increase animal productivity.

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## SEROPREVALENCE OF CAMEL BRUCELLOSIS IN PASTORAL AREAS OF AFAR, SOMALI AND OROMIA REGIONS, ETHIOPIA

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### Abstract

A cross-sectional study was conducted in the pastoral areas of Afar, Somali and Oromia regions of Ethiopia between October 2008 and May 2009 to determine the antibody prevalence and to identify risk factors for brucellosis in camels (*Camelus dromedarius*). Sera were collected from 1100 camels and 86 herds. Rose Bengal plate test (RBPT) was used to screen all serum samples and positive samples were subjected to confirmation by complement fixation test (CFT). Twenty six of these (2.36%) tested positive using the Rose Bengal plate test and 21 (1.91%) tested positive by the complement fixation test. The true seroprevalence of camel brucellosis as adjusted to the RBPT and CFT sensitivities and specificities was 5.71%. The highest prevalence (3.16%) was reported in Afar however the difference was not significant ( $p > 0.05$ ) among regions. Age and sex were not found to be significant in the occurrence of brucellosis. Univariable logistic regression model showed that adult camels in the age group of 4-6 years had significant impact on camel seropositivity to brucellosis ( $P < 0.05$ , odds ratio (OR), 4.56; 95% confidence interval (CI), 1.38-15.04). Herd size recorded significant association with seropositivity of brucellosis ( $P < 0.05$ , medium herds OR, 5.51; 95% CI, 1.80-16.91 and large herds OR, 1.85; 95% CI, 0.46-7.48). The authors recommend the implementation of well-organized disease control and prevention methods to mitigate the economic losses and public health hazard caused by the disease.

**Key words:** brucellosis; camel; complement fixation test; Rose Bengal plate test; seroprevalence;

## SÉROPRÉVALENCE DE LA BRUCELLOSE CHEZ LES DROMADAIRES DANS LES ZONES PASTORALES DE AFAR, SOMALI ET OROMIA REGIONS, ETHIOPIE

### Résumé

Une étude transversale a été menée dans les zones pastorales d'Afar, de Somali et Oromia régions de l'Ethiopie entre Octobre 2008 et mai 2009 afin de déterminer la prévalence des anticorps et d'identifier les facteurs de risque de la brucellose chez les dromadaires (*Camelus dromedarius*). Les sérums ont été recueillis chez 1100 animaux dans 86 troupeaux. Les tests de Rose Bengale (RBPT) a été utilisé à l'écran tous les échantillons de sérum et des échantillons positifs ont été soumis à la confirmation par le test de fixation du complément (CFT) ont été utilisés. Vingt-six animaux (2,36%) ont testé positif au test Rose Bengale 21 (1,91%) à la fixation de Complément. La séroprévalence de la brucellose vraie ajusté à la sensibilité RBPT et CFT et des spécificités était de 5,71%. La prévalence la plus élevée (3,16%) a été signalé dans l'Afar mais la différence n'était pas significative ( $p > 0,05$ ) entre les régions. L'âge et le sexe n'ont pas été de facteurs significatifs dans la survenue de la brucellose. Modèle de régression logistique univariée a montré que les chameaux adultes dans la tranche d'âge de 4-six années ont eu un impact significatif sur la séropositivité de chameau à la brucellose ( $P < 0,05$ , odds ratio (OR), 4,56, intervalle de confiance 95% (IC), de 1.38 à 15.04). La taille du troupeau enregistrées association significative avec la séropositivité de la brucellose ( $P < 0,05$ , moyenne ou troupeaux, 5,51, IC 95%, de 1.80 à 16.91 et de grands troupeaux OR, 1,85; IC 95%, 0,46 à 7,48). Les auteurs recommandent la mise en œuvre du contrôle de la maladie bien organisé et les méthodes de prévention pour atténuer les pertes économiques et des risques de santé publique provoqués par la maladie.

**Mots clés:** brucellose; chameaux; test de fixation du complément; Rose essai à la plaque du Bengale; séroprévalence

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## Introduction

Camels are a subset of huge livestock resource in Ethiopia with the population estimated to be over 800,000 (CSA, 2010). Nearly all camels are reared by pastoral communities under extensive methods of husbandry primarily for milk production followed by transport, cash income by sale and meat production. The eastern part of Ethiopia is considered as the heartland for camel production, which is the home of two-third of the nations camel population (Getahun and Bruckner, 2000). Brucellosis is a disease of high economic and public health importance. It is one of the most serious diseases of livestock, which constitute a major impediment for livestock trade. The magnitude of this disease in developing countries is more severe due to lack of appropriate control measures (Abbas and Agab, 2002). Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species.

Camels are frequently infected with *Brucella* organisms, and infection rate in camels depends upon the infection rate in primary host animals especially large and small ruminants in contact with them (Radwan *et al.*, 1992; Agab *et al.*, 1994). Serological evidence for *Brucella* infection in camels has been reported from Asia and Africa (Radwan *et al.*, 1992; Musa *et al.*, 2001). It is prevalent in Ethiopia (Domenech, 1977; Teshome *et al.*, 2003; Megersa, 2004) and surrounding countries such as Sudan, Kenya (Waghela *et al.*, 1978), and Somalia (Ghanem *et al.*, 2009). Brucellosis prevalence in animal reservoirs presents a constant hazard of human infections to occur mostly through the consumption of unheated milk (Madkour, 1989).

Brucellosis in animals is characterized by abortion in females, epididimitis and orchitis in males and infertility in both sexes (Omer *et al.*, 2000; Radostits *et al.*, 2000). The relation between *Brucella* infection and abortion in camels has been recorded (Al-Majali *et al.*, 2008; Dawood, 2008). Biotypes of *B. abortus* and *B. melitensis* have been isolated from fetuses, genital discharges, urine and milk

and were implicated as the cause of camel brucellosis and respective abortion by many researchers (Abbas and Agab 2002). *Brucella melitensis* biotype 3 was the most common isolated *Brucella* strain from small ruminant (Al-Majali, 2008).

In Ethiopia, several investigators have established the epidemiology of brucellosis in cattle, sheep and goats and the available information on brucellosis clearly showed that the disease occurrence is endemic and wide spread with significant economic importance with a seroprevalence of up to 22% has been reported across different livestock management systems (Yilkal *et al.*, 1998; Abay *et al.*, 2000; Berhe *et al.*, 2007; Mekonnen *et al.*, 2010).

The complexity of disease epidemiology and the lack of exact camel population concerning detailed demographic data are among the major factors that have constrained disease control in Ethiopia. Research on the epidemiology of camel brucellosis is very scarce. The objective of this study was to determine the seroprevalence of brucellosis and associated risk factors in camels in the eastern and southern pastoral areas of Ethiopia.

## Materials and Methods

### Study areas

The study was conducted in pastoral areas of Ethiopia; Afar, Somali and Oromia regions (Figure 1). These regions are the major areas where camel husbandry is widely practiced and the livelihood of pastoral communities is certainly ensured by camels used as the main source of milk and meat (Vossene, 1991). The Afar region constitutes the northeast range lands of Ethiopia, which is featured by its hot tropical climate of Africa. High temperature and low rain fall generally characterize the region. It has annual rain fall of 561 mm on the western edge of escarpment and 225 mm on the lava plain and volcanic ash, where only camels and goat can be sustained in fragile pastoral existence. The lowest and highest annual temperature is 18°C and 35°C, respectively (National Metrology Agency,

2007). Three zones namely; zone 1, 3 and 5 were selected for this study.

Borena zone of Oromia region is located between 3°36'N latitude and 6°38' N and 41°40'E longitude. Over 62% of the zone is below 1500m altitude, with a semi arid climate. The rain pattern is bimodal with short and long rainy seasons. The annual rainfall ranges from 440 to 1100 mm and the annual temperature ranges from 10-25°C (National Metrology Agency, 2007). Camels in Borena zone were considered for this study. The lowland areas with semi-arid climate occupy the greater portion of Somali region and 85% of the population of the region makes up pastoralist communities. Three pastoral zones of Somali; Shinele, Jijiga and Liben were selected for this study. The average annual rain fall is about 560mm. The annual daily minimum and maximum temperature ranges from 13°C to 28°C (National Metrology Agency, 2007).

#### Study design

A cross-sectional study was conducted during the period from October 2008 to May 2009 using serological procedures. A total of 1100 unvaccinated camels in 86 herds were examined. The serological survey was carried out with the intention of determining individual animal- and herd-level prevalence. Blood sampling was performed from accessible individual camels of both sexes, aged 1 year and over. The blood was allowed to clot and the sera were separated by centrifugation and stored at -20°C until tested. Information of each camel sampled were obtained including; its location, herd size, sex, age, health status and whether reared individually or with other species.

#### Sample size determination

The true representatives of the study population were selected by combination of simple random and cluster sampling methods. A total of 86 herds were established as a sampling frame and 10% of herds were considered for sampling. We assumed an expected prevalence of 6% (Teshome et al.,

2003) with 95% confidence interval and 2% absolute precision. This gave us a total of 550 camels. However, in the absence of between cluster variance data, it was stated that inflating the sample size two to four folds can account for the potentially large variation that may occur among clusters (Thrusfield, 2005). We inflated two folds and obtained a total of 1100 camels. Division of the total number of animals (1100) to the number of herds (86) gave us 12.8. Therefore, we sampled camels from 86 herds. All camels were sampled in herds with herd size of 13 or less. Random selection was carried out in case the herd size was more than 13 animals.

#### Serological testing

The Rose Bengal plate test (RBPT) was used as a screening test for detection of *Brucella* agglutinins and samples giving positive results were then confirmed by the Complement Fixation Test (CFT). Both tests were carried out at the laboratory of National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia.

#### Rose Bengal plate test

All sera collected were screened for the presence of antibodies against *Brucella* using the standard RBPT, employing stained *B. abortus* antigen (Institute Pourquier, Montpellier Cedex 5, France) and known positive and negative reference sera. *B. abortus* antigen was heat inactivated and 0.5% phenol adjusted to pH 3.65 and colored with Rose Bengal. For the RBPT the procedure described by Staak et al., (2000) was followed. Magnifying glass was used to detect micro-agglutination. Reactions were identified as 0, +, ++ and +++; 0 = no agglutination, + = barely perceptible agglutination (seen using magnifying glass), ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive. Positive sera were then subjected to complement fixation test.

#### Complement Fixation Test

RBPT positive and inconclusive

serum samples were further tested using CFT. Preparation of the reagents was performed according to OIE protocols (OIE, 2000). A titration of hemolysin and antigen was performed before the test. The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. According to OIE (2004) sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive. Camels with both positive RBPT and CFT results considered *Brucella* seropositive camels. According to the manufacturer, the sensitivity and specificity of RBPT are 89 and 97%, respectively. CFT has a sensitivity of 88.1% and a specificity of 100% (Blasco, 2006).

#### Statistical analysis

The *Brucella* seroprevalence was estimated by adjusting the apparent prevalence to the sensitivities and specificities of the two serological tests (Dawood, 2008) using the following formula:

$$TP = \frac{Ap - (1 - Sp1)(1 - Sp2)}{Se1 - (1 - Sp1)(1 - Sp2)}$$

Where TP is the true prevalence; AP is the apparent prevalence, Sp1 and Sp2 are RBPT and CFT tests specificities, respectively; Se1 and Se2 are RBPT and CFT sensitivities, respectively (Noordhuizen, 1997). Association between the occurrence of *Brucella* infection and the potential risk factors on both herd and animal level were investigated using logistic regression. The odds ratios (OR) were computed using Stata software package, version 8 (StataCorp, 2004) to estimate the risk of brucellosis being associated to these variables.

## Results

### Seroprevalence of brucellosis in camels

A total of 1100 camel sera were examined from the three pastoral regions of Ethiopia. Out of the 1100 camel sera tested, 26 (2.36%) were positive by RBPT. When tested by CFT, 21 (1.91%) out of the 26 RBPT positive sera were positive by CFT (Table 1). Hence, the true seroprevalence of camel brucellosis in the study area as adjusted to the RBPT and CFT sensitivities and specificities was 5.71%. Out of the 86 herds investigated, 18 (20.9%) had at least one positive camel. Although not statistically significant, higher antibodies prevalence was recorded from camels in Afar region (3.16%).

The distribution of infected camels among different age groups and between sex groups is shown in Table 2. Despite high proportion of females (74%) as compared to males (26%), there was no statistically significant ( $p > 0.05$ ) difference in seroprevalence of brucellosis between male (2.46%) and female (1.72%) camels. The same table shows that 81% of the seropositive camels were adult (>4 years old) and the remaining 19% were young, ranging from 1-3 years old. However, the difference among age groups was not significant ( $P > 0.05$ ).

### Univariable and multivariable logistic regression analyses of risk factors for brucellosis

The differences between brucellosis seroprevalence in camels per each risk factor categories as well as their associations are summarized in Table 3. During the statistical analyses of all risk factors, the first level of each independent variable was used as a reference category. Univariable logistic regression model showed that adult camels in the age group of 4-6 years had significant impact on camel seropositivity to brucellosis ( $P < 0.05$ ). Adults were more likely at risk (OR, 1.03-4.56) of acquiring brucella infection as compared to young (1-3 years). Herd size recorded significant association with seropositivity of brucellosis ( $P < 0.05$ ). Camels in medium herds (OR, 5.51; 95%CI, 1.80-16.91) and large herds (OR, 1.85; 95%CI,

**Table 1:** Seroprevalence of camel brucellosis using RBPT and CFT tests

Region	No. of serum samples tested	No. positive with RBPT <sup>a</sup>	Prevalence (%)	No. positive with CFT <sup>b</sup>	Prevalence (%)
Afar	285	12	4.21	9	3.16
Somalia	493	9	1.83	8	1.62
Oromia	322	5	1.55	4	1.24
<b>Total</b>	<b>1100</b>	<b>26</b>	<b>2.36</b>	<b>21</b>	<b>1.91</b>

<sup>a</sup>RBPT,  $\chi^2 = 4.90$ , df = 2, P>0.05; <sup>b</sup>CFT,  $\chi^2 = 2.40$ , df = 2, P>0.05**Table 2:** Brucella reactors according to sex and age based on CFT

Region	Age group	Female	Reactors (%)	Male	Reactors (%)	Total	Reactors (%)
Afar	1-3	63	1(1.59)	21	1(4.76)	84	2(2.38)
	4-6	32	2(6.25)	18	2(11.1)	50	4(8.0)
	7-9	46	2(4.35)	19	0	65	2(3.08)
	>9	66	1(1.52)	20	0	86	1(1.16)
	total	207	6(2.90)	78	3(3.85)	285	9(3.16)
Somali <sup>b</sup>	1-3	120	0	33	1(3.03)	153	1(0.65)
	4-6	60	3 (5.0)	14	0	74	3(4.05)
	7-9	77	0	33	1(3.03)	110	1(0.91)
	>9	110	2(1.82)	46	1(2.17)	156	3(1.92)
	total	367	5(1.36)	136	3(1.47)	493	8(1.62)
Oromia <sup>c</sup>	1-3	79	1(1.27)	27	0	106	1(0.94)
	4-6	43	2(4.65)	9	0	52	2(3.85)
	7-9	53	0	23	1(4.35)	76	1(1.32)
	>9	66	0	22	0	88	0
	total	241	3(1.25)	81	1(1.24)	322	4(1.24)
<b>Grand total</b>		<b>815</b>	<b>14(1.72%)</b>	<b>285</b>	<b>7(2.46)</b>	<b>1100</b>	<b>21(1.91)</b>

<sup>a</sup> $\chi^2 = 4.37$ , df = 3, P>0.05; <sup>b</sup> $\chi^2 = 3.60$ , df = 3, P>0.05; <sup>c</sup> $\chi^2 = 1.58$ , df = 2, P>0.05**Table 3:** Summary results of the univariable and multivariable logistic regression (LR) analyses of risk factors with dependent Brucella seropositivity in camels in pastoral areas of Ethiopia.

Risk factors	Category	n*	Prevalence No.+ve(%)	Univariable LR analysis results			Multivariable LR analysis results		
				P-value	OR	95%CI OR	P-value	OR	95%CI OR
Region	Somali	493	8(1.62)	-	-	-	0.870	1.04	0.63- 1.71
	Afar	285	9(3.16)	0.166	1.97	0.75-5.18			
	Oromia	322	4(1.24)	0.938	0.95	0.31-2.94			
Sex	Male	285	7(2.46)	-	-	-	0.513	0.73	0.29-1.83
	Female	725	15(1.84)	0.524	0.74	0.30-1.84			
Age	1-3	343	4(1.17)	-	-	-	0.664	0.92	0.65-1.31
	4-6	176	9(5.11)	0.013	4.56	1.38-15.04			
	7-9	252	6(2.38)	0.421	1.72	0.45-6.48			
	>9	329	3(0.91)	0.956	1.03	0.25-4.19			
Herd size	Small	505	4(0.79)	-	-	-	0.783	1.03	0.82-1.29
	Medium	326	14(4.29)	0.003	5.51	1.80-16.91			
	Large	269	4(1.49)	0.384	1.85	0.46-7.48			

n\*: total number of camels tested; Herd size: small (1-8camels), medium (9-15camels), large (&gt;15camels)



0.46-7.48) were more likely at risk of getting brucella infection as compared to small herds. The multivariable logistic regression model (Table 3) showed that location, sex, age and herd size were not significantly associated with camel seropositivity. However, OR for location and herd size was greater than one.

## Discussion

Seroprevalences of camel brucellosis have been established at different times from various regions of the country (Teshome *et al.*, 2003; Megersa, 2004). In the present study the overall prevalence of camel brucellosis using CFT was found to be 1.91%. This finding is in agreement with Megersa (2004) who reported 1.8% prevalence in Borana zone, Oromia region and Baumsan and Zessin (1992) who indicated 1.9% and 0.3% in Somali using seroagglutination and CFT, respectively. However, the present prevalence is lower than the previous studies in pastoral areas of Ethiopia (Domenech, 1977; Teshome *et al.*, 2003) who reported 4.4% and 5.2% in Afar and 2.8% in Somali regions. The lower prevalence in this study can be due to sample variation, difference in study sites, husbandry and management conditions, and the rate of transmission, and the virulence of organisms (Radostits *et al.*, 2000).

The long inter-calving interval in combination with the late age at first calving in dromedaries kept under extensive production system (Wilson, 1998) limit the transmission within the herd and subsequently among herds (Abbas and Agab, 2002). On the other hand the relatively lower prevalence in Somali and Oromia region may be associated with the existing tradition of isolating calved and aborted females from the animals in the herds that might reduce the risk of infection. Similarly lower prevalence was also reported from some countries with seroprevalence ranging from 2-5% in extensively kept camels (Abbas and Agab, 2002).

Although the difference was not statistically significant, the prevalence of *Brucella* antibodies in camels was found higher in Afar as compared to Somali and

Oromia regions. The large number of different animal species; cattle, small ruminants and camels raised in Afar in communal pastures and watering areas may contribute for the increased prevalence of brucellosis in the area. Ashenafi *et al.*, (2007) has reported 5.8% seroprevalence of *Brucella* infection in goats and 3.2% in sheep in Afar region and 1.6% *Brucella* seroprevalence in sheep and 1.7% in goats in Somali region. This indicates that other species of animals may also play a great role in the transmission of the disease to camels.

The seroprevalence difference in age and sex in this study was not significant. This is in consistent with previous report of Teshome *et al.*, (2003). However, the higher proportion was recorded in the adult camel than in young in both present and previous studies. It had been reported that brucellosis is essentially a disease of sexually matured animals (Quinn *et al.*, 2002). Sexually matured and pregnant animals are more prone to *Brucella* infection than sexually immature animals (Radostits *et al.*, 2000). On the other hand it is true that younger animals are more resistant to infection and frequently clear established infection, although the latent infection can occur (Walker, 1999). This may be the result from the fact that sex hormones and erythritol which stimulate the growth and multiplication of *Brucella* organism tend to increase in concentration with age and sexual maturity (Radostits *et al.*, 2000).

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## EFFECT OF NUTRITIONAL STATUS ON CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I IN MILK FROM HOLSTEIN COWS

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### Abstract

The effect of diet on concentrations of milk insulin-like growth factor-I (IGF-I), and their relationships with plasma IGF-I, and with somatic cell count (SCC) were evaluated in two trials. In Trial 1, 32 multi-parous Holstein cows at 4 to 5 wk of lactation received 4 different diets formulated to provide high (H) or low (L) dry matter intake (DMI) with H or L metabolisable energy (ME) density for 5 wk. Dietary treatment did not affect milk IGF-I concentrations, but concentrations were repeatable in individual cows. The intraclass correlation coefficients were  $0.78 \pm 0.05$  and  $0.73 \pm 0.06$  in a.m. and p.m. samples, respectively, when concentrations were measured for 7 consecutive days. The association between milk IGF-I and plasma IGF-I concentration was weaker ( $R^2 = 0.140$ ;  $P = 0.035$ ) than the association between milk IGF-I concentrations and the natural logarithmic value (Ln) of SCC ( $R^2 = 0.249$ ;  $P = 0.004$ ). The effects of body condition score (BCS) at calving and level of grain supplementation in early lactation on IGF-I concentrations in milk were evaluated in Trial 2 with 21 Holstein cows that grazed pasture. The BCS at calving did not affect milk IGF-I concentrations; neither did supplementation with 1 or 6 kg of grain. Milk and plasma concentrations of IGF-I were not associated ( $R^2 = 0.002$ ;  $P = 0.830$ ), whereas the association between milk concentrations of IGF-I and Ln SCC was significant ( $R^2 = 0.342$ ;  $P = 0.005$ ). Concentrations of IGF-I in milk were not a sensitive measure of dietary changes in lactating pasture-fed Holstein cows.

**Keywords:** Insulin-like growth factor-I, milk, dietary factors, somatic cell count

## EFFET DU STATUT NUTRITIONNEL SUR LES CONCENTRATIONS D'INSULINE DE CROISSANCE DANS LE LAIT DE VACHES HOLSTEIN

### Résumé

L'effet du régime sur les concentrations d'insuline de croissance dans le lait et leurs relations avec le nombre des cellules somatiques (CCS) ont été évalués dans deux essais. Dans l'essai 1, 32 vaches Holstein multipares de 4 à 5 semaines de lactation ont reçu 4 régimes différents formulés pour fournir de haute (H) ou faible (L) ingestion de matière sèche (IMS) avec une densité d'énergie métabolisable H ou L pendant 5 semaines. Le traitement alimentaire n'a pas affecté les concentrations d'insuline de croissance dans le lait, mais les concentrations étaient les mêmes chez les vaches individuelles. Les coefficients de corrélation intra classe de  $0.78 \pm 0.05$  et  $0.73 \pm 0.06$  respectivement le matin et le soir ont été calculées quand les concentrations ont été mesurées pendant 7 jours consécutifs. La relation entre la concentration d'insuline et le nombre de cellule somatique était plus faible ( $R^2 = 0,140$ ,  $P = 0,035$ ) que celle sa valeur logarithmique naturelle ( $R^2 = 0,249$ ,  $P = 0,004$ ). Les effets de la note d'état corporel au vêlage et le niveau de suppléments des céréales en début de lactation sur les concentrations d'insuline dans le lait ont été évalués lors de l'essai 2 avec 21 vaches Holstein. Le la note d'état corporelle au vêlage n'a pas affecté la concentration d'insuline pas plus que la suppléments

avec 1 ou 6 kg de céréales. Les concentrations d'insuline de croissance dans le lait ne sont pas une mesure sensible de changements alimentaires en lactation des vaches Holstein nourris au pâturage.

**Mots-clés:** Concentration de l'insuline de croissance le lait, le régime alimentaire des facteurs, le nombre de cellules somatiques

## Introduction

Insulin-like growth factor-I (IGF-I) is a small peptide hormone of approximately 7kDa molecular mass (Hwa *et al.*, 1999) that plays important roles in mammary gland development, differentiation and milk production in ruminants (Akers *et al.*, 2000). Its primary source is the liver (Rosen and Pollak, 1999) and it may exert an endocrine effect on the mammary gland. There is also evidence for local synthesis and secretion of IGF-I and IGF binding proteins (IGFBPs) by the mammary gland of lactating cows (Sharma *et al.*, 1994). The relative contributions of systemic as compared to locally synthesized IGF-I to bovine mammary differentiation, growth and lactogenesis have not been resolved. Similarly, the contribution of each of these two sources to IGF-I concentrations in milk is unclear (Baumrucker and Erondur, 2000).

The concentrations of IGF-I in milk vary with stage of lactation in dairy cows. They decline at the approach of parturition, remain low in early lactation and then increase gradually through lactation (Sejrsen *et al.*, 2001). There are some reports on the pattern of changes in milk IGF-I concentrations in dairy cows during early lactation (Skaar *et al.*, 1991; Vega *et al.*, 1991; Sejrsen *et al.*, 2001). These studies have been conducted using dairy cows fed completely balanced, total mixed rations (TMR). Limited information exists on the relationship between plasma and milk concentrations of IGF-I.

Plasma concentrations of IGF-I are affected by nutritional status (Thissen *et al.*, 1994). These changes can be used as a sensitive monitor of energy balance in dairy cows fed TMR during the postpartum period (Beam and Butler 1999). They are also a sensitive measure of dietary changes in cows in pasture-based systems during early lactation (Obese *et al.*, 2008b). There

is a lack of information on nutritional effects on milk concentrations of IGF-I, as well as, the associations between milk and plasma concentrations of IGF-I among cows in pasture-fed dairy herds.

The objectives of the present study were to evaluate: (i) the effects of varying either level of DMI or ME density during early lactation on milk concentrations of IGF-I; (ii) the effects of BCS at calving and different levels of grain feeding in early lactation on milk concentrations of IGF-I; (iii) the day- to-day variation in IGF-I concentrations in milk of individual cows; and (iv) the relationships between milk concentrations of IGF-I with plasma concentrations, and milk SCC.

## Materials and Methods

### *Animals and Experimental Procedures*

Two studies were conducted in Victoria, Australia in 2000 and 2001. Trial I was conducted at the Ellinbank Dairy Research Centre, Victorian Department of Primary Industries during August and September, 2000 (Obese *et al.*, 2008b). Thirty-two multiparous Holstein cows 4 to 5 wk postpartum were randomly assigned to one of four treatments receiving different diets. Briefly, these diets were formulated to provide high (H) or low (L) DMI with H or L ME density for 5 wk (comprising a 3- wk adaptation period followed by 2 wk of intensive sampling). The treatments were LL: 16.6 kg of DMI and 174 MJ of ME; HL: 17.3 kg of DMI and 181.1 MJ of ME; LH: 15.4 kg of DMI and 183.1 MJ of ME; HH: 17.9 kg of DMI and 213.3 MJ of ME. The diets comprised freshly cut ryegrass-clover pasture, meadow hay and pelleted barley grain to achieve the two different levels of DMI and ME. The cows were milked at 06:00 and 15:00 h daily before being individually offered their allocated diet at 09:00 h and 16:00 h for 5 h. Water was available between the feeding and milking times.

Trial 2 was conducted between June and November 2001, at the Kyabram Dairy Centre, Victorian Department of Primary Industries. Twenty-one Holstein cows were managed to calve in three BCS groups of 4 (3.5 to 4.5), 5 (4.6 to 5.5) or 6 (5.6 to 6.5) on an 8-point score (Earle, 1976). There were 9, 4 and 8 cows in BCS groups 4, 5 and 6 respectively. The cows grazed pasture after calving at a pasture allowance of 35 to 40 kg DM/cow/day and were provided with either 1 or 6 kg of wheat grain energy concentrate daily for 10 wk (Stockdale, 2004). The concentrates were individually fed immediately after each milking before the cows returned to pasture to graze. They were milked at 06:00 and 15:00 h daily, and water was available only at milking times.

#### *Milk and Blood Sampling and Assays*

Milk samples were hand drawn (fore strippings) weekly from each cow during the 5-wk study in Trial 1 into 10 mL plastic vial tubes at 06:00 h. Samples were also collected daily for 7 consecutive days at 06:00 h and 16:00 h in Wk 4 to estimate within day variation in milk concentrations of IGF-I. Each sample was kept on ice and centrifuged shortly after collection at 1500 g for 15 min at 4°C. The fat was removed after centrifugation and the "fat-free" samples stored at -20°C until assayed for IGF-I concentration.

Milk samples were collected from the 21 cows in Trial 2 at 5 and 10 wk of lactation. The samples were processed as for Trial 1. Blood samples were also taken weekly from the coccygeal vessels of cows into 10 mL heparinized vacutainer tubes in both trials 1 and 2. The samples were centrifuged shortly after collection at 1500 x g for 15 min at 4°C. Plasma was aspirated and stored at -20°C for subsequent analysis for IGF-I concentration.

The IGF-I concentrations in milk and plasma were measured with the DSL-10-2800 ACTIVE TM Enzyme-linked Immunosorbent assay (ELISA) commercial kit (Diagnostic Systems Laboratories Inc, Webster, TX, USA) which had been validated against an IGF-I radioimmunoassay (RIA) (Obese et al., 2008a). Validation results showed low coefficient of

variation (CV) values (intra-assay and inter-assay CVs for the milk IGF-I assay were 2.0 % and 3.5 %, and for the plasma IGF-I assay were 2.3 % and 4.1 %). Minimum detection limit was 10 ng/ mL for the milk and plasma assays.

The SCC was determined in weekly milk samples in both trials using a Milkoscan (Foss Electric, Denmark).

#### *Statistical Analyses*

The IGF-I concentrations in milk and plasma, and SCC of individual cows in each week were averaged over the 5-wk period of the study in Trial 1 and values used in the evaluation of diet on milk IGF-I, plasma IGF-I and SCC. The above data were analyzed by the Kruskal-Wallis One Way analysis of variance on ranks in SPSS version 11.5 (SPSS Inc., 2002). The Kruskal-Wallis one-way analysis of variance is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way analysis of variance with the data replaced by their ranks (measurement observations are converted to their ranks in the overall data set). It is an extension of the Mann-Whitney U test (McDonald, 2009). The association between IGF-I concentrations in a.m. and p.m. milk samples from the 7-day daily sampling period during Wk 4 was estimated using linear regression analysis. The day-to-day variation in IGF-I concentrations in a.m. or p.m. samples over the same 7-day measurement period was evaluated using intraclass correlations (ICC; Snedecor and Cochran, 1980) and Kendall's coefficient of concordance (SPSS Inc., 2002). Values for milk and plasma concentrations of IGF-I and SCC for the individual cows (n = 21) at 5 and 10 wk of lactation in Trial 2 were averaged and the data used in assessing the effect of BCS at calving (4, 5 and 6) on milk and plasma concentrations of IGF-I, and SCC. Analyses were also by the Kruskal-Wallis One-way analysis of variance on ranks. The effect of level of grain feeding on these three variables was by Mann-Whitney One-way analysis of variance on ranks in SPSS v 11.5. Data on SCC were transformed to natural logarithmic values (Ln) and linear regressions

were used to assess the relationships between milk and plasma concentrations of IGF-I and Ln SCC.

## Results

### *Milk Concentrations of IGF-I in Trial 1*

The milk IGF-I concentrations were low compared to plasma IGF-I concentrations. The IGF-I concentrations in weekly milk samples in the 4 dietary groups generally peaked at d14, followed by declines of varying magnitude (Figure 1). The average concentrations of milk IGF-I for the 32 cows in the 4 treatment groups were only  $7.5 \pm 1.8$  and  $5.9 \pm 1.7$  ng/mL at d 0 (the beginning) and d35 (the end) respectively, compared to corresponding values in plasma of  $58.1 \pm 4.6$  and  $60.4 \pm 4.3$  ng/mL. Dietary treatment did not affect milk concentrations of IGF-I or SCC when ranked in cows, while the plasma concentrations of IGF-I were significantly affected by diet (Table 1). Cows in the HH-dietary group had the highest mean ranking, while those in the LL-dietary group had the lowest.

### *Concentrations of IGF-I in a.m. and p.m. milk samples in Trial 1*

The intraclass correlation (used as a measure of the proportion of the total variance that was due to between cow variance) was high and significant for a.m. and p.m. samples, as well as, the average for a.m. and p.m. samples (0.73 to 0.78; Table 2). The Kendall's coefficient of concordance calculated as a measure of agreement among rankings of the cows over each of the 7 d of milk IGF-I were also high (a.m. = 0.76;  $P < 0.001$ ; and p.m. = 0.71;  $P < 0.001$ ). There was a strong association (Table 3) between IGF-I concentrations in a.m. and p.m. samples on each of the 7 d as well as the overall mean for each animal over the 7-d period. High milk IGF-I concentrations in some a.m. and p.m. samples were associated with high SCC ( $> 100,000$ ) in individual cows.

### *Milk and Plasma Concentrations of IGF-I, and SCC in Trial 2*

Body condition scores at calving were not associated with differences in the concentrations of milk and plasma IGF-I, or with SCC when cows were ranked. Level of grain feeding did not influence IGF-I concentration in milk (Table 4). By contrast, cows supplemented with 6 kg of grain per day, had higher mean ranking for plasma IGF-I than those on the 1 kg grain supplement (Table 4). Level of grain feeding did not affect the mean rankings for SCC.

### *Relationships among concentrations of IGF-I in milk and plasma, and SCC*

The relationships between milk and plasma concentrations of IGF-I were weaker than those between milk IGF-I and Ln SCC in both trials (Table 5).

## Discussion

Neither the IGF-I concentrations in milk nor SCC were influenced by dietary treatments used in Trials 1 and 2 in contrast to plasma concentrations of IGF-I. Concentrations of IGF-I in milk samples were not affected by BCS at calving (Trial 2). Information on the nutritional effect on milk IGF-I concentrations in the postpartum period is limited, but concentrations of IGF-I in mammary gland extracts were not influenced by level of feeding in prepubertal Holstein heifers (Weber *et al.*, 2000).

The mean concentrations of IGF-I in milk for the dietary treatments in the present study were within the range of concentrations previously reported in milk from Holstein cows in other studies (Daxenberger *et al.*, 1998; Taylor *et al.*, 2004).

Although milk IGF-I concentrations differed among cows, the concentrations were repeatable for individual cows. This consistency contributed to the high ICC of 0.78 for IGF-I in a.m. and 0.73 for p.m. milk samples, as well as the high Kendall's coefficients of concordance of 0.76 for IGF-I in a.m. and 0.71 in p.m. samples. The high association with respect to milk IGF-I concentrations in a.m. and p.m. milk samples on each day of the 7-day sampling may partly

**Table 1:** Effect of diet on ranked milk and plasma IGF-I concentrations and somatic cell count in weekly samples in Trial I (mean  $\pm$  se)

Group	Na	Milk IGF-I		Plasma IGF-I		SCC <sup>b</sup>	
		Concentrations (ng/mL)	Mean Rank	Concentrations (ng/mL)	Mean Rank	Concentrations (ng/mL)	Mean Rank
LL <sup>c</sup>	8	6.4 $\pm$ 2.1	14.69	44.7 $\pm$ 7.5	10.88	87.98 $\pm$ 51.28	16.75
HL <sup>d</sup>	8	9.3 $\pm$ 4.9	14.63	51.7 $\pm$ 5.9	13.50	240.71 $\pm$ 191.1	20.25
LH <sup>e</sup>	8	9.2 $\pm$ 3.9	16.69	64.2 $\pm$ 10.3	17.13	101.23 $\pm$ 59.82	15.88
HH <sup>f</sup>	8	11.0 $\pm$ 3.3	20.00	74.6 $\pm$ 7.9	24.50	49.27 $\pm$ 23.05	13.13
P <sup>g</sup>			0.629		0.023		0.502

<sup>a</sup>number of animals in each treatment group; <sup>b</sup>somatic cell count; <sup>c</sup>high DMI and high ME; <sup>d</sup>high DMI and low ME;

<sup>e</sup>low DMI and high; <sup>f</sup>low DMI and low ME; <sup>g</sup>Probability value

**Table 2:** Intraclass correlations at exact 95% confidence intervals for milk IGF-I concentrations in a.m. and p.m. milk samples during a 7-day consecutive sampling period in Trial I

Time	Intraclass correlation	P-value	R <sup>2</sup>	Exact 95% confidence intervals
a.m	0.78 $\pm$ 0.05	<0.001	0.809	0.68 to 0.87
p.m	0.73 $\pm$ 0.06	<0.001	0.764	0.62 to 0.83
Average, a.m. and p.m. For individual cows	0.77 $\pm$ 0.05	<0.001	0.796	0.67 to 0.86

**Table 3:** Association between a.m. and p.m. milk IGF-I concentrations in milk samples for 7 consecutive days in Trial I (mean  $\pm$  se)

Day	Milk IGF-I (ng/ mL)		Intercept $\pm$ se	Slope $\pm$ se	R <sup>2</sup>
	a.m.	p.m.			
1	11.7 $\pm$ 2.0	11.1 $\pm$ 2.1	0.548 ( $\pm$ 0.587)	1.003 ( $\pm$ 0.037)	0.961***
2	7.9 $\pm$ 2.0	6.6 $\pm$ 2.0	1.433 ( $\pm$ 0.529)	0.979 ( $\pm$ 0.041)	0.951***
3	4.9 $\pm$ 1.6	4.0 $\pm$ 1.6	0.343 ( $\pm$ 0.550)	1.130 ( $\pm$ 0.065)	0.911***
4	7.3 $\pm$ 2.1	6.6 $\pm$ 2.0	0.560 ( $\pm$ 0.580)	1.013 ( $\pm$ 0.044)	0.946***
5	7.7 $\pm$ 2.0	6.5 $\pm$ 2.0	1.703( $\pm$ 0.722)	0.923 ( $\pm$ 0.055)	0.904***
6	7.4 $\pm$ 2.3	6.2 $\pm$ 2.0	0.506 ( $\pm$ 0.463)	1.108 ( $\pm$ 0.036)	0.970***
7	9.1 $\pm$ 2.2	8.2 $\pm$ 2.4	1.629 ( $\pm$ 0.698)	0.905 ( $\pm$ 0.045)	0.931***
Overall	8.00 $\pm$ 1.9	7.0 $\pm$ 1.8	0.630 ( $\pm$ 0.314)	1.045 ( $\pm$ 0.026)	0.982***

\*\*\* P <0.001

reflect the effect of IGF-BPs prolonging the half-life of IGF-I in milk (Hadsell *et al.*, 1993). This suggests that IGF-I concentrations could be measured in milk samples recovered at any time of the day on a weekly basis. A similar suggestion was made for taking samples to measure plasma concentrations of IGF-I, as the overall intraclass correlation for plasma samples obtained from the cows enrolled in Trial I was 0.77 and the Kendall's coefficient of concordance was 0.84 (Obese *et al.*, 2008b).

The observed concentrations of IGF-I in milk were low compared with those in plasma samples from the same cows in both trials. Low milk IGF-I concentrations compared to plasma levels in the postpartum period has been reported for dairy cows (Skaar *et al.*, 1991; Vega *et al.*, 1991; Taylor *et al.*, 2004). The different patterns of milk and plasma concentrations of IGF-I as seen in this study suggest that, the regulation of circulating IGF-I differs to that in the mammary gland. Milk IGF-I levels may not simply reflect changes in



**Table 4:** Effect of diet on ranked milk and plasma IGF-I concentrations, and SCC in weekly samples in Trial 2 (mean  $\pm$  se)

Group BCS or Supp. level	N <sup>a</sup>	Milk IGF-I		Plasma IGF-I		SCC <sup>b</sup>	
		Concentra- tion	Mean Rank (ng/ mL)	Concentra- tion	Mean Rank (ng/ mL)	1000/ ml	Mean Rank
BCS effect							
4	9	8.7 ± 4.2	7.78	66.1 ± 7.6	13.33	31.61 ± 11.26	8.89
5	4	13.5 ± 3.5	12.50	51.5 ± 8.0	9.25	129.50 ±58.02	16.25
6	8	26.0 ± 8.2	13.88	53.7 ± 8.2	9.25	134.56 ±90.89	10.75
P-value			0.112		0.328		0.141
Supplementeffect							
1	13	15.7 ± 4.7	11.62	47.2 ± 4.2	7.69	107.35 ±57.81	11.73
6	8	16.9 ± 8.0	10.00	77.1 ± 6.4	16.38	60.37 ± 30.36	9.81
P-value			0.562		0.002		0.491

<sup>a</sup>number of animals in each treatment group.<sup>b</sup>somatic cell count.**Table 5:** Relationship of milk IGF-I concentration with plasma IGF-I concentration, and Ln SCC in weekly samples in Trials 1 and 2

Trial	Plasma IGF-I				Ln SCC <sup>a</sup>			
	intercept ( $\pm$ se)	slope ( $\pm$ se)	R <sup>2</sup>	P-value	intercept ( $\pm$ se)	slope ( $\pm$ se)	R <sup>2</sup>	P-value
1	-0.491( $\pm$ 4.559)	0.159( $\pm$ 0.072)	0.140	0.035	-5.340( $\pm$ 4.767)	8.650( $\pm$ 2.743)	0.249	0.004
2	18.505( $\pm$ 11.403)	-0.040( $\pm$ 0.183)	0.002	0.830	-9.502( $\pm$ 8.727)	16.716( $\pm$ 5.316)	0.342	0.005

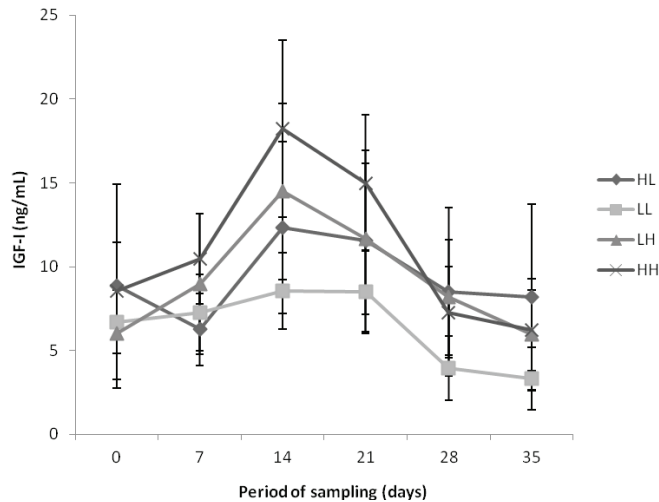
<sup>a</sup>natural logarithmic value of somatic cell count.

plasma IGF-I concentrations in the pasture-based management system used in Trials 1 and 2. This result is supported by other studies using more intensive management of dairy herds where increases in plasma concentrations of IGF-I occurred with concurrent decreases in milk IGF-I during early lactation in Holstein cows (Skaar *et al.*, 1991; Vega *et al.*, 1991; Taylor *et al.*, 2004). Although reasons for the weak association between plasma and milk IGF-I concentrations in this study were not apparent, they could be due to the influence of other factors in the local environment of the mammary gland including high SCC. It is remarkable that an individual cow may have consistent but contrastingly different concentrations of IGF-I in samples of milk and plasma obtained at consecutive milkings or on consecutive days.

Daxenberger *et al.*, (1998) found that SCC concentrations can influence milk concentrations of IGF-I in dairy cows. In the present study, high SCC were associated with

high milk IGF-I concentrations in some cows. For example, a cow with subclinical mastitis in Trial 1 had a high mean SCC (489,000/ mL) and a concurrently high mean milk IGF-I concentration of 40.7 ng/mL. Similarly, a cow in Trial 2 had a high mean SCC (274,000/ mL) and a high mean IGF-I concentration of 56.5 ng/mL in its milk. The release of Somatotropin during experimentally induced *E. coli* mastitis in periparturient cows has been reported (Shuster *et al.*, 1995; Burvenich *et al.*, 1999). The concentration of IGF-I in plasma did not change, whereas milk IGF-I increased significantly in milk from infected glands (Shuster and Kehrli, 1995). This increase was attributed partly to leakage of plasma IGF-I into the mammary gland and to de novo synthesis in mammary epithelial cells (Shuster *et al.*, 1995, Burvenich *et al.*, 2007). High IGF-I concentrations in milk may be associated with tissue repair or the healing process, but are equally likely to be due to leakage from blood as seen with associated





**Figure 1:** IGF-I concentrations (mean  $\pm$  se) in milk samples from 32 cows fed 4 diets (n=8 cows per dietary treatment; HH=High DMI and high ME; HL= high DMI and low ME; LH= low DMI and high ME ; LL= low DMI and low ME).

changes in blood constituents like sodium and albumin that are associated with clinical mastitis or sub-clinical mastitis (Burvenich, 1983). Based on the results from the two trials in the current study, the concentration of IGF-I in a composite milk sample from a herd is likely to be affected by the prevalence of high SCC associated with udder infections. This should be taken into account in studies in humans to measure associations between dietary and serum concentrations of IGF-I (Crowe et al., 2009).

## Conclusion

Concentrations of IGF-I in milk were generally low and variable between individual cows and were not affected by diet or BCS at calving. They were consistent for individual cows for milk samples obtained at consecutive milkings and on consecutive days. Associations between plasma and milk IGF-I concentrations were less than the associations between SCC and milk IGF-I concentrations. Milk IGF-I concentrations are not a sensitive measure for monitoring dietary changes and energy balance in pasture-fed Holstein cows. Plasma concentrations of IGF-I provided stronger relationships. The significance of IGF-I as a potential indicator of physiological status may be that IGF-I in milk reflects a

different aspect of partitioning of nutrients to that reflected by IGF-I in plasma. However, milk IGF-I may be a useful monitor of udder health during lactation.

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## PIG PRODUCTION SYSTEM, MARKETING CHAIN AND CYSTICERCOSIS AWARENESS IN THE GAMBIA AND SENEGAL

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### Abstract

Comme il y a très peu d'information disponible au sujet de la filière porcine et la cysticercose en Gambie et au Sénégal, cette enquête a été faite pour caractériser le système de production et le marché des porcs et d'étudier la connaissance de la cysticercose chez la population locale.

L'enquête a été faite dans 'Western region' et la Municipality de Kanifing en Gambie et dans les départements de Bignona, Kolda et Ziguinchor au Sénégal. Après un recensement des porcs dans ces régions un échantillon randomisé de 279 chefs de famille a été interviewé en utilisant un questionnaire au sujet de la gestion de l'élevage porcin, les ventes, la santé animale et la connaissance de la cysticercose. En plus des informations étaient rassemblées concernant la filière porcine, l'abattage et l'inspection des porcs.

La population porcine dans les 1.794 familles enquêtées était de 22.464. Quatre-vingt dix-neuf pourcent des 279 familles interviewées étaient des Chrétiens, et les 4 groupes ethniques dominants engagés dans l'élevage porcin étaient les Balanta, Jola, Mankagne et Manjago. Quatre-vingt dix pourcent des porcs dans ces familles appartenaient à la race locale et les autres étaient des croisés avec des races exotiques. Le marché du porc n'est pas organisé et l'infrastructure manque. Il existe un commerce de porcs vivants entre la Gambie, le Sénégal et la Guinée- Bissau.

La majorité des petits éleveurs de porc possèdent un petit nombre de porcs. Il s'agit d'un élevage de subsistance avec peu d'investissement dans la nourriture, le logement et la santé des animaux. Une minorité d'éleveurs est impliquée dans l'élevage commercial. Bien que la cysticercose porcine et humaine soient endémiques dans la région, seulement soixante quatre pourcent des interviewés connaissaient la cysticercose porcine. Personne ne savait comment la maladie est transmise du porc à l'homme. Sensibiliser la population sur la maladie peut minimiser sa transmission.

**Mots clé:** filière porcine, marché, cysticercose, Gambie, Sénégal

## SYSTEME DE PRODUCTION PORCINE, LA CHAÎNE DE COMMERCIALISATION ET DE SENSIBILISATION À LA CYSTICERCOSIS EN GAMBIE ET AU SÉNÉGAL

### Abstract

Publications on pig production and cysticercosis in The Gambia and Senegal are very scant. Hence, this survey was implemented to characterise the pig production systems and marketing chain, and to assess people's awareness of cysticercosis.

The survey sites were Western region and Kanifing Municipality of The Gambia; and the 'départements' of Bignona, Kolda and Ziguinchor in southern Senegal. Following a census of pigs in these sites, a random sample of 279 households were interviewed using questionnaire on pig management, sales, sanitation and knowledge on cysticercosis. Information on the pig market chain, pig slaughtering and inspection was also collected.

The pig population in 1,794 census households was 22,464. Ninety nine percent of 279 interviewed households are Christians, and the four predominant ethnic groups engaged in pig production are Balanta, Jola, Mankagne and Manjago. Ninety percent of the pigs in these households belong to the local breed, and the rest were crosses with exotic breeds. There is no organised pig market infrastructure. Trade in live pigs exist between The Gambia, Senegal and Guinea Bissau.

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The majority of small holder producers rear few pigs for subsistence investing low inputs in feeding, housing and health care. The minority produce pigs for commercial purposes with more inputs in feeding, housing and health care. Although porcine and human cysticercosis are endemic in the region, only sixty four percent of respondents were aware of porcine cysticercosis and none knew how it is transmitted between pigs and humans. Educating the population on the disease could help minimise its transmission.

**Key words:** pig production, marketing chain, cysticercosis, The Gambia, Senegal

## Introduction

Although not quite prominent as other livestock species, pig rearing plays an important socio economic role in households of non-Muslims in both The Gambia and Senegal. According to the FAOSTAT estimates for 2008, Gambia had 25,000 pigs and produced 650 tonnes of pig meat; and Senegal has 325,747 pigs and produced 10,606 tonnes of pig meat (FAO, 2009). Among 16 West African countries with pigs, Senegal had the sixth largest pig population and Gambia the least based on the 2008 estimates. In 2007, Senegal and The Gambia imported pork amounting to 129 tonnes at US\$366,000 and 10 tonnes at US\$35, 000; respectively (FAO, 2009).

Cysticercosis due to *Taenia solium* is one of the most important parasitic zoonotic diseases with high economic and medical importance. It is an endemic disease in Latin America, Africa and Asia where poor sanitation and intimate contacts between humans and pigs exist. Losses caused by condemnation of infected pork carcasses, high medical cost for diagnoses and treatment of human neurocysticercosis, and losses of functions associated with clinical neurocysticercosis are quite significant (Pawlowski *et al.*, 2005; Praet *et al.*, 2009; Schantz *et al.*, 1993). The estimated annual loss due to porcine cysticercosis in 10 West and Central African countries amounts to €25 million (Zoli *et al.*, 2003). The medical cost in monetary terms for managing 34,662 neurocysticercosis associated epilepsy cases in East Coast Province of South Africa in 2004 was estimated to be in the range of US\$ 18.6-34.2 million (Carabin *et al.*, 2006). Recent surveys have shown that porcine cysticercosis is endemic in Western Region of The Gambia whereas both porcine and human

cysticercosis is highly prevalent in some areas in Casamance, Senegal. Porcine cysticercosis seroprevalence of 4.8% in Western Region of The Gambia; 8.9% in Bignona, 13.2% in Kolda, and 6.4% in Ziguinchor in the Casamance area of Senegal were reported (Secka *et al.*, 2010b). A follow up study in Soutou village, Bignona 'département', Senegal found 11.9% of the population with circulating antigens of *Taenia solium* and 23.3% of the seropositives with neurocysticercosis (Secka *et al.*, 2011). A case control study in The Gambia found that 1.4% of epileptic people and 1.9% in the control group were seropositive to cysticercosis Ag-ELISA (Secka *et al.*, 2010a).

Pig production, marketing chains and general cysticercosis awareness in The Gambia and Senegal are not adequately investigated. Therefore, the objectives of this study were to characterise the pig production systems, marketing chain and determine people's awareness of porcine and human cysticercosis.

## Materials and Methods

### Study areas

Four sites were chosen to conduct this study. These are some parts of Kanifing Municipality and Western Region (name changed to West Coast Region in 2011) of The Gambia, and the 'département' of Bignona, Kolda and Ziguinchor in southern Senegal (Figure 1). The Gambia is divided into six administrative regions, one municipality and one capital city. Each region is in turn divided into several districts. The administrative divisions of Senegal consist of 'régions' at the top, which are subdivided into 'départements', then 'arrondissements', and then 'communes'



in the urban areas or 'communautés rurales' in the rural areas. The target study populations were domestic pigs and households at 'communes' and 'communautés rurales' engaged in pig production for subsistence or commercial purposes.

#### *Data collection and analyses*

A pig census at the four study sites was conducted in April-July 2007. The number of pigs in each household and type of confinement were recorded. A sample of 279 households with pigs in the four study areas was randomly selected and interviewed using a direct face-to-face questionnaire from October 2007 to January 2008. The questionnaire consisted of five parts: household information, pig management, sanitation and hygiene, cysticercosis knowledge and occurrence, and pig sales. Most of the questions were close-ended and few were open-ended. Household heads or representatives served as respondents to the author who administered the questionnaire in English, French, and local dialects: Wollof, Jolla and Manjago with the support of local translators. Information on the pig market chain, pig slaughtering and inspection were also gathered.

Collected data were collated in MS Access and analysed in MS Excel spread sheet and Intercooled Stata 8.0 utilizing logistic regression models and summary statistics. The pig density was calculated based on the area in square kilometre for four districts and one municipality in The Gambia and seven arrondissements in Senegal found in GIS vector files for African continent.

## **Results**

### *Household pig census*

Tables 1 and 2 show the pig populations and densities from selected areas in The Gambia and south Senegal. The total numbers of pigs in most of the households vary between 1 and 63, whilst few had between 110 and 197 pigs. The mean number of pigs per household for the total 1794 visited households with pigs in the four selected study areas was 12.3 with a standard

deviation of 13.1.

The distribution of very huge six classes of pig population by areas census is heterogeneous. The lowest class consist of areas with pig populations of 4 to 120 whilst the highest class contains areas with 983 to 2241 pigs.

### *Household questionnaire results*

Fifty five percent of the 279 interviewed household heads were farmers, and 45% were either retired or working in construction, transport, health services, education and trade sectors. The experiences of the households at rearing pigs were between 1 and 60 years, with a mean of 14.4 years and standard deviation of 10.8 years. The interviewed households and their pigs are categorised as in Table 3.

On cysticercosis awareness, 64.5% and 6.5% of the households knew porcine and human cysticercosis, respectively; but none knew its mode of transmission. Eleven percent of the households claimed to have seen cysticerci in slaughter pigs during the period 1987-2003. Persons reporting to have manifested epileptic seizures were present in 7% of the households. On sanitation, almost all households had toilets that were used regularly except for 5% that used the bush and 2% that used neighbour's toilet. Whereas 76% of the households practised seasonal confinement, pigs belonging to 49% of the households were reported to have access to human faeces.

### *Pig marketing chain*

There are no organised pig markets; However, data emanating from the questionnaire and pork market surveys showed the following scheme of trade on pigs and pork with the different actors involved in the process (Figure 2). Small holder pig producers that wish to improve their stock often buy good breeder pigs from other producers. One hundred twenty nine household heads (46%) out of 279 reported to have sold 635 pigs in 2007: 24 pigs (4%) were bought by butchers, 214 (34%) bought by consumers, 29 (4%) bought by pig

**Table 1:** Pig populations and densities from selected districts and municipality in The Gambia

Region	District	Total communities/ villages	Total households	Total pig owners	Total pigs	SC <sup>a</sup>	PC <sup>b</sup>	Pig Density per km <sup>2</sup>
Kanifing Municipality	None	7	103	112	1702	1002	700	25
Western Region	Kombo Central	5	54	65	995	135	860	5
Western Region	Kombo East	5	51	53	708	213	495	3
Western Region	Kombo North	10	85	96	1117	498	619	6
Western Region	Kombo South	17	268	279	4680	1669	3011	14
<b>Total</b>		<b>44</b>	<b>561</b>	<b>605</b>	<b>9202</b>	<b>3517</b>	<b>5685</b>	

**Table 2:** Pig populations and densities of three départements and seven arrondissements in south Senegal

Département	Arrondissement	Total villages/Commune	Total households	Total pig owners	Total pigs	SC <sup>a</sup>	PC <sup>b</sup>	Pig Density per km <sup>2</sup>
Bignona	Sindian	6	63	64	822	813	9	0.6
Bignona	Tendouck	5	69	69	585	565	20	0.7
Bignona	Tenghory	13	287	326	2870	2742	128	3
Ziguinchor	Niassia	12	289	295	1961	1952	9	5
Ziguinchor	Niaguis	9	450	495	4767	4220	547	6
Kolda	Dabo	2	3	3	17	0	17	0.007
Kolda	Dioulacolon	6	72	116	2240	2117	123	2
<b>Total</b>		<b>53</b>	<b>1233</b>	<b>1368</b>	<b>13262</b>	<b>12409</b>	<b>853</b>	

**Note:** SCa means pigs confined only during the rainy season (June–October), and PCb means pigs that are permanently confined

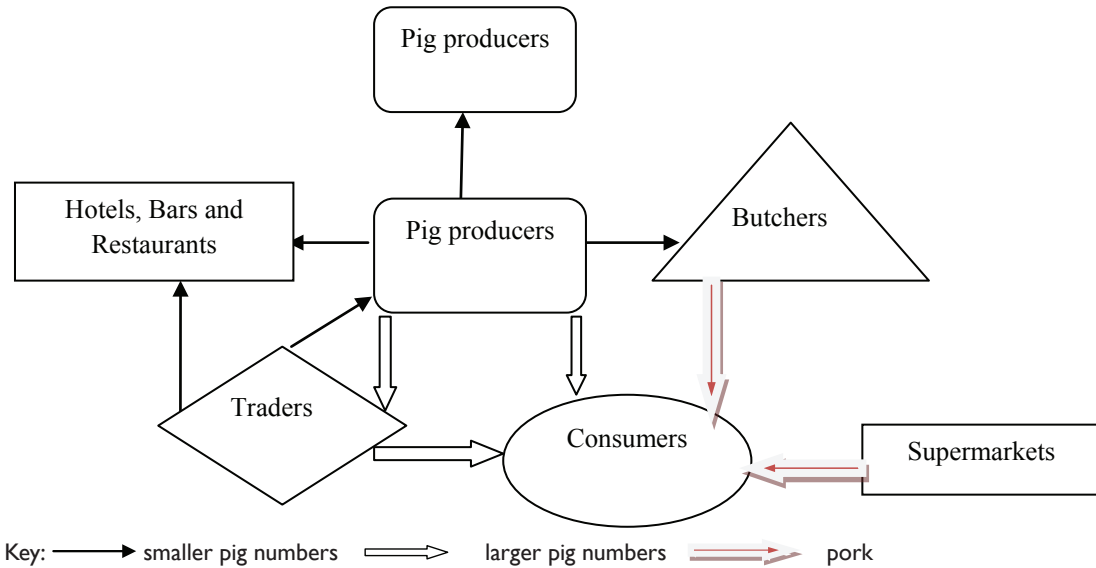
producers, and 368 (58%) bought by traders. The price of a live pig in both The Gambia and Senegal depends on its size, supply, and demand but generally vary from US\$40 for a pig with an average weight of about 20 kg to US\$80 for 40 kg pig and US\$150 for 80 kg pig. Many grower pigs are sold at 10–12 months old, but most producers prefer to sell older and bigger finishers at an age range of 18–30 months.

Market visits also showed that hotels, supermarkets, bar and restaurant operators are also components of the pig market chain. Supermarkets in The Gambia and Senegal sell imported pork, whilst bar and restaurant operators sell grilled pork of locally produced pigs. Butchery stalls also sell pork of local origin. The prices of pork per kilo in The

Gambia are 4 to 12 US\$ at Supermarkets, 4 US\$ at bars and restaurants, and 2.4 US\$ at butchery stalls (exchange rate: 1US\$=Dalasi 25.00). The origin of imported pork to The Gambia is mainly from Europe, USA and China, but the imported quantity decreased from 67 tonnes in 2007 to 3 tonnes in 2009 (GBOS). In south Senegal, the prices of pork per kilo are 6 to 10 US\$ at Supermarkets, 4.4 US\$ at bars and restaurants, and 2.7 US\$ at butchery stalls (exchange rate: 1US\$=450.00 F CFA).

#### *Pig housing, management and nutrition*

Pigsties vary from simple structures made of wooden lumbers with or without roof of thatched grasses or corrugated iron sheets for poor households to more

**Figure 2:** Marketing chain of live pigs and pork in the four study areas**Table 3:** Characteristics of interviewed households and their pigs in the four study sites

Category/study area	Western region	Bignona	Kolda	Ziguincho	Totals
Number of households	69	74	51	85	279
Religion:					
Christianity	100%	100%	100%	99%	
Islam	0%	0%	0%	1%	
Ethnic group:					
Balanta	7%	3%	41%	5%	
Jola	12%	88%	8%	62%	
Mankagne	1%	4%	37%	19%	
Manjago	70%	5%	4%	11%	
Others	10%	0%	10%	3%	
Pigs: Local breed	809	667	1240	778	3494
Local-Landrace crossbreds	222	40	0	130	392
Local-Large white crossbreds	0	0	0	0	0
Landrace breed	5	0	0	0	5
Male pigs ≤ 3 months	112	37	156	95	400
Male pigs 4-12 months	153	131	240	186	710
Male pigs > 12 months	66	72	115	39	292
Female pigs ≤ 3 months	204	59	173	134	570
Female pigs 4-12 months	271	178	289	259	997
Female pigs > 12 months	230	230	267	195	922

solid housing structures of cement blocks with an overhead corrugated iron roof for economically stronger households. The pigsties floors range from earthen to concrete floors that can be more easily cleaned. Two forms of pig confinement were

practised at the interviewed households. Obtained data showed that 15,926 (71%) pigs were confined only during the rainy season (seasonal confinement) and left on free-range system during the dry season; and 6,538 pigs (29%) were permanently confined. Pigs

under permanent confinement were fed at their pigsties, whilst the seasonally confined pigs scavenge for feed during the free-range periods but fed when confined. The locally available pig feeds are cereal by-products (millet/sorghum/rice bran), household food remnants, groundnut/sesame cake, cashew fruit residues, palm kernels, grasses and vegetable rejects. In most households, pig producers keep breeders, growers and finishers in the same house unit. All the pig categories in the same house unit receive the same feeds, and often the most aggressive ones in the group sharing feeding trough consume the greatest amount of feed as they push out the weaker ones. Pig producers are not specialised at either breeding or fattening, but combine them instead. All interviewed households except two in Western region consume pork in roasted or cooked form.

### Discussion

Household religion is a strong determining factor if pigs are raised or not. Results show that 99% of the households engaged in pig production are non-Muslims, a scenario very similar to many secular Islamic countries in the world. The reason why most Muslims are not engaged in pig production is the prohibition of pork consumption by the Islamic faith.

The indigenous 'local' pig breed is inadequately characterised in the literature. The phenotype of local pigs consist of long snout, short small erect ears, small to medium body size, straight back and tail, and white to black hairs. Local pig breeds in Senegal have been reported to have the following production indices: 12.78 months old at first farrowing, 1.81 farrowing per sow per year, an average litter size of 7.53, piglet mortality of 22.7%, and 10.53 weaned piglets per sow per year (Missohou *et al.*, 2001). This local breed constitutes 90% of the pigs present in the interviewed households. The remaining 10% are crossbreds of local pigs with Landrace, or Large White pig breeds, and few purebred Landrace.

Housing conditions of pigs in most

households are generally below the minimum standards for maintenance of healthy and productive animals. Pigs are not housed by class, whilst most pigsties have inadequate feeding/watering troughs, and are irregularly cleaned which results in an accumulation of faecal droppings. Household food leftovers/remnants are the major feed for pigs during confinement. Food remnants are collected from different households, fed to pigs directly or kept in containers as feed stock until needed. These food remnants are mixed with water when fed to pigs.

Pig marketing in the study areas is not well organised as there is no market infrastructure for pigs. This could be attributed to the lower demand for pork as compared with meat from other livestock species such as ruminants and poultry. Trade in pigs do occurs between The Gambia, Senegal and Guinea Bissau which implies that cysticercosis pigs could be traded within these three countries. Pork importation into The Gambia has shown a decreasing trend from 2007 to 2009 according to the data from The Gambia Bureau of Statistics (GBOS, 2007). This downward trend could be due to increasing local production of pork (FAO, 2009) and/or decreased demand for imported pork. There is an increasing trend of pig population in both The Gambia and Senegal (FAO, 2009). The higher retail price for imported compared with locally produced pork could also be a contributing factor for the downward trend of pork imports.

Although there is no pig abattoir in the study areas, both abattoirs in Dakar and Thiés regions of Senegal accommodate the slaughtering, scalding, carcass opening, washing, and inspection of pig carcasses. However, pigs destined to slaughter from the study areas are not brought to these slaughterhouses because of the long distance. Veterinary inspection of pork carcasses also takes place in Ziguinchor and the Greater Banjul Area, but since pig slaughter is not centralised, not all carcasses are inspected by the veterinary services.

It was not surprising to find that most household heads or representatives

interviewed were aware of porcine cysticercosis. However, very low proportions were aware of human cysticercosis, and none knew how the disease is acquired or transmitted. This finding agrees with the cysticercosis awareness level in many other endemic areas e.g. the state of Morelos, Mexico (Sarti *et al.*, 1997). As households slaughter their pigs themselves, they become familiar with the lesions of endemic diseases that affect pig carcasses. This is further buttressed by the fact that porcine cysticercosis is endemic and widespread in this study area (Secka *et al.*, 2010b). Whilst human cysticercosis seroprevalence is focalised and sporadic in The Gambia (Secka *et al.*, 2010a), hyperendemic foci of neurocysticercosis exist in the Casamance (Secka *et al.*, 2011).

There are many important risk factors for cysticercosis described in other African countries such as Southern highlands of Tanzania, North West Cameroun and Zambia (Boa *et al.*, 2006; Shey-Njila *et al.*, 2003; Sikasunge *et al.*, 2007) that are prevalent in these study areas. Absence of veterinary inspection of most pork carcasses, unawareness of cysticercosis mode of transmission, free-range management system of pigs, and consumption of roasted pork are potential risk factors for transmission of cysticercosis in the study areas. Pigs under seasonal confinement constitute 71% of the population, thus may ingest taeniid eggs as they scavenge for feed during the free-range periods. Five percent of the households that reported to be defecating in the bush might contaminate the environment with taeniid eggs if they are carriers of adult *Taenia solium*.

In conclusion, pig production is largely for subsistence utilizing well adopted local breeds by smallholders investing very low inputs in feeding, housing and health care. Few producers are engaged in commercial production utilizing crossbreds or pure exotic breeds at a semi-intensive production scale with more inputs in feeding, housing and health care. The absence of organized pig markets hampers the marketing of pigs. The practice of free-range pig management system, presence of households without

toilets and people that defecate in the bush, and pigs' access to human faeces in the study areas could perpetuate the transmission of cysticercosis. This situation is more aggravated by the fact that the mode of cysticercosis transmission in humans and pigs is not known. Educating the population on the disease could help minimise its transmission.

## Impact

Cysticercosis is a disease caused by the larvae of *Taenia solium* in pigs and humans. Infection results from the ingestion of the eggs of the parasite along with food or water. The larvae hatch inside the small intestine, cross the gut wall and are transported to various tissues and organs of the body via the circulatory system. Large numbers of encysted larvae in the humans could cause epileptic seizures, blindness and muscular pain. Infected pig carcasses are condemned during meat inspection. Studies have shown that the disease is endemic in the study areas affecting both pigs and humans. This study has shown that although many households are aware of the disease in pigs, they do not know its mode of transmission. The current pig production system which is dominated by free range system, pig's access to human faeces, uncontrolled pig slaughter and inspection, and cross border trade in pigs could perpetuate transmission of the disease. Educating the population on the disease could help minimise its transmission.

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questions.

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**SHORT COMMUNICATION****PREVALENCE OF FOREIGN BODY RUMEN IMPACTION IN CATTLE IN IBADAN, SOUTHWEST NIGERIA**

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The indigenous ruminant livestock industry in Nigeria represents a very important national resource, contributing immensely to national health and wealth through supply of protein and industrial raw materials.

The vast majority of livestock in Nigeria livestock are mostly kept by pastoralists from the sub-Saharan geographic zone of the north under traditional systems of management, with the major fodder resources comprising grasses, browse, crop residues and domestic waste (Adeloye, 1998; VanRaay and De Leeuw, 1974). The availability of these resources varies from season to season being scarce mostly during the long dry seasons (Alaku and Igene, 1983).

Malnutrition has long been identified by various workers as being the major constraint to livestock productivity in Nigeria (Gbodi *et al.*, 1990; Lamorde and Franti, 1975; Synge, 1980). In order to meet the ever increasing demand for meat and milk as major sources of animal protein, more effort has focused on improvement of productivity through the provision of good quality feed all the year round. This has caused desperate and indiscriminate movement of herds by pastoralists into non-conventional areas in search of food. In many cases, animals are left grazing at the outskirts of cities where they get exposed to heaps of refuse dump containing non-biodegradable materials.

Reports of ingestion of foreign materials leading to rumen impaction in cattle, sheep and goats are well documented in Northern Nigeria (Abdulahi *et al.*, 1984; Garba *et al.*, 1994; Remi –Adewunmi *et al.*, 2004). However, the reports have not received adequate attention because the

effect of impaction many times go unnoticed or wrongly diagnosed as it has similarities with other conditions. Many cases have been discovered at necropsy or slaughter (Lamorde and Franti, 1975; Remi –Adewunmi *et al.*, 2004). Although foreign body rumen impaction is seldom presents as an acute disease, cases of fatalities have been reported (Akinrinmade *et al.*, 1988; Elsa, *et al.*, 1995, Otesile and Akpokodje, 1991).

Prior to this investigation, foreign-body rumen impaction was widely believed to be uncommon in the southwest subtropic zone of Nigeria because of its relatively small livestock population and richer fodder resources. To the best of our knowledge, the prevalence of the condition has not been documented in this geographical zone despite the apparent massive movement of the pastoralists to the south. This is what this investigation sought to achieve.

The study was conducted at the central abattoir of Ibadan. It involved ante-mortem and post-mortem examinations of cattle slaughtered during the months of March, April and May of the year. Pre-slaughter physical examinations to determine the health status, sex, age, and breed were performed. Animals were also closely observed for signs of disease or pathological conditions through their general disposition, gait, and condition of hair coat, feces and visible mucous membrane. Those found to be unhealthy were held as suspects and noted for detailed post-mortem examination. Age estimation by teeth examination was done according to the method described by (Sisson and Grossman, 1975). With easily distinguishable color markings, breed and sex, a detailed systematic

examination of the fore stomach was carried out immediately after flaying and evisceration. The dry weight and composition of impacted materials were noted in affected animals.

Records of all findings were collated and subjected to statistical analyses. Values obtained were expressed as means and standard deviation ( $X \pm S.D$ ) *P* value less than or equal to 0.05 were considered significant using student *t*- test.

Out of 3031 animals examined, 327 (10.77%) were found to have foreign-body rumen impaction (Table I). A significantly high percentage (73.5%) of the cattle slaughtered were in poor body condition, moderately to severely emaciated with prominent ribs and the carcasses were fairly pale. The materials recovered from animals with impaction varied in weight (0.95 – 8.08kg) and composition with non-biodegradable polythene materials being the most prominent. The prevalence of rumen impaction was significantly higher ( $P < 0.05$ ) in females than males (Table 2). Majority (61.40%) of the animals slaughtered were less than 3 years of age and the prevalence of rumen impaction was significantly higher ( $P < 0.05$ ) in older (3 years above) than younger (3 years below) animals (Table 2).

The prevalence of 10.77% recorded in this study is slightly higher than previous reports from the north (Abdulahi *et al.*, 1984; Remi –Adewunmi *et al.*, 2004). In view of the

fact that a large proportion of the animals examined come from the north, this finding may suggest a worsening situation in the fodder resources.

The significantly higher prevalence in older animals agrees with the findings of previous workers (Abdulahi *et al.*, 1984; Garba *et al.*, 1994, Remi –Adewunmi *et al.*, 2004) and may be attributed to the slow, progressive mechanism of formation of the condition. Exposure of animals over a long period to poor husbandry practices with grossly inadequate feed intake, lack of prophylactic medication and mineral supplementation facilitate the development of foreign-body impaction in old animals.

Our observation of higher prevalence in females is also in agreement with previous reports from the north (Abdulahi *et al.*, 1984; Garba *et al.*, 1994; Remi –Adewunmi *et al.*, 2004). The slaughter of females early in their reproductive life may be due to poor reproductive performance consequent upon nutritional inadequacy rather than economic reasons. This portends serious clinical and economic implications to the growth of the indigenous livestock industry in Nigeria.

The result of this study have shown that foreign-body rumen impaction due to declining fodder resources has the potential to cause further decline in livestock productivity if not given prompt attention. It has also brought to consciousness, the environmental hazards posed to animals

**Table I:** Breed Distribution and Prevalence of Rumen Impaction in Cattle

Breed	Number of Cattle Examined	Number of Cattle with Rumen Impaction
Kuri	244(8.00)	15(0.49)
Red Bororo	772(25.50)	213(17.03)
White Fulani	816(26.90)	26(0.85)
Sokoto Gudali	971(32.20)	54(1.78)
Azawak	91(3.00)	8(0.36)
Keteku	139(4.60)	11(0.36)
<b>TOTAL</b>	<b>3031</b>	<b>327(10.77)</b>

\* Percentages in parentheses.

**Table 2:** Sex and Age Distribution of Rumen Impaction in Cattle.

Sex	Number of Cattle Examined	Sex Prevalence	Age	Number Of Cattle Examined	Age Prevalence
Male	1296(42.76)	101(3.32)	Below 2years	682(22.50)	29(0.95)
Female	1735(57.24)	226(7.45)	2-3 years	1178(38.90)	96(3.16)
			3-4 years	606(20.00)	98(3.23)
			Above 4 years	565(18.60)	104(3.43)
<b>Total</b>	<b>3031</b>	<b>327(10.77)</b>		<b>3031</b>	<b>327(10.77)</b>

\* Percentages in parentheses.

by improper waste disposal and the urgent need for proper legislation. Further studies to evaluate the specific role of nutritional/mineral inadequacies are recommended.

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## SHORT COMMUNICATION

## A TYPICAL ACTINOBACILLOSIS IN AN ADULT FRIESIAN COW

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Keywords: “Hippo-head” presentation, Sodium iodide, actinobacillosis

Actinobacillosis due to *Actinobacillus lignieresii*, has been reported in domestic animals including cattle, sheep, goats, buffalo, horses and dogs (Carmalt *et al.*, 1999; Kennerman *et al.*, 2006; Muhammad *et al.*, 2006; Brown *et al.*, 2007). The causative agent is a gram-negative aerobic rod and a normal inhabitant of the gastrointestinal tract of ruminants (Radostits *et al.*, 2007; Smith, 2009). The most frequent clinical presentation is granulomatous or pyogranulomatous lesion of the tongue or subcutaneous tissues in the head and neck region. A typical manifestation of the disease have been reported affecting other body tissues / organs usually associated from lacerations, dehorning, nose-rings, intravenous injections and lymphadenitis (Aslani, *et al.*, 1995; Holzhauer and Roumen, 2002). Outbreaks of the disease have also been reported (Campbell *et al.*, 1975; Nakazawa and Azuma 1977).

This paper outlines an atypical case of actinobacillosis of soft tissue swelling of the head in a cow without any exudation from the lesion, which is a challenge to confirming the etiological agent and treatment.

An adult Friesian cow had been referred to the Large Animal Clinic, University of Nairobi, Kenya with a history of a longstanding bilateral swelling of the face over a period of two months. The patient had been treated by a private veterinarian with unknown dosages of antibiotics without any success.

On clinical examination the patient had a bilateral swelling of the face (Figure 1) resembling a hippopotamus. The swelling had extended from the ramus of the mandible to the muzzle and upper jaw region. On

palpation, the swelling was firm and non-painful. The patient also presented with hypersalivation, increased nasal discharge and slightly swollen sub-mandibular lymph nodes. All vital parameters were within the normal ranges and she had a pregnancy aged 4 months.

Treatment regime involved administration of 300 milliliters of 10% Sodium iodide through the jugular vein three weeks apart, followed with seven days injection of 4g procaine penicillin and dihydrostreptomycin intramuscularly.

The lesion regressed progressively for up to a period of 3 months. Signs of iodism appeared during the course of treatment (Figure 2) and regressed once the treatment was discontinued.

Actinobacillosis in cattle is usually represented by the classical “wooden tongue” syndrome. However, other forms have also been described including the unusual form presenting with wart-like lesions on the dorsum of the tongue (Taghipour Bazargani, *et al.*, 2010). In this case, the soft tissue swelling presenting as a “hippo-head” was firm, non-painful with no discharge. A similar case had been reported previously (Milne *et al.*, 2001). However, in the case reported by Milne *et al.*, 2001 the facial swelling was accompanied by granulomatous swellings with mucoid discharge unlike in the present case where the swelling was smooth and without discharge. The prolonged nature of the case could not allow for an aspirate to be sampled as it has been shown that it’s difficult to grow the causal agent after a prolonged antibiotic treatment (Fubini and Campbell, 1983). However, a biopsy could be taken but

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**Figure 1:** Bilateral head swelling “hippo-head presentation” in a cow that had Actinobacillosis as was presented to the Large Animal Clinic, University of Nairobi.



**Figure 2:** Signs of iodism in a cow treated with 10% Sodium iodide for Actinobacillosis at the Large Animal Clinic, University of Nairobi.

was not attempted due to the severity of the lesion and the requirement for deep skin incisions when taking biopsies (Milne *et al.*, 2001).

Many treatments have been tried for actinobacillosis including surgical debulking followed by postoperative therapy with intramuscular penicillin/streptomycin and intravenous sodium iodide (Arora *et al.*, 1980), streptomycin/or dihydrostreptomycin (Prescott and Baggot, 1993) and other antimicrobials including tetracycline's and sulphonamides (Prescott and Baggot, 1993,

Radostits *et al.*, 2000; Milne *et al.*, 2001). In the present case sodium iodide was the treatment of choice due to the failure of earlier antibiotics therapy and resulted in success. However, it is important to look out for signs of iodism which signifies an end point to treatment with sodium iodide.

It is therefore important for veterinarians who come across such cases of swollen face to recognize this form of actinobacillosis which is different from the classical “wooden tongue” and to institute treatment with Sodium iodide before



condemning the animals.

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## CASE STUDY REPORT

### THE ROLE OF VETERINARY NURSES IN POST OPERATIVE ANIMAL CARE MANAGEMENT IN NIGERIA

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Surgery may be curative or palliative in nature and is a significant and stressful event (Lemone *et al.*, 2004). It may be categorized according to relative risk - minor or major (Price 2004). Whether minor or major, postoperative management of a surgery is very important in the care of patients either human or animal. The trend of postoperative management determines the success and/or failure of the surgery and the surgeon.

Generally, in both humans and animals, postoperative care begins at the time the surgeon completes the surgery. From the surgical suite, the client may be moved to the post anesthesia care unit (PACU) or intensive care unit (ICU) (Daniels *et al.*, 2007). Within the PACU setting, the patients receive specialized care until cardiovascular status, respiratory status, consciousness and activity levels are adequate (Barone *et al.*, 2004). Airway, breathing and circulation are highly important during this most vulnerable period. The vigilant postoperative nurse protects the client during such time when the client is unable to perform vital functions independently. Nursing assessment centers on internal and external measures of stability such as cardio-respiratory function and thermoregulation.

The patient who spends many hours in surgery undergoing major and complex surgical procedures will be more likely to need the specialized care. The postoperative period can be intense, both physically and psychologically, but the nurse is in a unique position to ensure the highest quality of care and the most desirable outcome. The need to provide efficient and qualitative postoperative management is imperative to predict the outcome of the surgery.

Nursing care in Veterinary practice is non existent in Nigeria (Okanlawon and Emikpe 2011). In developed countries where the training and the role of veterinary nurses is more appreciated, Veterinary nurses work alongside veterinary surgeons in order to provide a high standard of care for animals. They normally work within a veterinary practice or veterinary hospital and are involved in a wide range of care and treatment, including: providing skilled supportive care for sick animals, undertaking minor surgery, monitoring during anaesthesia, medical treatments and diagnostic tests under veterinary doctors' supervision (Okanlawon and Emikpe 2011). Veterinary nurses also play an important role in the education of owners on good standards of animal care (Hendrix *et al.*, 2005).

Veterinary nursing which is an integral aspect of critical care management is not an eight hours job as they cover 'on call' as well as their normal day and night shifts including weekends. Veterinary nurses covering nights and weekends are expected to take care of the hospitalized animals and assist the duty veterinary surgeon with any emergency consultations. They attend to range of cases such as caesareans, gastric torsions, status epilepticus, road traffic accidents and other urgent conditions to save the animals' life. They work as a team with veterinary surgeons to get animals stabilised whilst running tests, taking x-rays or ultrasounds and preparing theatre for surgery.

Veterinary nurses with appropriate training are permitted to carry out minor surgery not involving entry into a body cavity e.g. stitch ups, wart removals and wound management. They are involved in pre and

postoperative activities including intubation, sedation, induction and maintenance of anaesthesia with halothane and isoflurane, pulse oximeter, respiratory and cardiac monitors. They carry out postoperative assessment. Based on the type of surgery done on the animals, veterinary nurses do a head-to-toe visual assessment postoperatively to observe the surgical site and check the vital signs, i.e. heart rate, respiratory rate and quality, pulse oximetry and body temperature. They pay special attention to respiratory and circulatory stability. They deal with pain management and alleviate the stresses and symptoms of critically ill animals. Pain and stress, are said to contribute to sleeplessness, impair recovery and sensitize the central nervous system, causing "wind-up" and this further amplifies pain and stress and increases cardiac demand, vasoconstriction, blood viscosity, platelet aggregation and catabolism (Lamont *et al.*, 2000). According to one of the leading researchers in the ethics of human critical care, "alleviating the stresses and symptoms of critically ill patients will enhance the quality of their ICU (Intensive care unit) stay, which itself achieves an important beneficial and ethical outcome, an outcome that should be a priority of every intensivist" (Silverman 2002).

In order to assess the need to reduce complications after surgery, this study reviews some evidence-based cases from veterinary students surgical exercises at a university veterinary teaching hospital:

Gastrotomy was performed on a dog. Antibiotics (*Penicillin*, *Streptomycin*, and *Oxytetracycline spray*), analgesics (*Pentazocine*, *Diclophenac*), dextrose saline, chains and a muzzle were provided on request by the students. After the surgery, the dog was returned to the kennel after being placed on antibiotic therapy for three days (*Penicillin-Streptomycin*). Analgesics and *Pentozacine* were given for three days and *Oxytetracycline spray* was applied at the surgical site till healing occurred. A basket collar was put in place to prevent the dog from licking the surgical site.

A call roster was made to ensure

that a student was on duty for each day to observe the animal. Such student recorded the clinical parameters for each day. The next day after the surgery, it was noticed that the collar was torn and the *Oxytetracycline spray* at the surgical site was licked with a resultant breakdown of some of the sutures. This open wound was subsequently treated while the animal was given dextrose saline and a new basket collar was put in place.

A dog had wound dehiscence after repair of femoral fracture with an internal fixation with intramedullary pin. After the surgery, the dog was placed on antibiotics and analgesic therapy for three days. The surgical site was sprayed with *Oxytetracycline*. There was wound dehiscence probably due to licking and irritation from contact with the floor. The site was cleaned, flushed with normal saline and antibiotics and sutured back. Again, wound dehiscence was observed on the dog.

Three goats died of *Peste des petits ruminants virus (PPRV)* infection after rumenotomy which could have been triggered by the stress due to the surgery they were subjected to.

Finally, the pre-surgical state of the animals used for the surgical exercise could not be ascertained apart from the physical examination, packed cell volume and fecal examination. This further stresses the need for proper examination and quarantining of animals to ensure their fitness for being used for a surgical procedure.

#### *The role of nurses in ameliorating postoperative complications*

Client's recovery following a surgery to full consciousness and a satisfactory health status without complications depend largely on the continuity of care rendered by the nurses in charge (Barone *et al.*, 2004). Factors that could contribute to postoperative complications include pre-existing cardiac or respiratory pathology and extent of surgery or difficult surgical course (Aragon *et al.*, 2003). Inadequate postoperative care could lead to any of the following complications and higher mortality rate:

Respiratory system – respiratory depression, airway obstruction, airway spasms, aspiration and pneumonia.

Cardiovascular system – thrombi/emboli, hypervolaemia (fluid volume excess), hypovolaemia (fluid volume deficit), shock, complex dysrhythmias, tachycardia, bradycardia.

Thermoregulation – acute temperature alterations.

Neurological – altered mental status, pain, peripheral nerve trauma.

Gastrointestinal system – nausea, vomiting, constipation, ascites, paralytic ileus.

Genitourinary system – urinary retention, urinary tract infection.

Integumentary system – infection, wound dehiscence, evisceration, decubiti.

Musculoskeletal system – decreased range of motion, activity intolerance.  
(Adapted from Dixon, 2002).

Studies have shown that immediate and adequate specialized and individualised postoperative nursing care had reduced postoperative mortality in the recent past (Barone *et al.*, 2004; Daniels *et al.*, 2007). Research indicates that in the care of animals, delegating care to non-veterinarians and non-qualified or untrained veterinary nurses with questionable or unfamiliar credentials adds risk and exposure for veterinarians already managing a busy practice (Tracy and Lindquist 2003). This is revealed in case 1 as roster was made to ensure proper monitoring in order prevent potential risks hence it is important to give adequate recognition to the training and services of qualified and certified veterinary nurses in veterinary practice (Okanlawon and Emikpe 2011) especially in the postoperative management of animals.

As observed from the highlighted cases, wound dehiscence and suture

breakdown were the major complications while three goats died of Peste des petits ruminants, an endemic erosive stomatitis, pneumo-enteritis condition in sub-Saharan goats (Emikpe and Akpavie, 2011) which could be triggered by the surgical stress postoperatively.

All these suggest that wound dehiscence, suture breakdown could be managed and the mortality due to PPR could have been prevented if the animals could have been well ascertained preoperatively. As stated by Cleland (1975), 'one can do wonders with medicine and surgery, but it will be the pre-operative, postoperative and general nursing care which will determine the degree of patient comfort, duration of hospital stay and the level of health achieved'.

It can be concluded therefore, that animals too, like human beings, need efficient nursing care hence the need for the development of a veterinary nursing curriculum as integral to intensive or critical care management in Nigeria, since non-veterinarians and para-veterinary personnel in the teaching hospitals can not take the place of trained veterinary nurses in animal health care (Okanlawon and Emikpe 2011).

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# **BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA**

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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  
December 2011

### Preamble

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.

### Aims and scope

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 livestock industry in Africa and to better utilization of animal genetic resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

- Veterinary microbiology, epidemiology
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Authors are invited to submit electronically their manuscripts via attachment only at [bahpa@au-ibar.org](mailto:bahpa@au-ibar.org) (The use of an email submission speeds up the decision-making process, enables immediate distribution and allows authors to track the status of their own manuscripts) to the editor 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).

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2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion and References.
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.
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#### Examples of References

- Journal Articles: Ouyang D, Bartholic J, Selegan 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 J C, 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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