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THE NASAL BACTERIAL FLORA CHANGES IN EXPERIMENTAL PESTE DES PETITS RUMINANTS VIRUS AND ITS CO-INFECTION WITH MANNHEIMIA HEMOLYTICA IN GOATS.

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CHANGEMENTS DANS LA FLORE BACTÉRIENNE NASALE DANS LES EXPÉRIENCES VIROLOGIQUES DE LA PESTE DES PETITS RUMINANTS ET CO-INFECTION AVEC MANNHEIMIA HAEMOLYTICA CHEZ LES CAPRINS.

Résumé

Les études sur les changements dans la flore bactérienne nasale associée à la pneumonie virale commune et à la pneumonie bactérienne compliquée chez les caprins subsahariens et ses implications dans les programmes de traitement sont rares dans la littérature scientifique. Cette enquête faisait partie d'une étude plus vaste qui impliquait cinquante chèvres naines d'Afrique de l'Ouest (WAD)apparemment en bonne santé, âgées de six mois, qui ont été divisés en groupes A, B et C de 15 chèvres chacun, tandis que 5 chèvres ont servi de témoin. Les chèvres du Groupe A ont été infectées avec 1 ml de culture pure (1 X 10^9 UFC) de *Mannheimia haemolytica* (MH) A2, le groupe B avec 1 ml de culture pure $10^{6.5}$ TCID50 de virus de peste des petit ruminants (PPR) cultivés dans les cellules rénales des lignées de nouveau-né hamster et pour le groupe C , avec 1 ml de PPRV et avec 1 ml de A2 MH une semaine plus tard. Les écouvillons nasaux ont été prélevés sur les chèvres chaque semaine tandis que l'identification et le comptage de bactéries dans les isolats ont été effectuées en utilisant des méthodes standard. Le Test-t d'étude a été utilisé pour tester les différences significatives. Dans le groupe B, les colonies de Mannheimia hémolytique ont considérablement augmenté, a partir du pi de la troisième semaine tandis que celle du groupe C, a augmenté de manière significative a partir du pi de la deuxième semaine ($P < 0,05$). La prédominance de MH par rapport aux autres bactéries a confirmé en outre sa capacité à surmonter la réaction de l'hôte, la compétition de manière favorable et réaliser son accrochage. Celle-ci semble être la première étude qui décrit les raisons possibles de la fréquence d'association sur le terrain des infections PPRV et MH chez la chèvre et le calendrier de traitement possible aux antibiotiques de la pneumonie virale et bactérienne compliquée chez les caprins

Mots clés: Bactéries Nasales, PPR, co-infection, *Mannheimia Hemolytica*

Abstract

The study into the nasal bacterial flora changes associated with common viral and bacterial complicated viral pneumonia in subsaharan goats and its implications in the treatment plans is scanty in literatures. This investigation was part of a larger study that involved fifty apparently healthy West Africa Dwarf goats (WAD) of six months of age that were divided into groups A, B and C of 15 goats each while 5 goats served as control. Group A goats were infected with 1ml of pure culture (1 X 10^9 CFU) of *Mannheimia haemolytica* (MH) A2, group B with 1ml of pure cultured $10^{6.5}$ TCID50 Peste des petit Ruminants (PPR) virus grown in Baby hamster kidney cell lines and group C with 1 ml of PPRV and a week later 1ml of MH A2. Nasal swabs were collected from each goat weekly while the identification and bacterial count of the isolates were carried out using standard methods. Student t-test was used to test for significant differences. In group B, the colonies of *Mannheimia hemolytica* was significantly increased from the third week pi, while that of group C, increased significantly from the second week pi ($P<0.05$). The dominance of MH over the other bacteria further confirmed it's ability to overcome host-response, compete favourably and achieve attachment. This appear to be the first study that described the possible reason for the frequency of field association of PPRV and MH infections in goats and the possible antibiotics treatment timing in the viral and bacterial complicated *viral pneumonia* in goats.

Key word: Nasal bacteria, PPR, co-infection, *Mannhemia Hemolytica*

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Introduction

Various investigations on the bacteria *flora* of the respiratory tract of diseased domestic animals in various parts of the world have focused on cattle (Pandey and Sharma, 1987), camel (Al-Tarazi 2001), sheep and goats (Ikede, 1977; 1978; Abubakar *et al*., 1981; Adekeye, 1984, Ugochukwu, 1985; Emikpe *et al*., 2009) with fewer reports on sequential bacterial flora changes in normal and diseased nasal passage of small ruminants especially goats.

The most common viral and bacterial respiratory disease of subsaharan goats is *Peste des petit Ruminants* and *Mannheimiosis*. The other factors that have been incriminated in the *pathogenesis* of the two diseases include stress factors such as adverse climatic condition, low plane of nutrition, transportation and confinement (Durojaiye, 1984; Obi, 1984). Of these factors, the secondary microbial agents have been speculated to play a greater role in the *pathogenesis* and severity of the viral infection (Adetosoye and Ojo, 1983; Emikpe, 2009).

Available literatures indicated that the development of *pneumonia* in PPR is the consequence of the viral replication, although bacteria such as *Mannheimia hemolytica, Klebsiella spp, Echerichia coli* and *Staphylococcus pyogenes* have been isolated from complicated PPR pneumonia (Ikede, 1977, 1978; Ojo, 1980; Obi, *et al*., 1983). That PPR infection is potentiated by *Mycoplasma* spp infection has been previously reported (Onoviran *et al*., 1984).

Therefore, it was suggested that PPRV probably initiated pulmonary damage that provided entry to bacteria (Emikpe *et al*., 2010). Some of the incriminated bacteria are also known to be part of the normal *flora* of the upper respiratory tract of small ruminants (Megra *et al*., 2006). The *pathogenesis* and changes in the bacterial *flora* in the course of PPRV and co-infection with MH and the possible reasons for the implications of some bacteria in PPR have received little or no attention. This study is therefore designed to investigate the nasal bacterial *flora* changes in experimental PPR virus and its co infection with *Mannheimia hemolytica* in goats in order to understand the changes and possibly adduce reasons for the role played by the normal resident nasal bacterial flora in the *pathology* of viral induced *pneumonia* in goats.

Materials and Methods

Study Location

The small ruminant pens of the Veterinary *Pathology* Department, in the experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan were used for this study.

Experimental Animals

The experimental protocol has been previously described (Emikpe and Akpavie, 2010). Briefly, Fifty (50) apparently healthy West African dwarf goats (WAD) six months of age, of an average weight of 6kg were used. Fifteen (15) WAD goats were in each treatment group while five (5) uninfected goats served as control. The animals in each treatment group were housed in a separate well partitioned pen on concrete floor. They were conditioned for 14 days before the commencement of study. Wheat bran and water were provided ad libitum daily. During the conditioning period, the experimental animals were tagged and treated intramuscularly with a broad spectrum antibiotic (*oxytetracycline hydrochloride*) at lml/kg body weight/ per animal against bacterial infections. They were also treated subcutaneously with ivermectin (ivomec®) for 5 days against *parasitic* infestations at dose rate of 1ml/kg body weight of the animal. The nasal swabs of the animals prior to the experimental phase were negative for *Mannheimia haemolytica* by cultural isolation. The animals were also confirmed seronegative by agar gel precipitation test for antibody to Peste des Petit Ruminants virus (PPRV) according to Obi and Patrick (1984).

Route

Animals were infected intratracheally according to the method described by Davies *et al*., (1981a).

Infection: The goats were divided in three groups of 15 goats each, aged 6 months. They were inoculated with 1 ml of pure culture of a 4 hour log phase 1.0 x 10^9 CFU of MH serotype A2, (MH group), 1ml of the pure culture of $10^{6.5}$ (Tissue Culture Infective Dose) PPRV grown in baby hamster kidney cells (PPRV group), and 1 ml of PPRV followed a week later with 1 ml of MH (PPRV+MH group) and five goats served as controls.

Bacteriology

Nasal swabs were collected from each tagged goat weekly post inoculation. The inoculated brain heart infusion broths were incubated for 24 hours at 37^0C before being sub-cultured into the blood agar. Each plate was labeled properly according to the tag numbers of the goats. The inoculated plates were arranged in candle jar and placed inside the incubator at 37^0C for 18 – 24 hours. The isolates were later sub-cultured into another prepared culture media to obtain pure cultures from some that gave mixed colonies. Characterization and identification of the isolates were carried out using standard methods (Quinn *et al*., 1994).

The pour plating procedure was adopted for the bacterial count. Briefly, 0.5ml of the ninth dilution of each nasal swab sample in brain infusion broth were measured into the petri dish and the melted culture medium (*Blood Agar*) were poured on the base of the petri dish at an angle 450 to prevent contamination. These were allowed to solidify and the plates were incubated aerobically at 37^0C for 24-48 hours. The bacterial colonies were then counted using a colony counter.

Results

All the goats in the MH group before inoculation were negative for *M. hemolytica* by cultural isolation using *blood agar*. Three days post inoculation (pi), *M. hemolytica, Streptococcus pyogenes, Staphylococcus aureus*, were isolated in three goats and by day 7 pi all the goats yielded pure culture of *M. hemolytica*. The bacterial count was not attempted in the MH group.

In the PPRV group, the average weekly nasal bacterial count on *blood agar* showed that, the colonies of *Mannheimia hemolytica* were more than those of *Staphylococcus aureus and Streptococcus pyogenes* (Fig. 1). The colony count significantly increased from the third week pi for *Mannheimia hemolytica*, ($P<0.05$) while that of *Streptococcus pyogenes* was markedly decreased and that of *Staphylococcus aureus* was not altered within the same period.

In the PPRV +MH group, the average weekly nasal bacterial count (Fig. 2) showed that, the colonies of *Mannheimia hemolytica* were more than those of *Staphylococcus aureus* and *Streptococcus pyogenes*. The colony count for *Mannheimia hemolytica* significantly ($P<0.05$) increased in the first two weeks than that of *Streptococcus pyogenes* and that of *Staphylococcus aureus* was not significantly altered within the same period.

Fig 3 compared the MH count in PPRV and PPRV+MH groups with PPRV group having higher nasal bacterial count than PPRV+MH group.

Discussion

This investigation showed that in PPRV infection, the nasal bacterial flora counts increased over time with marked increase in MH and subsequent dominance of MH over other bacteria especially at the third week of infection. This increase may be associated with the *immunosuppressive* effects of the virus experienced between 10-14 days as reported by Rajak *et al.* (2005). The *immunosuppressive* effect of the virus may be associated with reduced mucociliary clearance and reduced *phagocytic* function of the macrophages in the alveolar region which in turn aid the establishment and the colonization of the bacteria.

The pattern of the nasal bacteria observed in PPRV was similar to that of PPRV and MH with, a significant increase in the bacterial flora count in the first two weeks pi and dominance of MH from the first week post innoculation. This increase may be as result of the experimental introduction of MH and the immunosuppressive effect of the PPR virus (given 7 days before MH challenge) (Rajak *et al.,* 2005). These findings may help to explain the severity of clinical features observed and the peak lung consolidation observed in the first week pi in PPRV+MH group

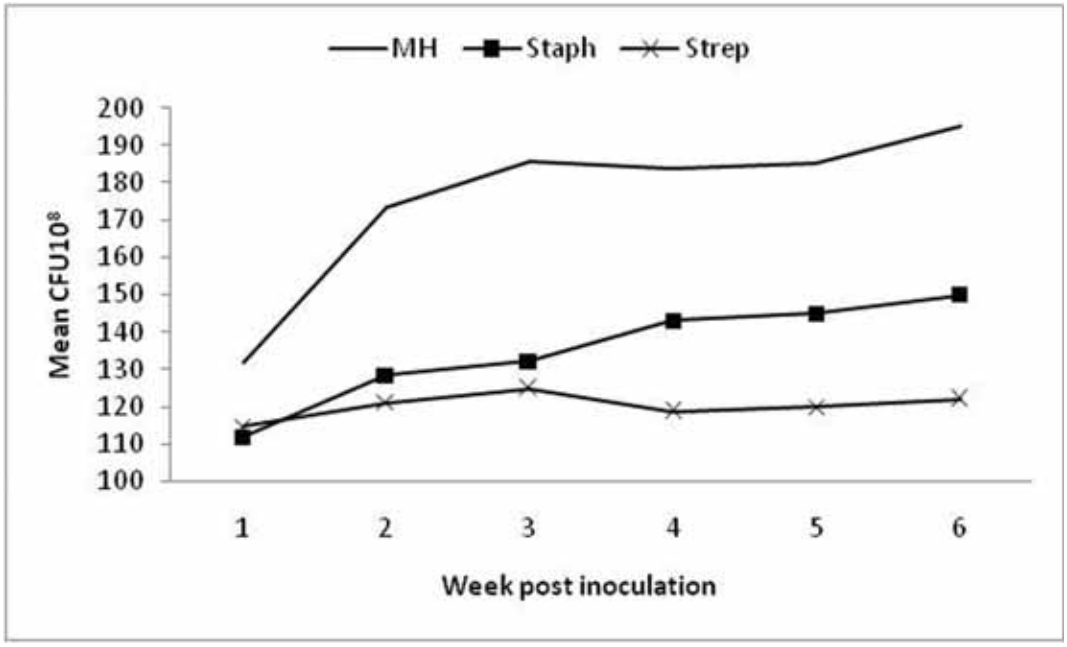

Fig 1: Weekly nasal bacterial count on Blood agar in experimental PPRV infection in West African Dwarf goats.

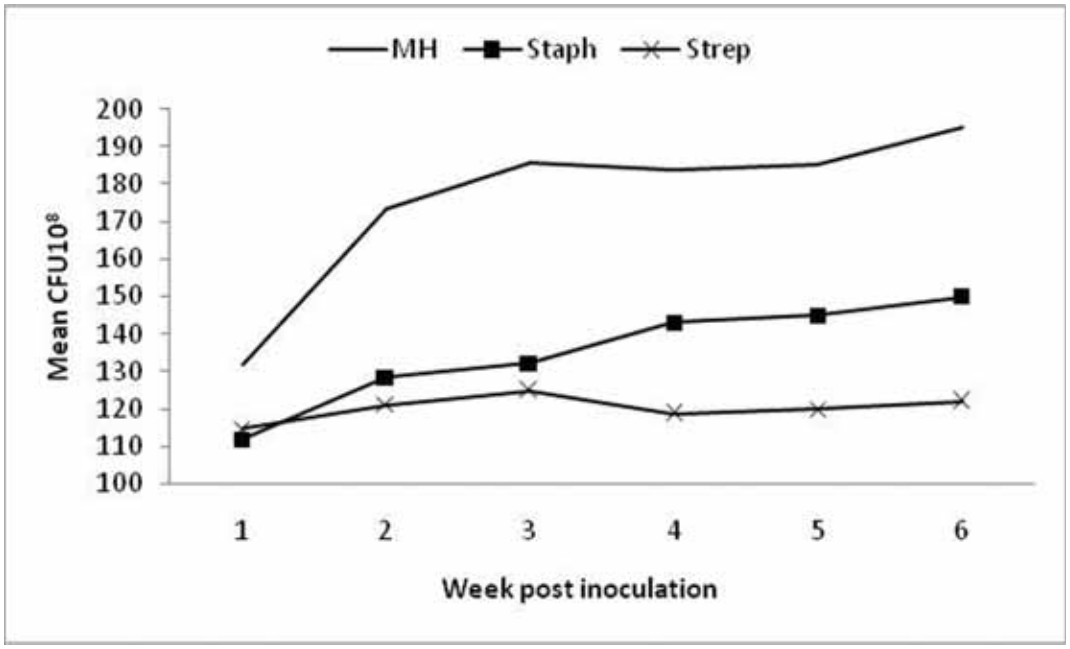

Fig 2: Weekly Nasal bacterial counts on Blood agar in Experimental PPRV +MH Infection in West African Dwarf goats
CFU- Colony forming unit

and third week in PPRV group (Emikpe 2009; Emikpe and Akpavie, 2010).

The major bacteria observed in PPRV and PPRV+MH groups alongside with *Mannheimia hemolytica,* which was introduced includes Staphylococcus aureus and Streptococcus pyogenes which were the agents that have been reported as major complicating bacteria in natural PPRV infection in small ruminants (Obi *et al*., 1983). The dominance of MH, over the other bacteria showed the ability of MH to overcome host-response, compete favourably with resident bacterial flora and achieve attachment (Rowe *et al*., 2001). This dominance especially in PPRV+MH may be associated with the use of the organism at its log phase as the log phase culture has been reported to produce *virulence* factors (Shewen and Wilkie, 1985) such as leukotoxins, capsule and lipolysaccharide which aid the bacteria to escape the host defensive mechanisms like the antimicrobial barriers such as beta defensin and anionic peptides found in the epithelial cells as well as resident and inflammatory cells (El Ahmer *et al*., 1996, 1999). This ability also enhances the colonization and invasion of the lungs (Confer *et al*.,

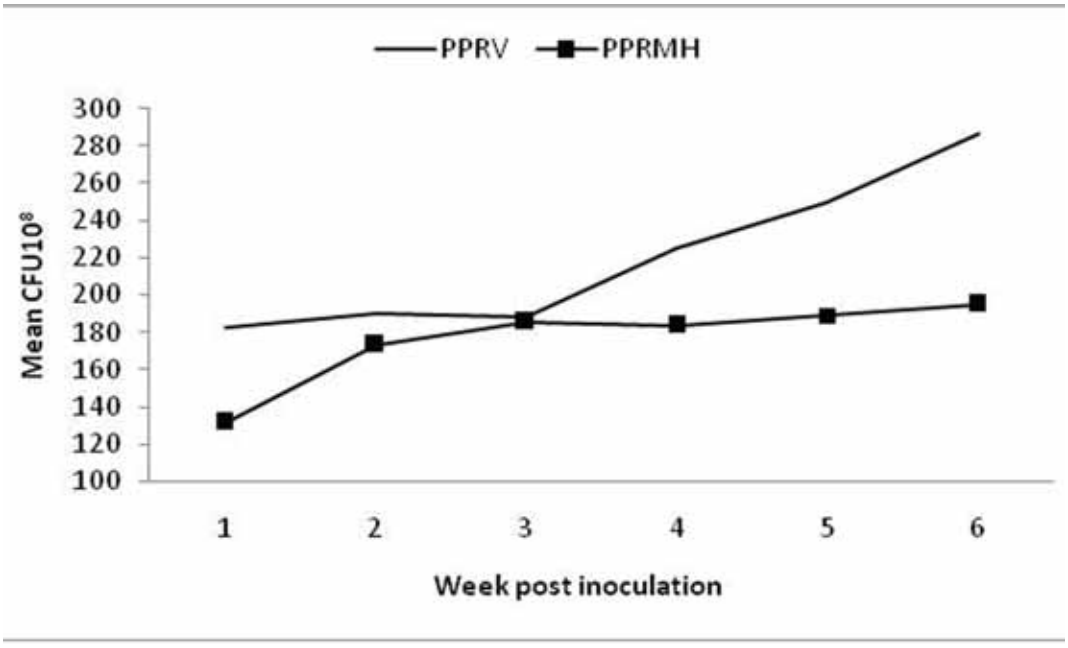

Fig 3: MH counts on Blood agar in experimental PPRV+MH and PPRV infection in WAD goats

1990; Brogden *et al*., 1998).

It had been well documented that serotype A2 which was employed and re-isolated in this study appears to be more efficient at surviving than serotype A1 as the length of survival time was longer and their resuscitation was observed to be quicker (Rowe *et al*., 2001). The dominance of MH may be associated with the rare ability of *M. haemolytica* to survive in low nutrient fluids by adopting a long-term survival strategy which is associated with the temporary or permanent change from normal size colonies to '*micro-colonies*' as observed on sheep blood agar (Rowe *et al*., 2001). This survival trait of MH has important implications for disease transmission as most ruminants pick up the organism from body fluids present in pasture, accommodation or in drinking water.

The reduction in bacterial count of *Streptococcus pyogenes* at 4-5 week pi in PPRV group and 3-4 week pi in PPRV+MH group may be associated with the fastidiousness of the bacteria as it requires a very rich medium to thrive and as the medium was depleted by the competing bacteria, so, its increase may not be sustained over time.

The implication of the dominance and the ability to escape the host defensive mechanisms displayed by MH may be the probable reason for the higher frequency of observed field association of PPRV and MH infection (Obi *et al*., 1983) as compared to other resident nasal bacteria.

The isolation of MH in PPRV infected goats in this study was in contrast with the reports of Davies *et al*. (1982), Sharma and Woldehiwet (1990) in sheep where MH was not isolated from nasal swabs of lambs infected with *Adenovirus* and BRSV (Write in full then abbreviate) respectively while there was a high recovery of MH in BRSV+MH infected group (Sharma and Woldehiwet, 1990) as observed in PPRV+MH group in this investigation. This observation further suggests that infection with PPRV enhances the capacity of MH to colonise the respiratory tract as it has been observed that the effect of viruses on *epithelial* cells of the respiratory tract increases the adherence of MH and *Pasteurella multocida* to expression of cell surface antigens (El Ahmer *et al*., 1996,1999) and the recovery of MH may be related to the time at which the virus was administered which may determines the level of colonization as reported by Rajak *et al*. (2005).

Comparing PPRV and PPRV+MH groups, the MH mean CFU obtained in PPRV was higher than PPRV+MH group. This observation may be connected with the difference in the level of carriage of MH in the animals used as the level has been reported to differ in animals (Magwood *et al*., 1969). The level of immunosupression experienced in PPRV group may be higher than PPRV+MH group as the increase in the mean CFU of MH in PPRV increased in the third week after the maximum immunosupression would have been experienced (10-14 dpi) (21) as compared to PPRV+MH where the bacterium was given 7dpi after PPRV administration which did not allow for a full immunosupression as described by Rajak *et al*. (2005).

These findings also showed that periodic nasal bacterial isolation and characterization could help in the detection of complicating bacterium/bacteria in viral infections and the antibiotic sensitivity of such isolates could inform the course of treatment. The marked increase in the nasal bacterial count experienced in PPRV group especially at 21 dpi reveal the probable appropriate time for antibiotic intervention in the treatment of subacute PPRV in goat in order to combat the possible effect of bacterial complication in the course of the disease.

The ability of *M. haemolytica* to adapt by deploying starvation/survival mechanisms requires further investigation. The areas of utmost importance are whether starved organisms resemble those that colonise the upper respiratory tract and if they are virulent (Rowe *et al*., 2001). The synergism associated with MH has been reported for some viral infections; *Para influenza* virus 3 infection (Rushston *et al*., 1979, Davies *et al*., 1981a, Davies *et al*., 1981b), Respiratory syncytial virus infection (Sharma and Woldehiwet, 1990; Fulton, *et al*., 2000), *Adenovirus* infection (Davies *et al*., 1982) and *Bovine viral diarrhea* (Ganheim *et al*., 2003) but none attempted to explain the probable reason of the synergy with the use of nasal bacterial count. It is therefore imperative that in the control of most viral respiratory infection in goats including PPR, there is need to include preventive measures for MH.

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Seasonal population changes of *Amblyomma lepidum* (Acari: Ixodidae) under field conditions in Damazin, Blue Nile State, Sudan.

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Résumé

Cette étude a été réalisée pour déterminer dans les conditions de terrain, le taux d'infestation saisonnière de la tique lepidum Amblyomma sur le bétail. La collecte des tiques a été effectuée sur une base mensuelle pendant deux années consécutives. La population, le stade d'évolution et le sex-ratio des tiques ont été enregistrés. Les résultats ont montré de fortes variations saisonnières sur le taux d'infestation des bovins par les différents stades de la tique Amblyomma lepidum. L'infestation par les tiques a commencé à augmenter au cours de la première pluie et a atteint un pic vers ou peu de temps après la fin de la saison des pluies. La population des tiques a diminué au cours de la saison froide et sèche pour devenir très faible ou presque nulle pendant la saison chaude et sèche. Par rapport à la première année, le nombre de larves de la deuxième année étaient significativement élevée ($P \leq 0,01$). D'autres étapes ont été presque constantes avec de faibles différences négligeables. La saison et la race de l'hôte ont affecté de manière significative le taux d'infestation ($P \leq 0,001$). L'effet de l'âge de l'hôte sur l'infestation par les tiques femelles était insignifiant, mais il affecté les larves et les mâles ($P \leq 0,01$) et les nymphes ($P \leq 0,05$). D'autre part, l'effet du sexe des animaux sur l'infestation par des larves a été insignifiante, tandis qu'il l'a affecté de manière significative ($P \leq 0,01$) d'autres étapes.

Mots clés: Amblyomma Lepidum, variations saisonnières, Soudan.

Abstract

This study was carried out to determine the seasonal infestation rate of *Amblyomma lepidum* on cattle under field conditions. Tick collection was carried out on a monthly basis for two consecutive years. Tick burden, stage and sex ratio were recorded. The results indicated marked seasonal variations on the infestation rate of cattle by the different stages of the tick *Amblyomma lepidum.* The tick infestation started to increase during the first shower and reached a peak towards or shortly after the end of the rainy season. Tick load declined during the cool dry season to become very low or almost nil during the hot dry season. Compared to the first year, larvae in the second year were significantly high ($P \leq 0.01$). Other stages were almost the same with only minor negligible differences. Season and breed significantly affected the infestation rate ($P \leq 0.001$). The effect of age on the infestation by female ticks was insignificant, but it affected the larvae and males ($P \leq 0.01$) and nymphs ($P \leq 0.05$). On the other hand, the effect of animal sex on the infestation by larvae was insignificant, while it significantly ($P \leq 0.01$) affected other stages.

Key words: *Amblyomma lepidum*, seasonal changes, Sudan.

Introduction

Twenty species of *Amblyomma* are known to transmit *Ehrlichia* ruminantium the causative agent of heartwater (Walker and Olwage, 1987), and about 175 million head of cattle are in countries where heartwater had been confirmed (Provost and Bezuidenhout, 1987). In the Sudan, A. lepidum was first incriminated on circumstantial evidence as vector of heartwater by Karrar (1960). Its ecology and host relationship in Kassala Province of the Sudan was studied by Karrar *et al.* (1963). Counts of tick numbers on livestock under conditions with no control measures are useful in the study of host – parasite relationships, and their seasonal *phenology*. It is also necessary for calculating losses in production caused by ticks, and hence in designing appropriate management strategies (Kaiser *et al.*, 1991). Although some authors found no seasonal variation on A. lepidum (Tatchell and Easton, 1986), but population changes of parasitic ticks were reported by many others (Yeoman and Walker, 1967; Yeoman, 1968; Pegram *et al.,* 1981). In Sudan, A. lepidum was studied earlier (Karrar, 1960; Karrar *et al.*, 1963; Karrar, 1968; Osman, 1978), but still data on this tick species is scarce. This study aimed at determiningthe infestation rate of the tick A. lepidum on cattle and its seasonal population changes as a basis necessary to formulate control polices.

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Materials and Methods

The Blue Nile State (33° 80°- 35° 80° E, 9° 30°- 12° 35° N) falls within the savannah belt. Its climate is charac terized by two main traits, sustained heat and marked seasonality of rainfall (Karrar and Partner, 1994). Most of cattle herds in the State are kept under nomadic system, but a considerable proportion, in and around towns, are under semi nomadic regime of husbandry. In Damazin (34° 48° N, 11° 21° E) cattle herds are let to graze freely in communal grazing pastures during the day and return in the evening when they are tethered in animals' pens. Four herds of cattle were chosen and five heads from each herd were selected for this study. Only farms that do not practice chemical acaricide control were selected for the study. Cattle comprised Kenana and *Umbararo* types. They were grouped according to their age as < 2, 2 - < 7 and ≥7 years old. Monthly meteorological data were obtained from the *meteorology department,* Damazin office. The rate of rains in the first year was higher than that of the first one and that was reflected by increased humidity and decreased temperature. All visible ticks were collected from the entire body (Macleod, *et al*., 1977) on a monthly basis for two consecutive years (May 1999 to April 2001). The same farms and the same animals were surveyed throughout the study. Ticks of each animal for each month were preserved in separate vials containing 70% ethanol. The vials were labeled indicating the animal number, farm and date of collection. Identification of ticks to the species level was carried out according to Hoogstraal (1956) and Walker *et al*., (2003). Tick burden, stage and sex ratio were recorded. Data collected were subjected to general linear model using SAS package. Analysis of variance (ANOVA) and mean square based on Ryan's range test (REGWQ) were performed according to Day and Quinn (1989).

Results

In the first year of the study *larvae* were not found on both cattle types during the period from January to May. *Larvae* first appeared with the first rains in June. They disappeared in August and steady increase was then noticed in September and reached the peak in November. *Nymphs* also were not encountered during the same period. The peak abundance of nymphs on Kenana cattle was in October, while on *Umbararo* it took place in November. Unlike the larvae and nymphs adult stages were found throughout the year, but with varied seasonal infestation rates; males always outnumbering females. The adult tick load was low during April, increased steadily during the rainy season and reached a pronounced peak in November, and thereafter they sharply declined. Umbararo cattle were found to carry significantly more ticks than Kenana cattle (Fig.1).

In the second year of the study the infestation followed the same pattern of the first year with the exceptions that larvae first appeared in May and the tick load was higher than that of the first year (Fig.2).

The mean tick load per animal according to the season and age is shown in table (1). Larvae were found to increase with the increase of animal age, and simultaneously with the sequence of the hot dry, wet and cool dry seasons. Unlike the other stages nymphs in the wet season, particularly in the mid age 2<7 were lower. The tick load of male ticks was similar to that of larvae, but females seemed to decrease with the increase of age.

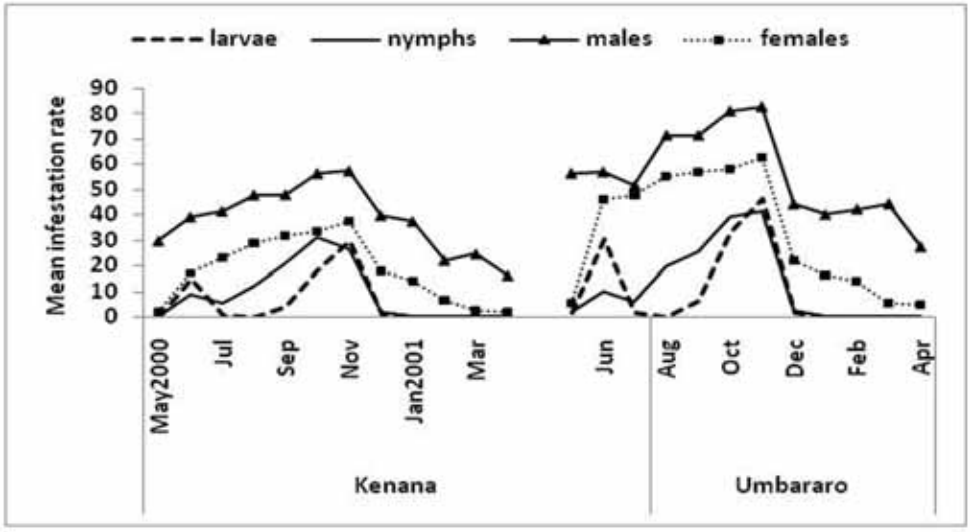

Fig 1.Mean infestation rate of different stages of *Amblyomma lepidum* under field conditions in Damazin, Blue Nile State, Sudan during May 1999-April 2000

Statistical analysis is shown in table 2. Larvae in the second year were significantly higher ($p \leq 0.01$), while the other stage showed no significant difference in the two years. The effect of seasonality on all the stages was highly significant ($p \leq 0.001$). Animal breed also had a significant effect on larvae, females and males ($p \leq 0.001$), but to a lesser extent ($p \leq 0.01$) on *nymphs*. Animal sex had no significant effect on larvae but it significantly ($p \leq 0.01$) affected the other stages. On the other hand, animal age had no significant effect on females but it significantly affected *nymphs* ($p \leq 0.05$) and *larvae* and males ($p \leq 0.01$).

Discussion

According to this study, marked seasonal variations on the infestation of cattle by A. lepidum were found. Although no seasonal variation in adults of A. lepidum in Tanzania was found (Tatchell and Easton, 1986), but other workers reported the peak abundance of the adult (Yeoman, 1968; Pegram *et al*.,1981) and nymphs Yeoman and Walker, 1967). However, differences in climatic conditions among different geographical zones affect the performance of ticks and hence their infestation rates. The overall mean tick burden of different stages was found to increase with age. Within the same age group, the infestation increased in the direction of the larvae to the adults. It is to be noted that some individual animals showed considerably lower tick burden compared to others in the same herd. This could be due to natural inherited resistance that needs to be investigated as an approach to be incorporated into an integrated control package aiming to reach a tick resistant cattle population.

The rate of infestation was found to be affected by the prevailing ambient temperature and relative humidity. Unlike the other two stages, adult A. *lepidum* were found on hosts throughout the year, but with varying seasonal infestation levels. The tick load was found to be low during the dry season; increased gradually during the rainy season and reached pronounced high peaks at the end of the rains and thereafter, it sharply declined. Similar results were obtained (Wolfgang and Jundidu, 1984). Exceptionally, larvae were found to disappear during the peak of rainfall. This finding was also observed by Wolfgang and Jundidu, 1984; Dipeolu, 1984). However, it could be speculated that larvae can not withstand excess of water and they are washed up during high rains, although immersing ticks in water for as long as 72 hours had no or little effect (Osman, 1978). Larvae were also absent during the hot dry season. This result agrees with Osman (1978) who stated that in hot dry season, when temperature ranges between 35 °C and 45°C, it is unlikely to support the existence of A. lepidum. Few larvae were recorded in May when the environmental conditions were very hostile. These larvae were collected from Umbararo cattle which were watered from water ponds, where water vapour and grass vegetation provide optimum microhabitats enabling ticks to survive the adverse conditions.

The current findings on seasonality, peak abundance, and the occurrence of adults throughout the year are in agreement with previous work (Oliveira *et al*., 2000). Punyua *et al*. (1985) stated that, in situations where there are marked seasonal variations in temperature, there might be long periods of the year, where some or all stages of the tick fauna are absent from the hosts. Adequate grass cover, moderate but sufficient relative humidity, absence of environmental modifications and large animal populations are considered as factors increasing tick burden (karrar, 1960). However, no single variable would explain the tick distribution pattern (Labruna *et al*., 2002).

Seasonal variations in tick activity are generally linked to the occurrence of well marked wet season, although the adults, at least, of many species maintain some degree of activity throughout the year (Punyua *et al*., 1985). In general adult ticks were found to be more numerous on their hosts during the wet season than during the dry season (Newson, 1978; Kaiser *et al*., 1988). In Tanzania the peak abundance of adults was found to occur shortly before or after the onset of rains (Yeoman and Walker, 1967). In Ethiopia A. lepidum occupies intermediate habitats between wet and dry zones (Pegram *et al*., 1981). However, the differences noticed between the results of the current study and those of the previous work in Africa, might be due to the variation in the environmental conditions of the different climatic zones. The seasonal variation of female ticks followed the same pattern of male ticks, but the latter were more abundant, which is in agreement of previous results (Karrar et al., 1963; Yeoman and Walker, 1967; Oliveira *et al*., 2000).

Compared to the first year, the infestation by larvae in the second year was significantly higher ($P \leq 0.01$). This difference could be attributed to the climatic changes when a decrease of 6 - 13% in humidity with increased temperature of 3 - 7 °C that occurred during the wet season of the second year, seemed to enhance the larval activity. The same conditions seemed to reduce the activity of nymphs but the latter finding was insignificant. It is obvious that the effect of seasonality is due to the varied ambient temperature and relative humidity. The effect of cattle type may be attributed to poor management of Umbararo cattle compared to Kenana. In relation to the age group, the effect on female ticks was insignificant, but the effect on other stages was marked where ($P \leq 0.001$), ($P \leq 0.05$) and ($P \leq 0.01$) for the *larvae*, *nymphs* and male ticks, respectively.

Conclusion

The results of the current study demonstrated marked seasonal variation on the infestation of cattle under field conditions with different stages of the tick A. lepidum. The peak abundance coincided with climatic conditions where temperature, rainfall and relative humidity provided the optimum requirements for their survival and development. Alternatively, ticks largely depend on the *microhabitat* at the ground level. Sex and age had little effect on tick burden, while management seemed to be a critical factor.

Impact

The findings of this study may address the cattle owners, stakeholders and policy makers to draw their attention to the critical period of the year when ticks are abundant. The occurrence of adult ticks during the dry periods implies that they withstand this period utilizing the *microhabitat*. Therefore, great efforts are needed to avoid, destroy this *microhabitat* if possible, or adopt a strategic tick control regime which can break the tick cycle to prevent subsequent population growth.]

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Laboratory Investigation of Three Outbreaks of Foot-and-Mouth Disease at Central Sudan and the Disease Type Situation

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Enquête en laboratoire des trois foyers de fièvre aphteuse au Soudan central et situation-type de la maladie

Résumé

Trois événements de maladie de la fièvre aphteuse (FA) dans le centre du Soudan ont été étudiés en 2008. Deux de ceux-ci ont été caractérisés comme étant de type "O" et le troisième comme étant de type "SAT2". L'isolement du virus a été réalisé par culture de cellule a partir de rein de veau (CRV) et la typologie a été effectuée en utilisant des préparations de référence de la fièvre aphteuse pour détection de l'antigène par les antisérums de référence ELISA (Laboratoire de Pirbright) dans un test de caractérisation en culture du (CRV). Les lésions de la bouche et la thermie ont été reproduites dans un veau de race locale, après la septième inoculation de passage CKC de culture pour l'isolation de SAT2. Les sérums post-inoculation et de "SAT2" de référence ont été utilisés pour calculer la valeur r1 entre les mêmes foyers des isolats "SAT2". Le système a été mis en place dans la culture du rein et les valeurs r1 de o.86 et 0,9 ont été respectivement obtenus.

La situation-type dans le centre du Soudan a été discutée en rapport avec les résultats obtenus et les dernières données sérologiques. L'activité constante des trois sérotypes du virus de la fièvre aphteuse: "O", "A" et "SAT2» est mise en exergue. "SAT1" montre une faible importance épidémiologique ou disparaîtra totalement du pays. Il montre une faible séroprévalence, actuellement et il ya 30 ans, et n'a pas été signalé au Soudan depuis 1976. Le type clinique "A" de la maladie a toujours été détecté à un taux inférieur à celui du type "O" quoique les deux types montrent une large diffusion par sérologie. L'augmentation du niveau de protection croisée entre les virus de sérotype "A" circulant sur le terrain par rapport à celle concernant les sérotypes "O" circulants sur le terrain au Soudan avait peu de chances d'être responsable de la situation décrite bien que les premières pourraient être plus diversifié au plan immunologique. La situation se conforme avec la plus large distribution connue du type "O" , ainsi qu'avec la limite établie de la classification du sous-type.

Mots-clés: Fièvre aphteuse de type soudano-culture, situation-CKC.

Summary

Three disease events of foot-and-mouth disease (FMD) in central Sudan in 2008 were investigated. Two were typed as type "O" and the third as type "SAT2". Virus isolation was carried out in calf kidney cell (CKC) culture and typing was effected using reference preparations of FMD antigen detection ELISA and reference antisera (Pirbright Laboratory) in a typing test in CKC culture. Mouth *lesions* and *thermia* were reproduced in one calf of the local breed, following inoculation of the 7th CKC culture passage of a SAT2 isolate. The post-inoculation and "SAT2" reference sera were employed to derive the r1 value between the same-outbreak "SAT2" isolates. The system was established in the kidney culture and r1 value of o.86 and 0.9 were respectively obtained. The type situation in central Sudan was discussed in relation to the obtained results and recent serological data. The maintained activity of three serotypes of FMD virus: "O", "A" and "SAT2" is stressed. "SAT1" is showing low epidemiological significance or is disappearing altogether from the country. It shows low *seroprevalence*, currently and 30 years ago, and has not been reported in Sudan since 1976. Type "A" clinical disease has always been detected at lower rate than that of type "O" though both types show wide dissemination by serology. Higher level of cross protection between serotype "A" circulating field viruses in comparison to that between serotype "O" circulating field viruses in Sudan was not unlikely to be responsible for the described situation although the former are expected to be more *immunologically* diverse. The situation fit with the known world widest distribution of type "O" and with the established limitation of the subtype classification as well.

Keywords: FMD-Sudan-type situation-CKC culture.

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Introduction

Foot-and-mouth disease virus (FMDV) is the type species of the *Aphthovirus genus* of the *Picornaviridae* family (Belsham, 1993). Seven serotypes of the virus have been identified *serologically*. They were designated "O", "A", "C", "Asia1" and the "SAT1, 2 and 3" (Grubman and Baxt, 2004). All of them were reported in Africa with the exception of "Asia1" and, apart from type "C", prevalent serotypes and topotypes in the continent are known for their *immunological* diversity (Vosloo *et al.*, 2002). *Serotypes* and *topotypes* cause indistinguishable clinical disease but severity may differ according to the strain within each *serotype*, species affected and previous exposure (Alexandersen and Mowat, 2005).

In Sudan, FMD was known to be existing since the early years of the last century. The clinical disease was reported in cattle only with type "O" as the most predominant followed at much lower extent by types "A" and "SAT1" while "SAT2" was relatively recently introduced in the country in 1977 (Abu Elzein, 1983). Since the mid 1980s, the need to concentrate efforts and resources on control of rinderpest led to abandoning the study of FMD in Sudan. These efforts were regenerated recently in 2004; samples from suspected FMD outbreaks being more regularly sent to the WRL and FMD diagnostic facilities were established at the local level. Recent serosurveillance indicated wide dissemination of three serotypes of FMDV in cattle species; "A", "O" and "SAT2" but very low activity for the serotype "SAT1" (Raouf *et al.,* 2009; Habiela *et al.,* 2010; Raouf et al, accepted for publication). It detected in cattle species, for the first time, higher prevalence of type "A" antibody than that of type "O" (Habiela *et al.,* 2010; Raouf *et al.,* accepted for publication) which was long known as the most frequent and most wide spread in Sudan.

The presented work reports the laboratory investigation carried out at the local level for three FMD events in the year 2008 in central Sudan. It highlights the type situation in the country on the light of obtained results and recent *serological* data. The advantages of establishing diagnostic facilities for FMD at local national laboratories which involved virus isolation and identification were stressed by Ferris and Donaldson (1992). Virus isolation is necessary for amplification of virus material in some samples, for *epidemiological* and vaccine selection studies and for development of new vaccines from local isolates.

Materials and Methods

Suspected FMD outbreaks

Reports of suspected FMD outbreaks in central Sudan usually occur during the cold season (December, January, February and March). Few other reports come in the late rainy season (September and October). During the year 2008, two suspected FMD outbreaks were reported in February in Khartoum State (at Al Rdowan in Omdurman and at Al Amel city in Khartoum) and a third in March in the neighboring Al Jaziera State (at Al kamleen). At Al Rdowan and Al kamleen cattle affected were of the cross breed. No age preference was observed and morbidity approached 40% at the first site. At Al Amel city both local and cross breed were affected. Morbidity and severity seemed to vary from one animal enclosure to another. In each outbreak recent history of introduction of new animals was reported. Prominent clinical signs were mouth lesion, salivation and general dullness. Erosion of the tongue *mucosa* was very extensive. *Lesions* frequently involved the gum and muzzle. No distinction in severity of these lesions could be drawn between the local and cross breeds. Ulcers in the udder were also observed. Lameness was a less prominent clinical sign.

Samples from suspected FMD outbreaks

Tongue *vesicular epithelium* was collected from recent cases of mouth lesion in transport media comprised of equal amounts of glycerol and 0.04 M phosphate buffer, 0.001% phenol red, antibiotics and antimycotics (PH 7.2-7.6). Samples were kept refrigerated till reaching the laboratory where they were transferred to -20 °C deep freezer.

Virus isolation

The collected tongue epithelium was wiped up *glycerol* and 10% suspension (W/V) was prepared in the usual manner in *Glasgo* minimum essential medium (GMEM) containing a double concentration of *antibiotics* and *antimycotics*.

For virus isolation calf kidney cell (CKC) was used (Bachrach *et al.*, 1955). Primary and secondary CKC was prepared according to methods described by Plowright and Ferris (1959) and Ferris and Plowright (1961). Outgrowth media was GMEM containing 5-10% newborn calf serum (NBCS) (SIGMA). Twenty four to forty eight hours old semi-confluent secondary monolayer flask cultures (surface area 25 cm^2) were washed 2-3 times with GMEM after removing the out-growth media, and then inoculated with 1-1.3 ml of the clarified epithelium suspension. Inoculated cultures were kept at 37 °C for one hour with tilting every 15-20 minutes. Thereafter inoculums were discarded; cultures washed once or none then received 5 ml of GMEM containing 2-3% NBCS and returned to the 37 °C incubator. Cultures were observed daily for developing of cytopathic effect (CPE) or blind passaged after 48 hours.

Identification by ELISA

The indirect sandwich ELISA for detection of antigens of FMDV (Roeder and Le Blanc Smith, 1987) was used for detection of virus serotype on epithelium suspension and on cell culture clarified supernatant. All ELISA reagents were prepared and supplied by the World Reference Laboratory (WRL) for FMD (Pirbright, UK) and were used according to the supplied protocol.

Micro-neutralization typing test

The micro-neutralization test for serological typing of FMD virus described by Rweyemamu *et al.,* (1978) was adopted using secondary CKC instead of baby hamster kidney (BHK) cells. Reference antisera were supplied by the WRL for FMD (Pirbright, UK). They were type "O", "A", "SAT1", "SAT2" and normal negative sera. All were inactivated at 56 °C for 30 minutes. Serum diluent consisted of GMEM containing 10% (V/V) tris-buffer (0.05 M). The test virus was used undiluted or as 10% dilution. The growth media for CKC was the same as serum diluent but contained in addition 10% NBCS.

The test was read microscopically after 48 hours or, occasionally, fixed and stained on the second or third day post-seeding with crystal violet vital stain then examined. The neutralizing titres were determined by the method of Karber (1931).

Virus passage and titration in CKC culture

The virus harvest of a whole frozen culture or collected fluid supernatants were clarified by centrifugation at 2000 rpm and used as inocula for the second passage level. Techniques of inoculation were as described before.

Virus *titration* was done in the *microtitre* system. Tenfold serial dilutions of virus were prepared in a diluent of GMEM containing 10% tris-buffer. Using microtitre pipette, 50 µl volume of each virus dilution was transferred into each of 8 wells (one column) of a flat bottomed *microtitre* plate. Each well then received 100 µl volume of secondary CKC suspension prepared in the media described for the *microtitre* system. The plate was shaken lightly, sealed with an adhesive tape and incubated at 37 °C with a source of humidity. Examination for CPE was carried out daily for 3 days under the inverted light *microscope*. Titres were calculated according to the method of Karber (1931).

Micro-neutralization assay

For screening of sera the standard procedure described in the OIE manual (2008) was adopted using secondary CKC culture. Following adaptation in CKC, stocks of local "SAT2" isolates were grown in *monolayer* cultures, clarified, titrated and stored in liquid nitrogen vapor. According to the result of titration, a dilution of virus that contains 100 TCID50 was employed in the test. A serum screened positive when both wells containing the final serum dilution of 1/32 were negative for CPE (*positive for neutralization*).

For deriving the r1 value a two-dimensional virus neutralization assay was used. The antiserum was "SAT2" reference antisera provided by the WRL. The tested viruses were "SAT2" isolates obtained from El Rdwan outbreak, one at the sixth and the other at the tenth kidney passage levels. The test was carried out as described by Booth *et al.,* (1978) but using CKC.

In both assays results were obtained 2-3 days post seeding without the need to fix and stain cultures. Neutralization titres were calculated according to the method of Karber (1931). The variance log SN50 was computed according to the methods described by Rweyemamu and Hingley (1984).

Disease transmission:

Calves of the local breed were screened for absence of antibodies against "SAT2" type of FMDV by the SN test. For reason of bio-security only one animal of the screened negative group was housed in a bio-secure facility and inoculated through the *intradermal-sublingual* route, with 2.9 and 3.9 $\log^{10}$ TCID50 of the local isolate designed SAT2-Kh 1/08 (at the 7th CKC passage level), in 0.1 ml of GMEM, each at two different sites. Rectal temperatures were recorded daily at a fixed time and clinical signs were observed. The animal was bled on day 14, 21 and 30; post-inoculation *sera* were separated and examined by SN test for the development of antibody response.

Results

Virus isolation in CKC culture:

A total of eleven samples were passaged in CKC culture (Table 1). Two isolates were obtained from each of the first and second outbreaks and one from the third.

Cytopathic effects in CKC culture usually appeared 24 hours post inoculation and were of focal nature. On primary isolation more irregular shapes were often seen on the monolayer. Figure (2) shows normal and inoculated CKC cultures.

Identification by the indirect sandwich ELISA and serum neutralization test

One outbreak was typed as "SAT2" and the other two as type "O" (Table 2).

Cell culture isolates at the 4th or 5th passage level were used for ELISA and serum neutralization tests. They were identified the similarly as epithelial suspensions (Table 2). Table (3) shows the neutralization titres obtained with the type specific reference *antisera* in the neutralization test. In each case the other type specific antisera showed titres less than $10^{1\cdot2}$ which is the positive threshold.

Table (1):Virus isolation in CKC culture

Orign of samples	Sample No	Primary isolation	Blind passage
Al Rdowan	1	+ve	
	2	toxicity	+ve on the 2nd passage after dilution of the original inoculum
	3,4,5	toxicity	-ve
Al Amel city	6	+ve	
	7	-ve	-ve
	8,9	-ve	
Al Kamleen	10	+ve	
	11	-ve	-ve

Table (2): Typing of epithelial suspensions and identification of *cell culture isolates*

Orign of samples	Typing by ELISA	Serial No. of isolates	Identification of cell culture isolates ELISA S.N.		Nomenclature
El Rdwan	Type "SAT2"	1	N.D	Type "SAT2"	SAT2-Kh 1/08
		2	Type "SAT2"	Type "SAT2"	SAT2-Kh 2/08
El Kamleen	Type "O"	6	N.D.	Type "O"	O-Kh 1/08
		7	N.D	N.D	
El Kamleen	Type "O"	10	Type "O"	Type "O"	O-Jaz 1/08

Table (3): Neutralization titres of typing test (cell culture isolates)

Virus isolates	Type specific reference antisera SN titre*
SAT2-Kh 1/08	2.25
SAT2-Kh 2/08	1.95, 2.4
O-Kh 1/08	1.8
O-Jaz 1/08	2.25

* log 10

Nomenclature

The isolates were referred to by its serotype, geographical origin within Sudan, year of isolation andorder of isolation from that origin (Table 2).

Experimental disease transmission:

Following inoculation of strain SAT2-Kh 1/08, clinical signs were detected 48 hours later. Mouth lesions were observed at the inoculation sites and in the gum coinciding with slight thermal reaction. No foot lesions were observed. Healing was evident by the eighth day and complete by the thirteen day.

Establishing the *serological* relationship between two "SAT2" isolates following multiple passages in CKC culture:

The log SN50 of the reference *antiserum* corresponding to 100 TCID50 for SAT2-Kh 1/08 and SAT2-Kh 2/08 were computed together with the regression slope (Table 4). The derived r1 value was 0.94.

Exactly 100 TCID50 from each isolate was used in a neutralization assay with post-inoculation *sera* of SAT2-Kh 1/08. Table (4) shows the neutralization titres and the resultant computed r1 values.

Discussion

The presented investigation of the three outbreaks of FMD in the year 2008 in central Sudan showed that two of them were of the type "O" and one was of the type "SAT2". Types "O" and "SAT2" were isolated rom other parts of the country during this same period (Habiela et al, 2010). All FMD suspected materials sent to the WRL between 2004 and 2007 were of these two types excluding one sample of the serotype "A" (Ferris, 2007). Recent *serosurveillance* revealed high prevalence of antibody against type "A" (78%-85%), followed by type "O" (69%-81%) then "SAT2" (44%-65%) and low prevalence for type "SAT1" (9%-20%) (Raouf *et al*., 2009; Habiela *et al*., 2010; These results taken together indicate the maintained activity of three serotypes of FMDV in Sudan; "O", "A" and "SAT2" but low or nullified activity of the *serotype* "SAT1".

All over Africa, types "O" and "SAT2" have shown considerable activity during the last 50 years (Vosloo *et al*., 2002; Aidaros, 2002). In Sudan, type "O" has always constituted more than half or at least half of the positively typed samples within reported periods; 1957 to 1981 (Abu Elzein, 1983) and 1981 to 1989 (Vosloo *et al*., 2002; Ferris, 2007). Similarly since its introduction in Sudan in 1977 (Abu Elzein and Crowther, 1979), type "SAT2", un-

like type "SAT1", was more frequently isolated while the latter *serotype* was lastly recorded in Sudan in 1976 (Abu Elzein, 1983). In two occasions; in 1980 (Abu Elzein *et al*, 1987) and recently in 2005 and 2006 (Raouf *et al*., 2009; Habiela *et al*., 2010), type "SAT1" showed low antibody prevalence (around 10%) i.e. no further increase in the rate of infection. It is not unlikely that it is disappearing altogether from the country.A case, that was not without its match in literature as demonstrated by the disappearance of type "C" from most part of the world (Vosloo *et al,* 2002). Throughout Africa, this *serotype* shows the least activity (apart from type C) causing about 14% and 10% of FMD outbreaks in Western and Eastern Africa respectively. It seems to be mainly maintained in the Southern part of the continent causing about one third of the outbreaks (Vosloo *et al*., 2002).

It worth reasoning in the described type situation, that type "A" clinical disease was detected at low rate despite the fact that it showed wide seroprevalence which was similar to that of type "O" or even higher. Recently, type "O" was recorded in the years 1999, 2004, 2005, 2007 and in this work (2008), whereas during this same period type "A" was detected only once; in 2006 (Ferris, 2007). In older reports type "A" showed also much lower frequency than that of type "O" (Abu Elzein, 1983; Vosloo *et al*., 2002). The described situation could be indicative of low cross protection levels between type "O" circulating field viruses in comparison to that between type "A" circulating field viruses. *Paradoxically*, almost all of type "O" isolates in Sudan were of the subtype O1 Manisa (Abu Elzein and Newman, 1980) whereas serotype "A" is known for its *antigenic* diversity. Accordingly and on other words, it seems that type "A" circulating field viruses were showing higher level of cross protection, that abate the clinical disease but not infection, than those of type "O" though they are expected to be more *antigenically* diverse than the latter. This supposed field situation fit more with the established limitation of the subtype classification as certifying protection of the field isolate by another isolate or a vaccine within this subtype. Instances were known that a vaccine within a subtype might not protect against all isolates within the subtype, but conversely a vaccine within a subtype might protect against isolates within different subtypes (Rweyemamu, 1984; Pay, 1985; Fenner *et al*., 1987; Kitching *et al*., 1989). After all, the r1 value derived between a field and a vaccine strain is an indication of serological relationship and not protection. That is for many reasons, among which is the variability of the in vivo immune response under different conditions (Ferris and Donaldson, 1992). On the other hand, quite relevant with the deduced low cross protection levels between type "O" circulating field viruses is that more "O" serotype antigen is always required in FMD vaccines than "A", "C", "Asia1" serotypes, to achieve an equivalent potency (Doel, 2003).

Diagnosis of FMD in this work was achieved by virus isolation and identification using typing microserum neutralization test and ELISA, and by transmission of disease experimentally. Fulfilling all these aspects was of particular significance when dealing with diagnosis of the condition for the first time at the local level. After multiple passages in CKC culture, strain SAT2 Kh 1/08, produced in one local breed calf, mouth lesion and slight thermal reaction 48 hours post inoculation. In comparison, strain O/UKG/2001 responsible for the 2001 FMD epidemic in the United Kingdom after 4 passages in CTY cells and swine kidney cell line, when inoculated in pigs, produced mouth and foot lesions, but no pyrexia, 2-3 days post inoculation (Aggarwal *et al*., 2002). As low as 100 TCID50 or less (Alexandersen and Mowat, 2005) was reported to establish infection through damaged epithelial lining. Nevertheless, since only one local breed calf was used in this work that could demonstrate the likely high susceptibility of our local breeds to this type of infection and at the same time showed that the virus has retained its pathogenicity for cattle through successive passages in CKC culture.

Two isolates were obtained from each of two outbreaks and one from the third. Obtaining more than one isolate from one FMD outbreak is indicated for vaccine matching studies (OIE, 2008). Clinical samples usually reach the national laboratory in good quality and in relatively shorter time in comparison with samples sent to a regional or international laboratory as expected Ferris and Donaldson (1992). The CKC culture was reportedly used for isolation of FMDV from epithelial samples (Bachrach et al, 1955; Sakamoto *et al*., 2002 OIE, 2008) and from oesophageal-pharyngeal (OP) fluid containing smaller amount of FMDV after treating with trifluorotrichloroethane (TTE) (Sutmoller *et al*, 2003). The present work shows that it could be satisfactory for isolation of FMD virus from epithelial samples of good quality. Secondary cultures rather than primary cultures were used in this

Table (4): *Serological* relationship between SAT2 isolates

Virus	SAT2 reference antiserum			SAT2-Kh 1/08 post-inoculation serum	
	Variance log SN50	r1 value	regression slope	Log 10 neutralizing titre with 100 TCID50	r1 value
SAT2-Kh 1/08	2.966		2.94	3.3	1
SAT2-Kh 2/08	3.15	0.94	2.77	2.85	0.86

work which could be an added advantage. The proposed nomenclature for these isolates (Table 2) is designed to be useful when applied to Sudan. It follows the system used for identification of isolates as they enter the WRL. It defines the serotype of these isolates and their geographical relatedness within Sudan. Future antigenic or genetic relatedness could be added to the designation. There is no an internationally accepted nomenclature for referring to strains of FMD (Kitching *et al.*, 1989). Generally speaking, useful method of nomenclature should define the serotype, history and beneficial relatedness (geographical, antigenic and genetic) of an isolate.

The reference test for *antigenic* profiling of field isolates is the VN test (Rweyemamu *et al.*, 1977; Rweyemamu, 1984). In the recent *pragmatic* approach that is endorsed instead of subtyping (Rweyemamu, 1984; Pay, 1985; Fenner *et al.*, 1987; Kitching *et al.*, 1989), it is absolute to derive the r1 value between different field isolates and then between representative field isolates and candidate vaccines. To establish the system in CKC culture, meanwhile bovine vaccinal sera (BVS) being produced, the r1 value was derived between the two isolates of the "SAT2" type of virus using reference and post-inoculation sera. Both isolates originated from one outbreak. The computed high r1 value of 0.9 and 0.86 indicated good repeatability of the system and also signified no evidence for mutational change that involved the structural protein (SP) during passage of these viruses in CKC.

Impact

One factor which adds to the complexity of the *epidemiology* of FMD is the multiplicity of types of virus. The manuscript demonstrated the activity of three types of FMDV in Sudan; "O", "SAT2" and "A". Further, it argued that the third type ("A") could largely be circulating unnoticed. These facts are of significance when considering control. That is on one hand; on the other hand the document exhibited the establishment of diagnostic facilities of FMD at the local level which is also indispensable for future control plans.

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OCCURRENCE OF CRYPTOSPORIDIUM SPECIES COPRO-ANTIGEN IN ASYMPTOMATIC CATTLE

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PRÉSENCE D'ESPÈCES DE CRYPTOSPORIDIUM COPRO-ANTIGÈNES CHEZ LES BOVINS ASYMPTOMATIQUES

Résumé

Un total de 341 échantillons de matières fécales ont été prélevés auprès des bovins ne présentant pas de diarrhée et sélectionnés au hasard, dans l'État d'Oyo, au sud-ouest du Nigeria. Les échantillons ont été classés en fonction de la tranche d'âge: - moins de 6 mois d'âge (69), entre 7-12 mois (71) et plus de 1 an (191). Chaque échantillon a été testé pour identifier la présence de copro-antigènes Cryptosporidium sp. à l'aide d'un kit d'essai d'immuno-absorption disponible dans le commerce pour les essais recouvert d'enzyme Cryptosporidium spécifiques (anticorps monoclonal) dirigés contre des antigènes de Cryptosporidium parvum. La prévalence globale des antigènes de Cryptosporidium dans l'échantillon était de 28,1% (96/341). La prévalence chez les veaux de moins de 6 mois (46,3%) était significativement plus élevée que celle observée (29,6%) chez ceux âgées de 6 à 12 mois (P = 0,0408) et celle de (22,5%) chez les bœufs de plus de 12 mois (P = 0,002). Les veaux de moins de six mois (<6 mois) étaient deux fois plus exposés au risque d'être infectés que ceux de 6 à 12 mois (OR: 2,059, IC 95%: 1,0 à 4,1) et 12 mois (OR: 2,977, CI 95%: 1,7 à 5,3) respectivement. En conclusion, la présente étude est la première à démontrer la présence d'antigènes d'espèces Cryptosporidium bovine dans des échantillons fécaux en provenance du Nigeria et révèle clairement que l'espèce Cryptosporidium sp. est répandue chez les bovins non-diarrhéiques. Par conséquent les bœufs non-diarrhéiques ainsi que ceux ayant la diarrhée doivent être pris en compte dans la conception des mesures de contrôle pour réduire le risque de transmission de la maladie à d'autres animaux et aux hommes.

Mots-clés: Cryptosporidium; bovins diarrhéiques et non-diarrhéiques, copro-antigènes, humains

Abstract

A total of 341 faecal samples were collected from randomly selected cattle without diarrhea from Oyo state, south western Nigeria. Samples were classified based on age range: less than 6 months of age (69), between 7-12 months (71) and greater than 1 year old (191). Each sample was tested for the presence of Cryptosporidium sp copro-antigens using a commercially available enzyme-linked immunosorbent assays kit coated with Cryptosporidium-specific (monoclonal) antibodies raised against antigens of Cryptosporidium parvum. The overall prevalence of Cryptosporidium antigens in the sample was 28.1% (96/341). The prevalence (46.3%) was significantly higher in calves < 6 months than (29.6%) in ages 6 -12 months (P = 0.0408) and (22.5%) in cattle greater than 12 months (P=0.002). Calves < 6 months were twice at risk of being infected than 6 – 12 months old (OR: 2.059; 95%CI: 1.0 - 4.1) and 12 months old (OR: 2.977; 95%CI: 1.7 - 5.3) respectively. In conclusion, the present study is the first to demonstrate the presence of Cryptosporidium species antigen in bovine faecal samples from Nigeria and also clearly reveals that Cryptosporidium sp. is prevalent in non-diarrhoiec cattle. Therefore non-diarrhoeic cattle as well as diarrhoeic ones should be considered in the design of control measures to reduce the risk of transmission of the dieases to other animals and humans.

Keywords: *Cryptosporidium*; *diarrhoeic*; non-diarrhoeic, Cattle; copro-antigen; humans;

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Introduction

ENTERIC cryptosporidial infection is a well-recognized cause of diarrhea in humans and animals, with increase in prevalence and severity of infection in immunodeficient humans, such as those with AIDS, as well as in neonates of some mammal species, such as ruminants (de Graaf *et al*., 1999; Caccio *et al*., 2005). Opportunistic infection with *Cryptosporidium* species remains a major threat to HIV-infected patients who do not have access to highly active *antiretroviral* therapy (HAART), especially in developing countries (Smith and Corcoran, 2004; Silva *et al*., 2005).

While there are still controversies over the taxonomic status of the *Cryptosporidium* sp that causes zoonotic and *anthroponotic* infection in humans (Hunter and Thompson, 2005), there are enough evidence to suggest that C. hominis and C. parvum are the species mostly recongnized in human (Alves *et al*.,2003; Hunter and Thompson, 2005; Sulaiman *et al*., 2005); with C. parvum from cattle as the major source of infection for humans and environmental contamination (Agus, 1990; Tzipori, 1983; Hunter and Thompson, 2005).

Cryptosporidium sp has been highly associated with diarrhea (Quilez *et al*., 1996; de Graaf *et al*., 1999) and has been described as the most common causative agent of intestinal *cryptosporidiosis* neonatal diarrhea syndrome in calves, lambs and goat kids (Bjorkman *et al*., 2003; de Graaf *et al.,* 1999; O'Handley *et al*., 1999; Santin et al., 2004; Xiao, 2010). Although, there are evidences to also show that the infection may not always be associated with diarrhea (Kaminjolo *et al*., 1993; Snodgrass et al., 1986) and that the diarrhea observed in cryptosporidiosis may be as a result of other enteropathogens (de Graaf *et al*., 1999).

With growing evidences that demonstrate the public health importance of C. parvum in developed nations like U.S. (Fayer *et al*., 2005) and England (Hayes *et al*., 1989; McLauchlin *et al*., 2000); as well as in some developing nations like Uganda (Tumwine *et al*., 2003), Kenya (Gatei *et al*., 2003,2006) and Malawi (Peng, 2003) (Peng et al., 2003); *Cryptosporidium parvum i*nfection is now recognized worldwide as an important emerging disease with zoonotic importance (Agus, 1990).

In Nigeria, reports of cryptosporidiosis in animals were based on microscopy of stained oocyst in feces (Ayeni *et al*., 1985; Ibrahim *et al*., 2007) and there is yet to any study on the use of immunoassay in the study of cryptosporidium infection in Nigerian cattle.

This study therefore aims at determining the prevalence of *Cryptosporidium* sp. copro-antigen in *asymptomatic* cattle from Oyo state of Nigeria, using an enzyme *immunoassay* technique. Data from this study will not only enable further assessment of the risk of this disease in cattle, but will also serve as a reliable basis for future molecular studies on the genotype of circulating species of the *protozoan* in cattle in Nigeria.

Materials and Methods

Sampling methods

Stool specimens were collected from 341 randomly selected white Fulani cattle without diarrhea from farm settlements in Oyo-state (south-western) of Nigeria. The sample size was determined using Win episcope software (Ortega *et al*., 1996). Taking an expected prevalence of 50%, a confidence interval of 95% and an error of 5% with an estimated cattle population of 3 million. Animals were classified based on their age range: less than 6 months of age (69), between 7-12 months (71) and greater than 1 year old (191).

A single faecal sample was taken from the rectum of each animal by disposable plastic bag and emptied into wide mouthed disposable plastic container. The faecal samples were transported to the laboratory and kept at – 4°C without preservative until processed.

Detection of *Cryptosporidium parvum* copro-antigen

Cryptosporidium sp copro-antigen was detected using a commercial

ELISA kit for stool samples (RIDASCREEN Cryptosporidium, R-Biofarm, Germany) according to manufacturer's instructions.

In the RIDASCREEN® *Cryptosporidium* test, specific antibodies are used in a sandwich-type method. *Cryptosporidium*-specific (monoclonal) antibodies against antigens of *Cryptosporidium* parvum are applied to the surface of the well in the microwell plate. A suspension of the stool sample to be tested and the controls are pipetted into the wells of the microwell plate. Next, antibodies conjugated with *peroxidase* against the antigens of *Cryptosporidium* parvum are added and the plate incubated at room temperature (25 -30°C). In the presence of Cryptosporidium antigens in the sample, a sandwich complex forms which is made up of the immobilised antibodies, the Cryptosporidium antigens and the conjugated antibodies. Unattached enzyme-labelled antibodies are removed during the washing phase. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. On adding the stop reagent, the colour changes from blue to yellow. The extinction is proportional to the concentration of *Cryptosporidium* antigen present in the sample.

Statistical analysis

Data were computed using Prisms and *Winepiscope* (statistical) software. X^2 test and one-way Anova was used to compare the differences in prevalence of *Cryptosporidium* oocysts between age-groups of cattle at 5% level of significance. Associations between variables were computed with 95% confidence intervals on overall prevalence.

Results

The result of the enzyme immunoassay is presented in table 1. 28.1% (96/341) stool samples were positive for *Cryptosporidium sp antigen* (O.D > 0.332). Calves of < 6 months old had the highest infection rate of 46.3 % (32/69), while those between 6 – 12 months and greater than 12 months had infection rates of 29.6% (21/71) and 22.5% (43/191) respectively. The prevalence of *Cryptosporidium* infection in the age groups was significant different (P = 0.0008), with the infection rate decreasing with increase in age. Cryptosporidium infection was significantly higher in calves < 6 months than ages 6 -12 months (P = 0.0408) and in cattle greater than 12 months (P=0.002). Calves < 6 months were twice and about thrice at risk of being infected than 6 – 12 months old (OR: 2.059; 95%CI: 1.029 - 4.120) and 12 months old (OR: 2.977; 95%CI: 1.681 - 5.270) respectively. No statistically significant difference (P > 0.05) was observed between calves of 6 – 12 month and the adults greater than 12 months and also among the different sexes.

Discussion

Cryptosporidium sp. has been highly associated with diarrhea, especially in calves (Quilez *et al*., 1996; de Graaf et al., 1999). However, the result of this study showed that Crypt*osporidium sp c*opro-antigen was found in 28.1% (96/341; 95% CI: 23.4–32.9) of cattle without diarrheoa surveyed in Oyo state, South western Nigeria. This study supports earlier studies that suggested that cryptosporidium sp. infection is not always associated with diarrhea (Bjorkman *et al*., 2003; Kaminjolo et al., 1993; O'Handley et al., 1999 ;Snodgrass et al., 1986).

Table 1 The Result of ELISA examination for detection of *Cryptosporidium parvum* in different age groups of cattle without diarrhea.

Age range	No positive(O.D > 0.332) / Number examined	Infection rate (%)	95%CI
< 6months	32/69	46.3	0.3431- 0.5844
6 – 12 months	21/71	29.6	0.1870 – 0.4046
> 12 months	43/191	22.5	0.1654 – 0.2849
Total	96/341	28.1	0.2335 – 0.3295

Diarrhoea in Cryptosporidium sp. infection may often be as a result of the presence of other diarrhea-causing enteropathogens in cattle, especially in calves (de Graaf *et al*., 1999), newborn and unwean calves (Castro-Hermida *et al*., 2002; Lefay *et al*., 2000; Lorenzo-Lorenzo *et al*., 1993; Scott *et al*., 1994; Quilez *et al*., 1996).

The reason for the observed relatively high prevalence of Cryptosporidium copro-antigen in cattle without diarrhea is not known; however, the finding may be associated with factors like the type of husbandry employed (free range grazing), herd immunity and absence of concurrent infection, further work is required to study the underlying factors.

The public health significance having a 28.1% prevalence of *Cryptosporidium* copro-antigen in asymptomatic cattle from an ELISA kit coated with Cryptosporidium-specific (monoclonal) antibodies raised against antigens of *Cryptosporidium parvum* cannot be overemphasized, since cattle has been implicated as a major source of C. parvum in pasture run off; believed to be responsible for environmental contamination for human infection either by direct contact or indirect through faecal contamination of food or water for human consumption (Tzipori, 1998). Cryptosporidium parvum infection may therefore constitute a much greater health challenge than envisaged in Nigeria and most developing nations, since cattle often come in close proximity to human source of water and farms during grazing due to the management systems that is mainly free range.

Furthermore, the study affirms that oversight can exist in the study and control of *Cryptosporidium parvum* in livestock, since attention may be given only to cattle with diarrhea, whereas infected ones without diarrhea may continue to serve as reservoir, thus constituting potential hazard to public health.

Conclusion

The present study to the best of our knowledge is the first to demonstrate the presence of *Cryptosporidium* sp copro-antigen in faecal sample from cattle in Nigeria. It has also clearly demonstrated that Cryptosporidium sp is prevalent in Nigerian cattle without diarrhea. Therefore measures to control and prevent Cryptosporidium infection should be considered in both *symptomatic* and *asymptomatic* cattle to reduce the risk of *zoonotic* infection to human.

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EQUINE HELMINTHIASIS IN AND AROUND ASSELA, ARSI ZONE OF OROMIA REGIONAL STATE

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HELMINTHIASE ÉQUINE DANS ET AUTOUR DE LA LOCALITE D'ASSELA, ZONE D'ETAT DE L'ARSI, REGION DE L'OROMIA EN ETHIOPIE

RÉSUMÉ

L'étude a été menée sur un total de 384 étables chacune comprenant 167 chevaux et ânes et 50 mulets au travers d'un échantillonnage aléatoire pour évaluer les niveaux de l'helminthiase équine dans et autour de Assela, Etat régional d' Arsi-Oromia, Ethiopie ; en tenant compte des espèces, du sexe et de l'âge des animaux. L'examen coprologique de qualité fécale a été utilisé pour les œufs d'helminthes. Au total, 345 (89,8%) des animaux examinés étaient positifs pour au moins un helminthes où 93,4%, des ânes, 85,0% des chevaux et 94,0%, des mulets ont été trouves respectivement positifs. Une prévalence de 94,4% chez les jeunes et 89,6% chez les adultes selon l'âge, et 88,9% chez les mâles et 91,5% chez les femelles selon le sexe a été observée. Dans tous les cas, des différences non significatives de la prévalence ont été observées entre les espèces, entre les groupes d'âge et de sexe (P> 0,05). L'analyse qualitative des œufs fécaux a révélé des taux de 71,1% de Strongles spp, 30,73% d'equorum Parascaris (P. equorum), 7,29% de Strongloid westeri, 5,7% des espèces Anoplocephala (spp), 4,43% Oxyuris equi et 1,04% de Fasciola spp. chez les chevaux. Sauf pour les espèces P. equorum, Strongloid westeri et Anoplocephala spp. qui montrent une différence significative d'infection (P <0,05) chez les espèces équines, la prévalence d'autres helminthes particulières parmi les espèces équines, et entre les groupes d'âge et les sexes montrent une différence insignifiante (P> 0,05). Les plus grandes co-infections des équidés par les espèces de Strongles spp. et de P. equorum (17,7%) ont également été observées. Le taux d'infection mixte avec au moins deux helminthes ont été observées dans 20,4% d'ânes, 22,2% de chevaux et 56,0% de mulets, respectivement ; ce qui montre des différences significatives (P <0,05) chez les équidés. Ces helminthes ont un grand impact sur la santé et la productivité des espèces équines, en fonction de l'écologie, des pratiques d'élevage et de l'impact des maladies. Il est important de développer la prévention et le contrôle intégré et durable des maladies par le déparasitage stratégique, la rotation des pâturages et l'identification des espèces de parasites pour un dosage correct des anti-helminthes.

Mots clés: Assela, chevaux, coprologie, helminthes, prévalence

ABSTRACT

Study was conducted on a total of 384 equine comprising 167 each horses and donkeys, and 50 mules by randomly sampling to assess the statues of equine helminthiasis in and around Assela, Arsi-Oromia regional state, Ethiopia by considering species, sex and age of the animal. Qualitative *coprological fecal* examination was used for eggs of *helminthes*. A total 345 (89.8%) of examined equine were found positive for at least one *helminthes* where 93.4%, 85.0% and 94.0% of donkey, horses and mule are positive, respectively. A prevalence of 94.4% in young and 89.6% in adults by age, and 88.9% in male and 91.5% in female by sex were observed. In all circumstances, insignificant differences in prevalence were observed among species, between age groups and sex groups (P>0.05). Qualitative fecal egg analysis revealed the rate of 71.1% *Strongyles* spp, 30.73 % *Parascaris equorum* (P. equorum), 7.29 % Strongloid westeri, 5.7% *Anoplocephala* species (spp), 4.43 % *Oxyuris equi* and 1.04% *Fasciola* spp in equine. Except for *P. equorum*, *Strongloid westeri* and *Anoplocephala* spp which shows significant difference infection (P<0.05) among equine species, the prevalence of other particular helminthes among equine species, and between age and sex groups show insignificance difference (P>0.05). Highest co-infection of equines with Strongyles spp and P. equorum (17.7%) were also observed. The rate of mixed infection with at least two *helminthes* were observed in 20.4%, 22.2% and 56.0% of donkey, horse and mule, respectively showing significant differences (P<0.05) among equine species. These *helminthes* have great impact on health and productivities of equine, depending up on the ecology, husbandry practices and impact of the diseases, it is important to develop sustainable integrated diseases prevention and control through strategic deworming, rotational grazing and parasite species identification for proper dosing of *antihelminthes*.

Key Words: *Assela, Equine, coprology, helminthes*, prevalence

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Introduction

Highest equine population in Africa is found in Ethiopia mainly Arsi Zone of Oromia Regional State where they play an important role in rural communities providing power and transport at low cost (Tegegne *et al*., 1999). They can be used for various agricultural and social operations such as ploughing, planting and weeding. They also provide the much needed transport in rural areas for activities such as carrying water, building materials, agricultural products and people (CTA, 1997). Although horses and mules are faster and more powerful animals for work, they are more costly to buy and maintain them than a donkey (Pearson *et al*., 2003). Despite its huge population, equine remains marginal in health, production, productivity and activity performances due to presence of malnutrition, management constraints and diseases like parasites.

Parasitism represents a major obstacle to development of the livestock sector and hampers the poverty alleviation programs in livestock farming system in the Ethiopia (Hednrix and Charles, 2006; Jobre *et al*., 1991). Parasitic diseases dominated by gastrointestinal parasitosis are serious health hazards, contributing to poor body condition, reduced power output, poor reproductive performance and short life span (Pearson *et al*., 2003). Horses, donkeys, mules and other equidae are hosts for large numbers of internal parasites. The vitality and well-being of equines of all ages are threatened by a variety of internal parasites. The most common and important equine internal parasites are large strongyles, small strongyles, *Parascarids*, pin worms (*Oxyuris equi*), and equine bots such as *Gastrophilus intestinalis*, *Gastrophilus nasalis* and *Gastrophilus haemorrhoidalis*. Additionally, Hednrix and Charles (2006) and Pearson *et al*. (2003) indicates less important infection of cestodes, lung worms, trematodes and the intestinal thread worms (strongyloids) in equine. Although few studies have been conducted on helminthes infections of equine in some other areas of Ethiopia (Hednrix and Charles, 2006; Gebreab *et al.,* 1997; Shiferaw *et a*l., 2005; Shiferaw *et al.,* 2001), such study no has not been conducted in and around Assela town where highest equine population are found in the country. Hence, it is necessary to examine the status and impact of these diseases and existing control measures with a view of directing control strategies.

Material And Methods

The study was conducted from November 2008 to March 2009 in and around Assela the capital of Arsi zone of Oromia region located 175 Km from Addis Ababa, capital city of the Ethiopia. *Topographically*, the area is known to be a high land ecosystem with altitude ranging from 2000 to 3000 meter above sea level, annual rain fall 1300-1350 mm, average temperature range of 18-25^{0}C during dry season and 5-10^{0}C during wet season. Most of the sampling sites were humid high land area with long rainy season. The main farming system was a mixed one in marshy communal grazing lands with horses, donkey, cattle, sheep, goat and mule. A simple random sampling with 50% expected prevalence and precision of 5% was used by considering species, age and sex group of horses, donkeys and mules in the selected study area. The age of the selected donkeys was determined by dentition (MAFF, 1977). A total of 384 animals were sampled. Of these, 167 were horses, equal number donkeys, and 50 mules (Table 1).

The population of mules is low and most of them are kept indoor with few grazing period during wet season. Faecal samples were taken directly from the rectum with strict sanitation sterile using hand groves and placed in air and water tight sample vials, and then brought to the laboratory for direct microscopic examinations (Soulsby, 1982; Urquhart *et al*., 1996). Qualitative coprological examination was used for identification of eggs of helminthes using egg morphology, shape and other visible structures (Anon, 1977, Soulsby, 1982). The data collected during the study period were entered in MS-Excel and analyzed using Stata 0.7 and SPSS- 0.5.11 for prevalence and significance of associations between various variables. The Chi-square (x2) was calculated and the significance of association between and among the variables determined using P-value.

Table 1: Simple random sample distribution study *equine* in age and sex groups

Species	Age groups (Numbers)			
	Young (18)		Adult (366)	
	Sex		Sex	
	Male	Female	Male	Female
Donkey (167)	7	1	97	62
Horses (167)	1	9	109	48
Mules (50)	0	0	29	21
Total (384)	8	10	235	131

Results

As shown in Table-2, out total of 384 equine species examined during the study period, a total of 345 (89.8%) were found positive for at least one *helminthes* parasite. The *helmenthes* prevalence of 93.4%, 85.0% and 94.0% were observed in donkey, horses and mule, respectively. Similarly, the *helminthes* prevalence of 94.4% in young and 89.6% in adult equine was observed. With regards to the sex of animal, 88.9% of male and 91.5% of female were found positive for *helminthes*. Insignificant differences in prevalence of equine *helminthes* were observed among species, between age groups and sex

Table 2: Prevalence of *helminthiasis* among various *Equine* species by age and sex

Risk factors		Total No. examined	Total (%) Positive No.	(%) positive No of parasite observed					
				Strongyles spp	P. equorum	O. equi	Stron.. westeri	Anoplocephala species	Fasciola species
Total		384	345 (89.8)	273 (71.1)	118 (30.7)	17 (4.4)	28 (7.3)	23 (5.7)	4 (1.04)
Species	Donkey	167	156 (93.4)	119 (71.3)	54 (32.3)	4 (2.4)	18 (10.8)	5 (3.0)	0 (0)
	Horses	167	(85.0)	(68.3)	(24.6)	(7.2)	(3.0)	(5.4)	(1.798)
	Mules	50	(94.0)	(80.0)	(46.0)	(2.0)	(10.0)	(18.0)	(2.0)
			x2	2.583	8.6747	5.3291	8.1088	15.5772	3.1262
			P-value	0.275	0.013*	0.070	0.017*	0.000*	0.209
Age	Young	18	(94.4)	(722)	(44.4)	(11.1)	(11.1)	(5.5)	(0.0)
	Adult	366	(89.6)	(71.1)	(30.1)	(4.1)	(7.1)	(6.01)	(1.09)
			x2	0.0117	1.6689	1.9941	0.4075	0.0063	0.1988
			P-value	0.914	0.196	0.158	0.523	0.937	0.656
Sex	Male	243	(88.9)	(71.2)	(30.5)	(4.5)	(6.5)	(4.9)	(0.41)
	Female	141	(91.5)	(70.9)	(31.2)	(4.3)	(8.5)	(7.8)	(2.127)
			x2	0.0032	0.0238	0.0155	0.4898	1.2990	2.5493
			P-value	0.955	0.877	0.901	0.484	0.254	0.110

groups. With regards to the prevalence of particular helminthes parasites observed in current study, Strongyles spp were 71.1%, *P. equorum* 30.7% Oxyuris equi 4.4% Strongyloid westeri 7.3% *Anoplocephala* species 5.7% and Fasciola species 1.04%. Only significant differences in the prevalence of *P. equorum, Strongyloides*. westeri and *Anoplocephala* species were observed among the equine species (P<0.05). However, insignificant differences in prevalence of particular helminthes parasite were observed between age groups and sex groups (P>0.05).

The infection rates of *helminthes* in different age and sex groups within equine species are shown in Table 3. For instance, prevalence of *Strongyles* spp was 85.7% in male young donkeys and 95.24% in female adult mule while that of *P. equorum* was 71.4 in male young donkeys and 55.17% in male adult mules.

Mixed helminthes infections were also observed in equines at the study area (Table 4) where the highest co-infection were observed with Strongyles spp and P. equorum (17.7%) and less with Strongyles spp and fasciola (0.26%). Of all equines examined in present study, 34/167 (20.4%), 37/167 (22.2%) and 28/50 (56.0%) of donkeys, horses and mules were found to be infected at least with two *helminthes*, respectively showing significant differences (P<0.05).

Discussion

The present study using *microscopic fecal* examination showed that *helminthiasis* was an important

Table 3. Prevalence of *helminthes* within equine species by age and sex.

Species	Age	Sex	No examined	Strongyles spp	P. equorum	O. equi	Stron. westeri	Anoplocephala species	Fasciola species
Donkey	Young	Male	7	6	5	0	0	0	0
		Female	1	0	0	0	1	0	0
	Adult	Male	97	73	16	0	2	0	0
		Female	62	40	20	2	5	0	0
Horses	Young	Male	1	1	1	0	0	0	0
		Female	9	6	2	2	1	1	0
	Adult	Male	109	73	23	8	4	8	0
		Female	48	34	15	2	0	0	3
Mules	Young	Male	0	0	0	0	0	0	0
		Female	0	0	0	0	0	0	0
	Adult	Male	29	20 (68.96)	16 (55.17)	1 (3.45)	0 (0)	3 (10.34)	1(3.45)
		Female	21	20 (95.24)	7 (33.33)	0	5 (23.81)	6 (28.57)	0 (0)

Table 4: Mixed infections of *helminthes* among study *equine*

Types of Parasites	No. examined	Positive No (%)
Strongyles spp and *P. equorum*	384	68 (17.7)
Strongyles and *Oxyuris equi*	384	4 (1.04)
Strongyles and *Anoplocephala species*	384	9 (2.34)
Strongyles and *Fasciola species*	384	1(0.26)
Strongyles and *Strongyloid westeri*	384	8 (2.08)
P. equorum and *Anoplocephala species*	384	7 (1.8)
P. equorum and *Strongyloid westeri*	384	8 (2.08)
Oxyuris equi and *Anoplocephala species*	384	2 (0.52)
Strongyles, P. equorum and *Strongyloid westri*	384	3 (0.78)

equine health significant disease/condition in the study area. The high helminthes prevalence (89.8%) observed in present study across the various equine species sampled is similar with the reports of Fikru *et al.* (2005) in Wonch, Mulate (2005) in South and North Wollo zones, and Soulsby (1982) in Western highlands of Oromia in equines. This might be due to susceptibility of all equine species to currently observed helminthes parasites, similarity of ecology of the study area which favors the maintenance of parasites mainly *Strongyles* spp and *P. equorum*. The insignificant difference and similarity in prevalence of helminthes parasite burden between age groups and sex groups within in and among equine species is similar with work of Fikru *et al.* (2005). This could be due to equal exposure of all groups of equine in a mixed grazing system in the study area. Similar reasoning was given by (Francisco *et al.*, 2009; Radostits *et al.*, 2007; Soulsby, 1982) in which the management systems such as mixed grazing one of the risk factor for exposure and infection helminthes.

The prevalence of Strongyles spp is higher (71.1%) in present study than all other parasite observed in the present study. This might be due to suitability of marshy environment in the study area. Similar reasons were given by Urquhart *et al.* (1996) in that marshy environments are suitable for developments of large strongyles in their long pre-patent period which ensures that larvae acquired in one grazing season where it only reach maturity during the next season. Although the present study is direct microscopic, the current Strongyles spp prevalence was in accord with the work of (Ayele *et al.*, 2006; Fikru *et al.*, 2005; Mulate, 2005) using McMaster egg count who's indicated that fecal worm egg counts begin to rise to severe levels during the wet season in Ethiopia. The 30.7% *P. equorum* infection in equine species is in agreement with (Mulate, 2005) who reported 43.8% in south and north Wollo provinces in Ethiopia. But it is in-agreement with 17.3% findings of Fikru *et al.* (2005). This could be due to similarity in the topography and ecology of the study area. Heavy infections of *P. equorum* causes impaction and perforation leading to fatal peritonitis (Ayele *et al.*, 2006; Urquhart et al., 1996). The 4.4% Oxyuris equi prevalence among was similar with 6% that reported by Ayele *et al.* (2006) at Dugda Bora Worada in donkeys. The present 7.3% prevalence of Strongyloides. westeri was among equine is lower than the 32% reported by Ayele *et al.* (2006) in donkeys as determined by coproculture in Dugda Bora Worada in the same season. This difference could be due to difference in techniques of *helminthes* identification, pre-patent and the study areas. Similar reasoning was given by Soulsby (1982). The current 5.7% prevalence of Anaplocephala species is found to be similar with 7.6% reports of Ayele *et al.* (2006) in donkeys. This could be due to the seasonality of vectors which is the Orbited mites. Soulsby (1982) indicated that the occurrence Anaplocephala species is associated with the vector prevalence. Similarly, the 1.04% Fasciola species recorded in the present study was comparable with 1.5% Fasciola species observation in donkeys at Dugda Bora Worada by Ayele *et al.* (2006). Compared to other parasites observed in present study, the prevalence of *Fasciola* species were lower. This might be due to the unsuited ecological condition for the development of and/or low occurrence of intermediate host snails. The high altitude agro ecology of in and around Assela does not permeate snail development. As Hammami and Ayadi (1999) have reported that permanent dampness, suitable luminosity, basic pH of the soil and water and temperature contribute to multiplicity of snails in a given ecology.

The significantly high rate of mixed infection with at least two *helminthes* was observed in mule (56.0%) compared to that of in donkeys and horses in present study. This could be due to susceptibility of mule than others equines under the study since they have low exposure due to their management in the area. In addition to this most of mules are in the study area are kept indoors with dry feed and few grazing period during wet seasons. Similar reasoning was given by Radostits et al. (2007; Soulsby (1982) in that exposure animal to the infected/contaminated grazing area has high risk of infection which results in high prevalence of helminthes. The highest co-infection of equine species in the study area with Strongyles and *P. equorum* in present study could be from agro ecology of the study area which favors the parasites. Similar idea was also generated by Radostits *et al.* (2007) and explained that horses, donkeys, mules and other equines are host for high number of internal parasites. The horse is susceptible to more than 60 internal parasites, and may harbor several species of worms at any one time (Stoltenow and Purdy, 2003). Moreover, (Hednrix and Charles, 2006) suggest that *strongyles* are ubiquitous parasites in the world.

In general, all sex and age groups of equine species are more susceptible to *strongyles* infection than other parasites species observed in the present study area. All helminthes parasites observed in present study have greater

impact directly or indirectly on equine health and production. Mainly mixed infection indicates the burden of parasites in mixed grazing system in and around Assela. Of primary importance are the large strongyles namely *Strongylus vulgaris*, S. edentatus and S. equines because adults are voracious blood suckers and cause anemia, weakness, diarrhea, and damage of the intestinal lining, and larvae, migrate to the branches of the intestinal mesenteric arteries where they may cause damage, irritation and *parasitic aneurysm*. Hence, strategic de-worming using broad spectrum *antihelmintics* drugs and rotational grazing program shall be implemented to reduce pasture contamination and infection of susceptible hosts. Additionally, parasite identification to the species level shall also be recommended to take proper control and preventive measurers.

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A RETROSPECTIVE STUDY OF BRUCELLOSIS SEROPREVALENCE IN COMMERCIAL AND SMALLHOLDER CATTLE FARMS OF ZIMBABWE

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UNE ÉTUDE RÉTROSPECTIVE DE LA SÉROPRÉVALENCE DE BRUCELLOSE DANS LES ÉLEVAGES DE BOVINS ET DE PETITS EXPLOITANTS COMMERCIAUX DU ZIMBABWE.

Résumé

Une étude rétrospective portant sur 10 années (1995 - 2004) a été menée afin d'évaluer la séroprévalence et les tendances de la brucellose auprès des petits exploitants commerciaux élevant des bovins laitiers et de viande au Zimbabwe ; en utilisant les données de surveillance du Laboratoire Central Vétérinaire à Harare. Les cas positifs ont été analysés en fonction du secteur agricole, de la province administrative, des tendances mensuelles et annuelles. La prévalence et la séroprévalence générales de la brucellose aux niveaux individuel et du troupeau ont été estimées à 2,9% (2417/183990) et à 17,8% (505/2833), respectivement. La séroprévalence de brucellose a varié considérablement entre les huit provinces du pays ($p < 0,05$) mais pas par secteur agricole. La séroprévalence de brucellose était plus faible dans les zones où les laiteries commerciales étaient prédominantes et où le contrôle de la brucellose a été appliquée. Un modèle de régression logistique a identifié la taille du troupeau, l'année et la province administrative comme étant des facteurs indépendamment liées à la probabilité accrue de la séropositivité, mais la saisonnalité n'a pas été démontrée. Ces résultats ont démontré que la brucellose est d'importance égale tant dans les fermes commerciales que dans les petites exploitations. Les déplacements accrus du bétail entre les deux secteurs de l'agriculture a permis de mettre en évidence l'augmentation de la brucellose dans les domaines des petits exploitants. C'est ainsi que, l'application des mesures de contrôle est nécessaire pour réduire la séroprévalence de la brucellose.

Mots clés: séroprévalence de la brucellose, bovins de production mixte de lait et de viande de bœuf, Zimbabwe.

Abstract

A retrospective study covering 10 years (1995 – 2004) was conducted to investigate *brucellosis seroprevalence* and patterns in commercial and smallholder mixed dairy-beef cattle in Zimbabwe using surveillance data from the Central Veterinary Laboratory at Harare. Positive cases were analysed according to farming sector, administrative province, monthly and annual trends. The overall individual and herd- level *brucellosis seroprevalence* were estimated at 2.9% (2417/183990) and 17.8% (505/2833) respectively. *Brucellosis seroprevalence* varied significantly among the eight provinces of the country ($p<0.05$) but not by farming sector. *Brucellosis seroprevalence* was lower in areas where commercial dairies were predominant and where brucellosis control was enforced. A logistic regression model identified herd size, year and administrative province as independently associated with increased odds of seropositivity, but seasonality was not demonstrated. These results demonstrated that brucellosis is of equal significance in both commercial and smallholder farms. Increased cattle movement between the two farming sectors has allowed build up of brucellosis in smallholder areas. Thus, the enforcement of control measures is required to reduce *brucellosis seroprevalence*.

Key words: *Brucellosis seroprevalence*, mixed diary-beef cattle, Zimbabwe

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Introduction

Bovine brucellosis is a disease of both economic and public health significance in many countries in the world. The disease is caused by biovars of *Brucella abortus,* a Gram-negative, coccobacillary and facultative intracellular bacterium. World-wide, bovine brucellosis is mainly caused by B. abortus biovar 1, while biovar 2 is less common (Quinn *et al*., 1999). The other biovars are infrequently isolated and are believed to be rare causes of the disease (Quinn *et al*., 1999). However, there are no proven differences in the *pathogenicity* of the *biovars* of B. abortus (Nicoletti, 1980).

Bovine brucellosis was first reported in Zimbabwe in 1913 (Bevan, 1914). The disease was closely monitored using the milk ring test (MRT) as well as *serological* tests (Mohan *et al.*, 1996) and later gazetted as notifiable (Anon., 1995). Cattle production in Zimbabwe is broadly divided into commercial and smallholder sectors. Commercial cattle farmers usually keep large herds (>100 cattle per herd) and use exotic breeds (Bos taurus), supplemented with local breeds (Bos indicus). Under this sector there are beef and dairy farms or mixed dairy-beef farming enterprises. Dairy cattle are usually intensively managed and kept in smaller farms compared to beef cattle that are often kept in ranches but may also be intensively raised in feedlots. In the smallholder sector, mainly beef cattle of the Bos indicus origin are kept usually as small household units (median size 15 cattle per household) which are kept in pens during the night but often mix with other cattle from the village during the day when grazing and watering (Matope *et al*., 2010). In some areas, smallholder dairy co-operatives have been established using Bos taurus breeds purchased from commercial farms (Matope *et al*., 2010). Previous studies showed higher herd brucellosis seroprevalence ranging between 10-53% in commercial cattle farms in different regions of the country compared to 0-25% in the smallholder sector (Madsen, 1989; Manley, 1969; Matope *et al*., 2010; Mohan *et al*., 1996). However, in recent years, the country has faced many challenges that include reduced delivery of veterinary services following the economic depression that has affected the country since the year 2000. Further, the introduction of the land reform programme in the same year brought about increased movement of cattle between the commercial and smallholder farming sectors (Matope *et al*., 2010). These factors could have resulted in the spread of brucellosis to new areas that were previously free from the disease. But there is lack of information on brucellosis seroprevalence in both commercial and smallholder cattle farming sectors in the whole country. This study was carried out to investigate brucellosis seroprevalence and establish temporal trends in commercial and smallholder mixed dairy-beef cattle farms from all the eight administrative provinces of Zimbabwe, using data collected on routine annual *brucellosis* testing from 1995 to 2004. Such data could be a convenient and inexpensive source of information that can be sued to study the *epidemiology* of *brucellosis* in Zimbabwe.

Materials and Methods

Study areas

Zimbabwe is located in Southern Africa, between 25 - 33^{0}E and 16 - 22^{0}S, with a subtropical climate that is characterised by seasonal rainfall (November to March) and a dry period (April to October). The country is divided into eight administrative provinces that are further split into several districts. According to land use, the country is demarcated into five agro-ecological regions (designated I-V) based on rainfall pattern, topography and terrain that in turn determined human settlement and agriculture. Most of the commercial agriculture (both cropping and animal husbandry) is carried out in regions I to III that are characterised by good soils and moderate to high rainfall (800-1200mm per annum). The smallholder farming is predominantly carried out in communal areas that are mainly located in agro-ecological regions III to V. These areas are characterised by low and erratic rainfall patterns (<600mm per annum). A summary of the cattle production systems in the different provinces is summarised (Table 1).

Sampling

Records of the annual *brucellosis serological* testing of cattle from each of the eight administrative provinces of Zimbabwe were obtained for the years 1995-2004 from the Department of Veterinary Services. The participating herds were selected by the Department of Veterinary Services for an annual brucellosis serological surveillance programme. The *serological* testing was coordinated and supervised by state chief animal health inspectors in each of the respective eight provinces and was not influenced by cattle owners. From each of the selected herd, all cattle ≥ 2 years were eligible for serological sampling and testing. Since the definition of a herd is difficult to fulfil in smallholder farming areas due to mixing of animals, all cattle from one village were regarded as one herd.

Laboratory tests

All the laboratory tests were conducted at the state-owned Central Veterinary Laboratory in Harare. A serial testing programme (Madsen, 1989), was used to analyse all sera. Briefly the Rose Bengal test (RBT) (Alton *et al*., 1988), was used to screen the sera for anti-brucella antibodies and positive sera were further tested using the serum agglutination test (SAT) (Brinley Morgan, 1967). All the doubtful reactors to the SAT were confirmed by the complement fixation test (CFT) (Alton *et al*., 1988). The antigens for all tests were sourced from the Onder-

Table1. Agro-ecological regions and cattle production systems for each of the provinces of Zimbabwe

Province	Agro-ecological regions	Cattle production system
1. Mashonaland Central	II & III	Intensive beef production and dairy
2. Mashonaland East	II & III	Intensive beef production and dairy
3. Mashonaland West	II, III, IV & V	Intensive and semi-intensive beef production and dairy
4. Manicaland	I, II & III	Intensive beef production and dairy
5. Midlands	III, IV & V	Intensive and semi-intensive beef production and dairy
6. Masvingo	III, IV & V	Intensive and semi-intensive beef production and dairy
7. Matabeleland South	IV & V	Semi-extensive cattle grazing and extensive cattle ranching
8. Matabeleland North	IV & V	Semi-extensive cattle grazing and extensive cattle ranching, few dairies

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Statistical analysis

Statistical analysis was performed using STATA/SE 10.0 for Windows (Stata Corp. College Station, Texas, USA). Individual animals were classified as positive if they tested positive either on the RBT and the SAT with titres of more than 1:80 or SAT titres less than 1:80 but with titres of ≥ 1:4 on the CFT as suggested by Madsen (1989). A herd was classified as positive if at least a single positive reactor animal was identified.

The mean brucellosis seroprevalence for each farming sector was calculated by considering the total number of sera submitted during the study period. To assess spatial variation in seroprevalence during the study period, samples submitted during the study period were separately pooled for each of the eight provinces. Similarly, to assess temporal trends, the samples received from all the provinces were pooled for the respective years. To assess the effect of herd on brucellosis seroprevalence, we arbitrarily categorized herds into groups; small (<51 cattle), moderately large (51 – 200 cattle), large (201 – 300), and very large (> 300 cattle).

A multivariable logistic regression model was used to investigate the association between herd-level variables and *brucellosis seroprevalence* in the eight provinces as well as between two different cattle farming types (commercial and smallholder). A Fisher's exact test was used for univariable testing of the association between the brucella seropositive status of herds (no seropositive animals = 0, ≥1 seropositive animal =1) and potential categorical risk factors while a *Kruskal-Wallis* test was used for herd size as a continuous variable. Since this variable ws skewed to the right, categorising was necessary to correct for linearity problem. The predictor variables were assessed for collinearity by cross tabulations using the two-sided Fisher's exact test. Variables with p-values ≤ 0.25 and with counts ≥5 in each cell were offered to the multivariable logistic regression model.

The logistic regression model was built with the *binomial* herd-level data, *brucella seropositive* status (no seropositive animals = 0, ≥1 seropositive animals =1) as the outcome and the explanatory variables identified in the univariable analyses. The model was manually constructed by a forward selection process as described (Dohoo et al., 2003). The evaluation for goodness-of-fit was done using the *Hosmer-Lemeshow* test while model sensitivity and specificity were assessed using the receiver operating characteristic (ROC) curves (Dohoo et al., 2003).

Results

The mean individual animal-level brucellosis seroprevalence for all the provinces in smallholder cattle was 2.9% (485/16759) compared to 1.2% (1932/161603) for commercial cattle, but this was not significantly different ($p>0.05$). Of the herds tested, the mean brucellosis seroprevalence was 17.8% in both the smallholder areas (140/786) and commercial farms (365/2047) from all the eight provinces. The spatial distribution of brucellosis seropositive individual sera and herds from all the eight provinces are shown (Table 2). The individual animal-level brucellosis seroprevalence ranged between 0.5 - 12.8% (Manicaland and Matebeleland north), and 0.5 – 13.3% (Manicaland and Masvingo) for smallholder and commercial cattle respectively (Table 2). The distribution of seropositive sera within each farming sector differed significantly ($p<0.05$) among the study provinces.

At herd-level, brucellosis seroprevalence ranged between 5 – 53.8% (Mashonaland west and Matebeleland north) and 12.5 – 23.2% (Matebeleland south and Matebeleland north) for smallholder and commercial cattle respectively (Table 2). Similarly, the distribution of seropositive sera within each farming sector differed significantly ($p<0.05$) among the study provinces.

There has been a general decrease in the number of samples submitted for brucellosis serology, dropping from 51565 in 1996 to 1306 in 2004. The first five-year period (1995 – 1999) recorded a significantly ($p<0.05$) higher mean annual number of samples submitted (mean = 30550.2) compared to the second five-year period (2000 – 2004) (mean = 6247.8). The numbers of herds investigated for brucellosis serology dropped from 492 in 1996 to only 64 in 2004 (Fig. 1). Similarly, the first five-year period (1995 – 1999) recorded a significantly ($p<0.05$) higher mean annual number of herds investigated (mean = 413.2) compared to the second five-year period (2000 – 2004) (mean = 153.4).

Table 2. The individual animal- and herd-level Brucella seroprevalence (raw estimates) in the eight provinces of Zimbabwe (1995-2004).

Province	No. of herds tested (Cattle tested)		% Brucella seroprevalence			
	Smallholder	Commercial	Smallholder		Commercial	
			Individual-level	Herd-level	Individual-level	Herd-level
MSC	46 (915)	223 (11340)	4.7	19.6	3.8	22.9
MSE	166 (3908)	423 (83131)	3.5	12.7	0.8	13.7
MSW	139 (1274)	801 (16365)	1.8	5.0	1.7	16.6
MAN	72 (5796)	142 (17397)	0.5	11.1	0.5	19.0
MSV	96 (827)	97 (625)	4.5	16.7	13.3	21.7
MID	170 (2962)	229 (27015)	2.8	20.6	1.2	20.5
MTN	58 (799)	108 (5135)	12.8	53.8	0.9	23.2
MTS	39 (278)	24 (595)	11.2	33.3	1.5	12.5
Total	786 (16759)	2047 (161603)	2.9	17.8	1.2	17.8

MSC = Mashonaland central, MSE = Mashonaland east, MSW = Mashonaland west, MAN = Manicaland, MSV = Masvingo, MID = Midlands, MTN = Matabeleland north, MTS = Matabeleland south

Figure 2 shows the temporal trend of brucellosis seroprevalence in the study areas from 1995 to 2004. The individual-level *brucellosis seroprevalence* in the commercial farming sector was on average low (range; 0.4 - 3.5%) and remained fairly constant from 1995 to 2004. The individual-level *brucellosis seroprevalence* in the smallholder sector was comparable, but showed a marked increase after the year 2001 (Fig. 2). During the study period, for both commercial and smallholder cattle, herd-level *brucellosis seroprevalence* was higher than that obtained for individual animals. Although there was no significant difference in herd level *brucellosis seroprevalence* between the two farming sectors ($p>0.05$), the seroprevalence in the smallholder sector showed a gradual increase between 1995 and 2004 (Fig. 3) (trend line not shown).

When data was categorised into seasons for both farming sectors, there was no significant difference ($p>0.05$) in *brucellosis seroprevalence* at both the individual- and herd-level of aggregation between the wet (November to April) and the dry (May to October) seasons (Fig. 3).

Logistic regression analysis

All three variables (province, year, and herd size) offered to the logistic regression model were identified to be independently associated with herd brucellosis seroprevalence (Table 3). Herds from Matebeleland north had higher odds (OR =4.0; 95% CI: 2.7, 6.1) of being brucellosis seropositive compared to those from Mashonaland east. The odds of herd-level brucellosis seropositivity were observed to increase progressively with the years (1995 – 1997 vs 1998 – 2000, or 1995 – 1997 vs 2001 - 2004) and herd size (Table 3). There were no significant ($p>0.05$) interactions between the main effects and post-fit testing did not reveal major influence of outliers on the model. The Hosmer-Lemeshow goodness-of-fit test showed that the model fit the data ($X2 =6.5$, d.f.8, $p=0.6$. The model had a good predictive ability (area under the ROC curve = 0.6).

Discussion

The study investigated *brucellosis* and compared the spatial and temporal variations of *brucellosis seroprevalence* in commercial and smallholder cattle farming sectors of Zimbabwe. The results showed that brucellosis is widely spread in both commercial and smallholder cattle in all the provinces of the country. However, the overall within-herd prevalence was generally lower than herd-level prevalence, indicating that in most of the infected herds, only a small proportion of cattle were brucellosis seropositive. Although some farms, especially commercial dairy farms use B. abortus S19 vaccine in calves (3 to 6 months of age) but not in adult cows, and given the fact that the CFT which was used as confirmatory test, fails to discriminate B. abortus S19, the occurrence of confounding bias due to vaccinal antibodies was minimised by including cattle of at least 2 years of age. In calves vaccinated between 3 and 6 months of age, B. abortus S19 vaccinal antibodies are likely to fall below detectable levels from age 1.5 years (Cunningham, 1977; Madsen, 1989).

Generally, *brucellosis seroprevalence* was higher in Matebeleland, Mashonaland central, Midlands and Masvingo provinces compared to the other provinces. The reasons for this spatial distribution can not be explained by the present data, but appears not to be influenced by the type of cattle breed (beef or dairy) but rather by cattle management factors and other agro-ecological factors that promoted or restricted contact between herds (Matope et al., 2010; McDermott and Arimi, 2002). These risk factors include, characteristics of animal population (eg. herd size, population density), management practices (eg. livestock intermix, source of replacement heifers), and biological features (eg. herd immunity) that largely

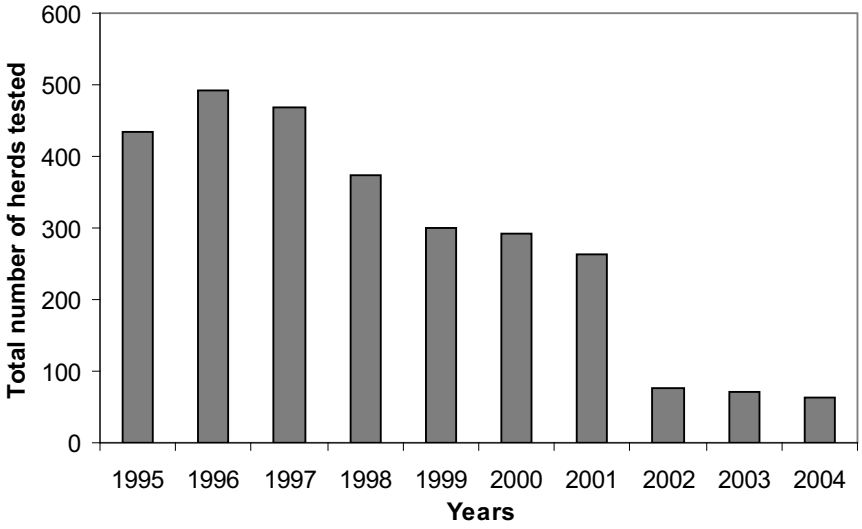

Fig. 1. The distribution of the total number of herds tested for *brucellosis* over the ten-year period from 1995 to 2004

influence the epidemiology of Brucella spp. (Crawford *et al*., 1990; Salman and Meyer, 1984). Although it was not possible to separate dairy from beef herds based on the available data, it is however noteworthy that commercial dairy cattle farming activities are predominantly in Manicaland, Mashonaland east, Mashonaland west and some areas in Mashonaland central provinces. In commercial dairy farms, a brucellosis accreditation scheme, which entails compulsory serological testing of herds and slaughter of brucellosis seropositive cattle has been gazetted (Madsen, 1989) and the possibility of the eradication of the disease from some of these farms where cattle movement is strictly regulated has been suggested (Mohan *et al*., 1996). In contrast, in areas where there is less restriction of movement of cattle co-mingling is likely to take place, for example through co-sharing of grazing facilities and during cattle sales. Comingling of cattle has been identified as an important risk factor not only for brucellosis (McDermott and Arimi, 2002) but also other infectious diseases such as bovine tuberculosis (Munyeme *et al*., 2008). While there are no previous records of brucellosis study in Matebeleland provinces,

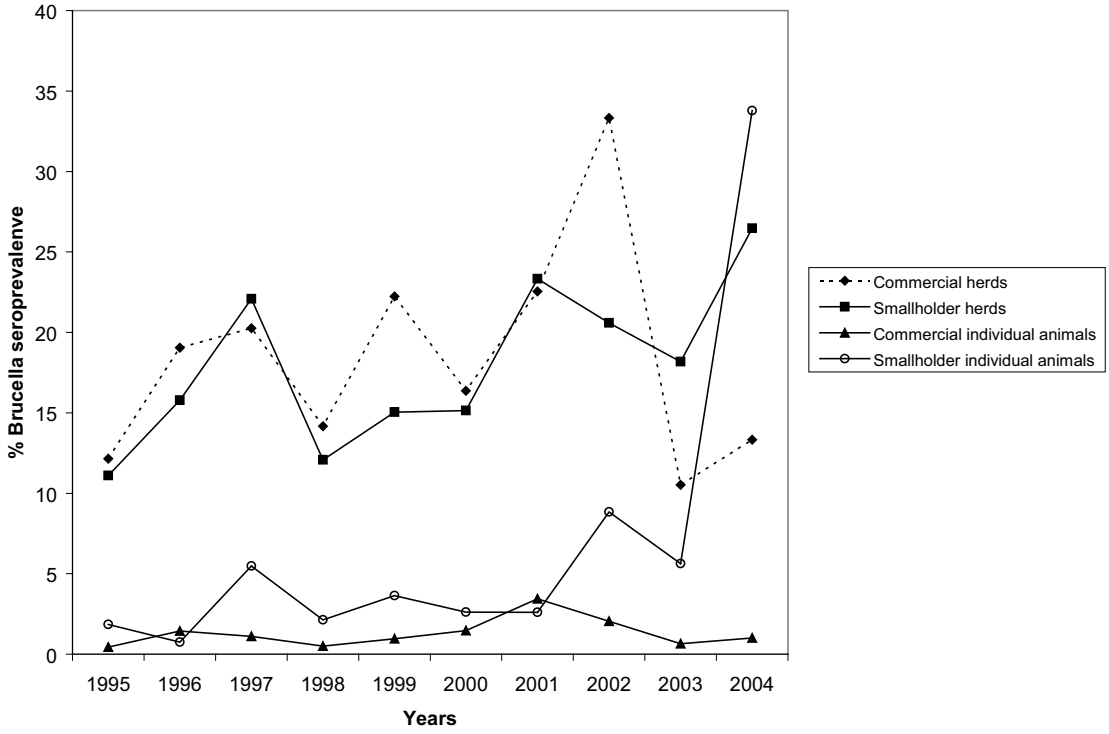

Fig 2. Annual individual- and herd-level brucellosis seroprevalence in commercial and smallholder herds from the eight provinces of Zimbabwe (1995-2004

our results are similar to those of previous studies especially for Manicaland province where brucellosis seroprevalence has been reported to be low (Bryant and Norval, 1985; Matope *et al*., 2010; Mohan et al., 1996).

The logistic regression model revealed that administrative province, year of study and herd size were independently associated with brucellosis seroprevalence. However, we noted a drastic drop in the number of individual sera and herds tested for brucellosis during the ten-year period. This decline could be reflective of reduced budgetary allocations by the Department of Veterinary Services for disease surveillance or could be indicative of a change in the reporting system. The effect of the economic depression that affected the country after the year 2000 could not be ruled out. There has been a general decline in the number of samples submitted to the Central Veterinary Laboratory for routine investigation of other infectious diseases (Anon., 2002). While the temporal trend of *brucellosis seroprevalence* could partly be explained by the decline in the number of samples investigated, the general increase of *brucellosis seroprevalence* in the smallholder areas could be a true reflection of the rise of the disease in the sector because in the commercial sector the disease has remained steadily constant over the years. In earlier years, brucellosis used not to be a problem in smallholder areas with some believed to be free from the disease (Bryant and Norval, 1985) but in the absence of stringent control measures, brucellosis has gradually been well entrenched in this sector. This is likely to be caused by interchange of cattle between these two farming sectors. Smallholder farmers often purchase cattle from commercial farms for the purpose of improving the genetics of their herds and this practice could have increased chances of contact with infected herds (Madsen, 1989). The purchase of animals has been reported to be an important risk factor for infection with *Brucella* spp. (Salman and Meyer, 1984).

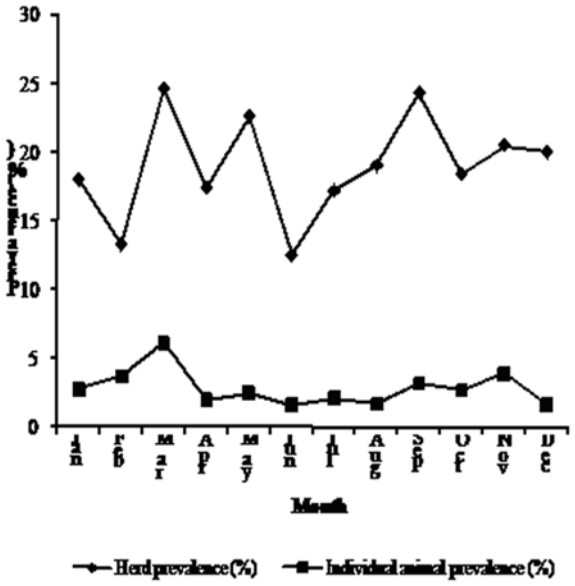

Fig. 3. Monthly individual- and herd level brucellosis seroprevalence in commercial and smallholder herds from the eight provinces of Zimbabwe (1995-2004).

Table 3. Crude description of herd-level factors for *brucellosis seropositivity* in cattle from commercial and smallholder herds in Zimbabwe (1995-2004)

Variable	Level	Herds positive/tested	Herd-level brucellosis seroprevalence (%)
Provincea	Mashonaland east	79/589	13.4
	Mashonaland central	60/269	22.3
	Mashonaland west	140/800	17.5
	Manicaland	35/214	16.4
	Masvingo	37/193	19.2
	Midlands	82/399	20.6
	Matabeleland north	56/166	33.7
	Matabeleland south	16/63	25.4
Yeara	1995 - 1997	240/1394	17.2
	1998 - 2000	161/694	16.7
	2001 – 2004	104/475	21.9
Farming type	Smallholder	140/786	17.8
	Commercial	365/2047	17.8
Herd sizea	≤ 51 cattle	302/1965	15.4
	51 - 200 cattle	116/616	18.8
	201 - 300 cattle	33/112	29.5
	> 300 cattle	54/140	38.6

[a]These variables had Fisher's exact p<0.25 and were presented to the multivariable logistic regression model

Table 4. Final multiple logistic regression model of herd-level factors for brucellosis sero-positivity in commercial and smallholder herds (n = 2833) in Zimbabwe (1995- 2004)[a]. bResults given with beta (b), standard errors (S.E.), and odds ratio (OR) with 95% confidence intervals (CI).

Risk factor	Level	Multiple logistic regressionb				
		b	SE (b)	P value	OR	95% CI
	b	SE (b)	P value	OR	95% CI	
Area	Mashonaland east	-	-	-	1.0	-
	Mashonaland central	0.8	0.2	0.000	2.3	1.6, 3.4
	Mashonaland west	0.3	0.2	0.061	1.3	1.0, 1.8
	Manicaland	0.2	0.2	0.48	1.2	0.8, 1.8
	Masvingo	0.6	0.2	0.006	1.9	1.2, 2.9
	Midlands	0.6	0.2	0.000	1.9	1.3, 2.7
	Matabeleland north	1.4	0.2	0.000	4.0	2.7, 6.1
	Matabeleland south	1.1	0.3	0.000	3.1	1.6, 5.7
Year	1995 - 1997	-	-	-	1.0	-
	1998 – 2000	-0.01	0.1	0.92	1.0	0.8, 1.2
	2001 - 2004	0.4	0.1	0.009	1.4	1.1, 1.9
Herd size	51 cattle	-	-	-	1.0	-
	51 - 200 cattle	0.4	0.1	0.003	1.5	1.1, 1.9
	201 – 300 cattle	1.0	0.2	0.000	2.8	1.8, 4.4
	> 300 cattle	1.6	0.2	0.000	4.7	3.2, 7.0

[a]Overall data of the model: LL = -1266.6, LR chi2(12 d.f) = 122.7, p = 0.0000, number of observations = 2833. Hosmer-Lemeshow X2 (d.f.,8) = 6.5, Prob > chi2 = 0.6, ROC = 0.6.

Further, the introduction of the land reform programme in the year 2000 brought about increased movement of cattle between the two sectors and this had a potential of changing the *epidemiology* of many infectious diseases, including brucellosis. Considering the public health importance of brucellosis in smallholder areas where the practice of consuming raw milk is a common practice, it is prudent that the disease be controlled to prevent further spread of brucella infections.

Despite the variability of defining what constitutes a large herd (Salman and Meyer, 1984) and the difficulties of identifying independent herds in smallholder farming systems, our study showed that brucellosis seroprevalence increased with increasing herd size. This is consistent with the biology of Brucella spp. where the conditions prevailing in large herds that are intensively managed tend to favour their transmission and maintenance (Bishop *et al*., 1994; Nicoletti, 1984). This perfectly conforms to the epidemiological rule of 'small herd-low incidence, large herd-high incidence' (Madsen, 1989). The practice of keeping large herds is often influenced by the availability of grazing pasture and also the farmer's resource endowment for investing into large cattle enterprises (Matope *et al*., 2010).

We observed that there was no seasonal pattern for *burcellosis seroprevalence* during the ten-year period and this supports the observations of other studies (Gul and Khan, 2007). However, in herds where controlled breeding is practiced, *brucella* abortions, since they occur mainly in the third trimester of pregnancy, tend to show a seasonal pattern predominating in periods around the calving seasons (Gul and Khan, 2007; Muma *et al*., 2007). Since Brucella spp. are mainly transmitted through ingestion of contaminated material-especially after abortions, the risk of infection is high particularly in herds with increased stocking density (Nicoletti, 1980). Since brucellosis is a chronic disease and once introduced into a herd, it is likely to be endemic unless strict control measures are implemented seropositive reactors are likely to be present in such herds throughout the season.

It is concluded that *brucellosis* is wide spread in all parts of the country, in spite of the variations in seroprevalence. The differences in brucellosis seroprevalence among the provinces is linked to differences in cattle management practices. In the absence of brucellosis control measures, the prevalence of the disease has tended to increase in smallholder cattle farming sector. In order to prevent further increase in brucellosis seroprevalence, strict disease control measures should be applied in all the cattle farming areas. Thus, the enforcement of the brucellosis eradication scheme, especially in the smallholder sector will halt further spread of the disease and reduce the public health risk of these resource-poor communities.

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POST- TREATMENT CYTO-ADHERENCE AND LYMPHOCYTES PROLIFERATION IN ONCHOCERCA GUTTUROSA INFECTED- ZEBU CALVES

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CYTO-ADHÉRENCE POST-TRAITEMENT ET PROLIFÉRATION DES LYMPHOCYTES CHEZ LES VEAUX INFECTÉS PAR ONCHOCERCA-ZÉBU GUTTUROSA.

Résumé

Cette étude a été réalisée pour évaluer et quantifier les changements post-traitements dans les réactions cellulaires chez les veaux infectés naturellement par des microfilaires (mmf) de l'onchocercose gutturosa. Les cellules sanguines périphériques (CSP) obtenues à partir de veaux infectés par l'onchocercose gutturosa - (n = 9), ainsi que de veaux non infectés (n = 3) ont été soumises à une cyto-adhérence invitro aux *microfilaires* (mmf) et à la prolifération des *lymphocytes* en réaction à deux antigènes différents. Les groupes atteints de trois veaux chacun, ont été traités soit par la chloroquine (200 mg i / m par jour pendant 7 jours), une dose unique s / c de l'ivermectine (150 ug / kg de poids corporel) ou une dose standard de l'artéméther (160 i mg / m pendant trois jours successifs). Deux semaines après ces traitements, les mêmes essais ont été répétés. Les résultats obtenus montrent que la cyto-adhérence des microfilaires était plus grande dans les cultures faites avec des sérums de veaux infectés, là où les cellules adhérentes couvraient de 17 à 20,9% de la surface du corps des mmf, comparativement à une faible adhérence aux mmf dans les cultures avec des sérums de contrôle de veaux sains (3,4%). Le traitement a entraîné une augmentation significative de l'adhérence a la surface des mmf (P <0,05) (dans l'ordre de 22,5 à 35%) . La prolifération des lymphocytes en réponse aux antigènes OV-ASP 2-clonés ou aux antigènes bruts des vers entiers de gutturosa O. a été plutôt faible dans les groupes infectés avec un indice de stimulation (SI) variant entre 1,70 à 2,15 ; alors que chez les veaux infectés elle était comprise entre 1,13 à 1,66. Après les traitements, la prolifération des lymphocytes n'a montré aucun changement dans l'indice de stimulation (SI) et celle-ci se situait entre 1,60 à 2,13. Il a été conclu que: le traitement par l'ivermectine, la chloroquine ou l'artéméther ont amélioré considérablement l'adhérence cellulaire a la surface des ffm sur les sérums stimulés alors que, son effet sur les réponses de prolifération des lymphocytes a été insignifiante.

Mots clés: onchocercose gutturosa, cellules sanguines périphériques, cyto-adhérence in vitro, veaux

ABSTRACT

This study was carried out to assess and quantify the post- treatments changes in cellular responses in calves naturally infected with microfilariae (mff) of Onchocerca gutturosa. Peripheral blood cells (PBC) from *Onchocerca gutturosa* - infected calves (n=9) as well as from uninfected ones (n=3) were subjected to an in *vitro cyto-adherence* to *microfilariae* (mff) and lymphocyte proliferation in response to two different antigens. Infected groups of three calves each, were treated with either *Chloroquine* (200mg i/m daily for 7 days), a single s/c dose of Ivermectin (150 µg / kg body weight) or standard dose of Artemether (160 mg i/m for three successive days). Two weeks post- treatments the same assays were repeated. Results showed that the cyto-adherence to mff was greater in cultures with sera from infected calves whereby the adherent cells covered 17 – 20.9% of the mff body surface, compared to a weak adherence to mff in cultures with control sera from uninfected calves (3.4%). Treatment resulted in a significant (P< 0.05) increase in adherence to the mff surface (22.5 - 35%). The lymphocyte proliferation in responses to OV-ASP2- cloned antigen or *O. gutturosa* whole worms crude antigens were rather weak in infected groups with a stimulation index (SI) ranging between 1.70 - 2.15 whereas that of uninfected calves ranging between 1.13 - 1.66. After treatments the lymphocytes proliferation showed no changes in (SI) and it ranged between 1.60 – 2.13. It was concluded that: treatment with Ivermectin, *Chloroquine* or Artemether significantly enhanced the cellular adherence to mff surface upon stimulated *sera* whereas, its effect on the lymphocytes proliferation responses was insignificant.

Key words: Onchocerca gutturosa, Peripheral blood cells, vitro cyto-adherence, calves

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Introduction

Antigen specific cellular immune responses in *onchocerciasis* depend on parasite density and the state of the infection. The attracted and activated macrophages as well as memory Th2-type *lymphocytes* and B-cells, may contribute to dermal immune responses associated with persistent low levels of *Onchocerca volvulus* mff (Fendt *et al.,* 2005). Moreover, the critical role of regulatory T cells in allowing *filarial* maturation is now well established (Taylor *et al*., 2005). Following a single dose of *Ivermectin*, the *cytokine* production by peripheral blood *mononuclear* cells (PBMC) changed towards a mixed Th1- and Th2 type profile (Soboslay *et al*., 1994). However, there is a major gab in knowledge between the recent advances in understanding the hyporesponsive state in filarial infections and the effect of chemotherapy on host susceptibility (Nfon *et al*., 2006 2007). Thus, there is an urgent need to investigate the relative duration of hyporesponsiveness after micro- and macrofilaricidal treatment in animal models in addition to humans. Studies with bovine model of onchocerciasis showed depressed lymphocytes proliferation and elevated antibody responses of chronically infected cattle (Graham *et al*., 2001). However, the down regulation of parasite- specific IL-2, IL-4 and IFN-y production reported by (Graham *et al.*, 2001) is highly informative.

The aim of this in vitro study is to quantify cell proliferation and eosinophil adhesion as effectors cells responsible for *cytotoxicity* prior to and during the skin mff clearance.

Materials and Methods

Experimental animals

A total of nine zebu calves naturally infected with *Onchocerca gutturosa* divided into three equal groups in addition to three uninfected calves as a control group. The animals were housed at the premises of CVRL, Khartoum and had free access to water and sorghum straw. The first group was treated with 200 mg Chloroquine (*Chloroquine – Phosphate* Base - France Lab.) daily for 7 days, the second group received a single dose of Ivermectin (Ivomec®, Merck Sharp and Dohme, New Jersy, USA) at 150 µg /kg body weight, whereas the third group was injected I/M with 160 mg Artemether (Artemedine, Kunming Pharmaceutical Corp, China) for three successive days.

*Collection of sera and cells***:**

Whole blood samples were collected in sterile heparinized vacutainers and were processed for separation of *lymphocytes* (for *blastogenic* responses) and eosinophils for determination of antibody mediated *cytoadherence* responses. Serum was obtained by centrifugation for 10 minutes at 1400 rpm and aliquoted into cryo- preservation tubes, labeled and stored at-20°C. The remaining blood was diluted into 1:1 with sterile Phosphate Buffer Saline (PBS with pH 7.2) in 15- ml sterile centrifuge tubes and mixed. Eight ml of the diluted blood were gently layered on top of four ml of Ficoll (Sigma diagnostics Lot. 114 H 6106, USA). The tubes were then centrifuged at 1400 rpm for 25 minutes. The buffy coat containing the PBMCs was carefully pipetted into another centrifuge tube, and then washed three times by suspension in seven ml of PBS, each wash followed by centrifugation at 1000 rpm for 10 minutes. Supernatants were discarded and cells were re-suspended in complete culture medium of RPMI- 1640 supplemented with 5% faetal calf serum (FCS), 50 µg/ml gentamycin, 100 mg/ml streptomycin, 100 iu penicillin and 0.05% HEPES. Cells were counted using the trypan blue exclusion technique where transparent cells were considered viable and dead cells stained blue (Leslie and Frank, 1991). The collected cells were immediately used for the cell proliferation assay.

Cyto- adherence Assay

Eosinophil- rich cell suspensions were prepared from heparinized blood collected from microfilariae- positive calves with high eosinophilia. Buffy coat containing the whole white blood cells suspended as 4-5 $x10^6$ cells/ml in complete RPMI 1640 medium. Serum samples were collected from each infected calf pre- and post-treatment with either drug as well as from the control group. The assay was carried out in 96 wells flat bottom culture plate in triplicates. A suspension of 100 mff in 50 µl complete RPMI 1640 medium were incubated at 37°C for one hour with 10 µL of (1: 5) diluted serum to allow the binding of antibodies to the microfilariae. Then 100 µL of cell suspension (4 -5 $x10^5$ cells) were added to each well. After three hours the degree of cell adherence to the surface of mff was assessed using an inverted microscope. At 24 hours of incubation smears of mff from each well were made on clean slides, fixed, stained with *Geimsa's* and examined for quantification of the degree of adherence. This was assessed as zero for non -adherance, and as percentages of the mff body surface being covered with adherent cells.

The lymphocytes proliferation assay (MTT- method)

lymphocytes proliferation assays were performed according to(Mosmann, 1983) Triplicate cultures were done in 96 wells flat bottom culture plates. From the cells suspension of each individual animal, 100 µl containing approximately ($6x10^5$ cell) were stimulated with either 40 µl (100 µg/ml) phytohaemoagglutination PHA (mitogen), or 40 µl (40 ug/ml) adult O. gutturosa soluble crude antigens, or OV-ASP-2 adult cloned antigen, or Maltose Binding Protein (MBP the fusion protein of the OV-ASP-2 antigen) as a blank. Three wells containing cells without any stimulant were kept as controls for each animal. The plates were incubated at 37°C in

CO_2 chamber for 48 hours, then 25 µl (5mg/ml) of MTT - (3- (4,5-Dimethylthiazolyl-2) - 2, 5- *Diphenyl tertyrazolium bromide, Sigma*) were added to each well, including controls, the plates were incubated for further six hours. When purple colour formed, the plates were read at a wavelength of 492 nm in ELISA reader as an Optical density (O.D). The stimulation index (SI) was calculated by dividing the mean (O.D) of each stimulated antigen or PHA (mitogen) by the mean (O.D) of the non- stimulated cells for each individual animal. Subsequently the same was done for cells obtained from the same animals after treatment.

Results

Cyto-adherence assay

In vitro culture of *Onchocerca gutturosa* mff with cells showed high degree of cell adhesion and immobilization of these mff within hours of incubation when using sera from naturally infected calves. The adherence of the cells in the presence of sera from uninfected calves showed a mean rate of 3.4% whereas it reached 17% -21% in the presence of sera from naturally infected untreated calves in the three groups of calves (Table 1) show that treatment with each of Chloroquine, Ivermectin and Artemether resulted in a significant ($P< 0.05$) increase in cyto-adherence rate to mff where it increased to 22.5%, 35% and 27.5%, respectively.

The lymphocyte proliferation Assay

Lymphocytes from infected calves showed relatively higher proliferation responses to both O. gutturosa crude antigens and OV- ASP-2 cloned antigen than lymphocytes from non-infected calves (Table 2).. However, after treatment with *Chloroquine*, *Ivermectin* or Artemether the proliferation responses were almost similar to the pre-treatment level for each group (Table 2). The stimulation indexes (SI) of the infected groups in response to the mitogen (PHA) above 2.0 whereas, it is less in the control uninfected group indicating that the culture system was technically adequate.

Discussion

The high degree of cyto-adherence to mff and their immobilization within 6 hours of incubation in the presence of sera from mff-infected calves compared to the weak or absence of adherent cells in cultures with sera from uninfected calves. These results are in agreement with those reported by (Mackenzie, 1980) who stated that mff and L3 larvae were killed in vitro when

Table 1: Pre- and post- treatment cyto-adherence (%) to the surface of mff of *Onchocerca gutturosa* in the control and treated groups

	Control group (n=3)	Chloroquine- treated group (n=3)	Ivermectin-treated group (n=3)	Artemether- treated group (n=3)
Pre-treatment	3.40%	20.90%	17.00%	19.20%
Post- treatment	----	22.50%	35.00%	27.50%
P- value	----	<0.05	<0.05	<0.05

% of mff body surface covered by adherent cells.

Table 2: Pre- and post- treatment *lymphocytes proliferation* expressed as stimulation index (SI) in the control and treated group

Stimulant	Control group (n=3)	Chloroquine-treated group (n=3)	Ivermectin-treated group (n=3)	Artemether- treated group (n=3)
		Pre-treatment (SI)		
PHA	1.66	2.02	2.01	2.15
OV ASP2	1.23	1.81	1.94	1.92
MBP	1.13	1.80	1.79	1.86
O.gutturosa crude antigen	1.20	1.70	1.93	1.82
		Post-treatment (SI)		
PHA	----	2.08	2.13	2.13
OV ASP2	----	1.94	1.95	1.83
MBP	----	1.67	1.79	1.73
O.gutturosa crude antigen	----	1.81	2.03	1.85

they were cultured in antibody positive sera with the presence of oesinophils. Moreover, (Titanji *et al*., 1996) concluded that IgG antibodies from *onchocerciasis sera* are implicated in the adherence to and killing of mff by pateint's leucocytes. The immobilization of mff shown early in this experiment, after 1-2 hours, of incubation system may probably be enhanced by the accumulation of degranulation products and toxic molecules released during the cellular reaction of adherent cells (mostly eosinophils). It has been stated by Gopinath *et al*., (2000) that the release of such degranulation products enhances further adhesion of eosinophils and neutrophils. Moreover, treatment with each of Ivermectin and Artemether resulted in a significant increase in cyto- adherence rate where it increased to 35 % and 27.5%, respectively compared to *Chloroquine* treated calves where the adherence rate was estimated as 22.5%. This confirms the observations of (Ali *et al*., 2003) who found that Ivermectin significantly enhanced antibody-mediated responses in patients with mild onchocerciasis which confirms the suggestions made by Soboslay *et al*. (1994)that Ivermectin therapy affects the immune system whereby it is involved in both killing and degeneration of mff. This concept is supported by the findings that Ivermectin is sometimes less effective in immuno-compromised patients (Ali *et al*., 2002)

In this study OV ASP2 antigen and adult worms of *O. gutturosa* (male and female) crude antigens, were used for *invitro* stimulation of peripheral blood mononuclear cells (PBMC). The mean values of stimulation index (SI) for PBMC from infected calves was less than 3.0 yet, it was higher than the mean SI for PBMC from mff negative calves. On the other hand, upon stimulation of PBMC from infected calves by T-cell mitogen (PHA) the responses were not significantly different from those stimulated by crude antigens of O. gutturosa or cloned OV ASP2 antigen. In all treated groups the stimulation index (SI) for each tested antigen did not change significantly. A decreased ability of *lymphocytes* to proliferate in vitro after stimulation with T cell (mitogen) has been described in *onchocerciasis* (Mackenzie, 1980) Poor proliferation was generally observed in this study to PBMC stimulated by either OVAg or O. gutturosa crude antigens. In immunized mice experimentally infected with O. leinalis, (Hogarth and Bianco, 1999)reported reduction or even absence of proliferation responses to embryonic stages and mff antigens compared to adult worm antigens. Other work indicates that the source of parasite antigen used for in vitro stimulation is an important determinant of proliferation unresponsiveness e.g. culture with antigen from mixed sex adult worms down-regulate proliferation responses while culture with adult male antigen alone had no such effect (Mahanty *et al*., 1996). In the present experiment the whole worm crude antigens contained adult worm antigens as well as uterine mff antigens which may imply the role of uterine mff in down regulation proliferation responses, as surface antigens of adult male and female should be homologous for most of them. In another work it was also shown that T-cell suppression is most pronounced in skin mff antigen response (Leiva and Lammie, 1989). In lymphatic filariasis, PBMC from *microfilarimic* patients produce large amount of IL -10 (invitro experiment), which induces suppression of antigen specific proliferation response (Mahanty *et al*., 1997). Generally, human filarial infection is characterized by dominant Th2 responses and defecti ve antigen specific T-cell proliferation responses (Sartono *et al*., 1999). A number of mechanisms have been proposed which would account for this hyporesponsiveness, nevertheless, the exact nature of the proliferation suppression is still not fully understood except that it is for the benefit of the parasite.

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Impact

Research on animal *Onchocerciasis* may add a valuable knowledge in controlling human *onchocerciasis* on the bases that *Onchocerca* volvulus only infects human. Other species of *onchocerca* in domestic animals may be suitable alternative models for in vitro and in vivo studies specially those related to treatment and eradication of the parasite.

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TRAITEMENT DE LA PERIPNEUMONIE CONTAGIEUSE BOVINE PAR L'OXYTETRACYCLINE LONGE ACTION ET TRANSMISSION EXPERIMENTALE DE LA MALADIE A PARTIR DE BOVINS TRAITES

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TREATMENT OF THE CBPP with OXYTETRACYCLINE LONG ACTION AND EXPERIMENTAL TRANSMISSION OF THE DISEASE FROM TREATED CATTLE

Summary

This study evaluated the effect of long-acting *oxytetracycline* (*Oxytetracycline* LA) in the treatment of cattle infested with contagious bovine *pleuropneumonia* (CBPP) and to determine the risk of disease transmission from animals treated. Experimental transmission was conducted by contacting 16 cattle clinically healthy and seronegative vis-à-vis the antibodies against M. *mycoides* subsp. *mycoides* Small Colony (MmmSC) 14 cattle naturally infected with the disease and treated with *oxytetracycline* LA. The experiment lasted 10 months during which all animals were monitored clinically and sampled at regular intervals for laboratory analysis. Post-mortem examinations were performed on all animals to detect lesions characteristic of CBPP and also take samples for laboratory analysis. The treatment of animals infected with *oxytetracycline* LA clinically cured a large majority of them (12/14). All 14 animals were *seroconverted* and post-mortem analysis showed the presence of *chronic* lesions including pulmonary sequestrations in 4 of them; MmmSC was only isolated from these receivers. However the 16 healthy animals in contact with these 14 animals remained clinically healthy throughout the experimental period, at autopsy no lesions characteristic of CBPP has been noted and laboratory tests were negative. The results of this study may have important implications in the control of CBPP in Africa.

Keywords: Contagious bovine *pleuropneumonia* - Antibiotic - *Oxytetracycline*-Experimental transmission - Pulmonary lesions - Mali

Résumé

La présente étude visait à évaluer l'effet de l'oxytétracycline longue action (oxytétracycline LA) dans le traitement des bovins atteints de péripneumonie contagieuse bovine (PPCB) et de déterminer le risque de transmission de la maladie à partir d'animaux traités. Une transmission expérimentale a été conduite par la mise en contact de 16 bovins cliniquement sains et séronégatifs vis-à-vis des anticorps contre M. mycoides subsp. mycoides Small Colony (MmmSC) avec 14 bovins naturellement infectés de la maladie et traités avec l'oxytétracycline LA. L'expérimentation a duré 10 mois pendant lesquels tous les animaux ont été suivis cliniquement et prélevés à intervalles réguliers pour analyses de laboratoire. Des examens post mortem ont été réalisés sur tous les animaux afin de déceler des lésions caractéristiques de PPCB et aussi de prélever des échantillons pour analyses de laboratoire. Le traitement des animaux infectés par l'oxytétracycline LA a cliniquement guéri la grande majorité d'entre eux (12/14). Tous les 14 animaux ont séroconverti et l'analyse post-mortem a montré la présence des lésions chroniques dont des séquestres pulmonaires chez 4 d'entre eux; MmmSC a été seulement isolé à partir de ces séquestres. Toutefois les 16 animaux sains mis en contact avec ces 14 animaux sont demeurés cliniquement sains durant toute la période d'expérimentation; à l'autopsie aucune lésion caractéristique de la PPCB n'a été notée et les analyses de laboratoire sont restées négatives. Les résultats de la présente étude peuvent avoir des implications importantes dans le contrôle de la PPCB en Afrique.

***Mots-clés*:** Péripneumonie contagieuse bovine - Antibiothérapie - Oxytétracycline -Transmission expérimentale - Séquestres pulmonaires - Mali

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Introduction

La péripneumonie contagieuse bovine (PPCB) est une maladie infectieuse majeure des bovins caractérisée par une pleuropneumonie exsudative et fibrineuse. Elle est causée par Mycoplasma mycoides subsp. mycoides Small Colony (MmmSC). La PPCB fait partie de la liste des maladies notifiables à l'OIE en raison de sa grande capacité de contagion et de diffusion transfrontalière, ainsi que de son impact sur les échanges internationaux(Provost, *et al.*, 1987). Elle représente en Afrique tropicale l'une des maladies les plus importantes causant d'énormes pertes économiques à l'élevage(Tambi *et al*., 2006). Les facteurs favorisant la dissémination de la maladie dans le contexte africain sont le caractère extensif de l'élevage *et la* transhumance(Massiga *et al*., 1996).

Dans le passé, la maladie a sévi en Europe et en Amérique. Elle a cependant pu être éradiquée sur ces continents à travers notamment une politique rigoureuse de restriction des mouvements du bétail, de l'abattage et des indemnisations consécutives. Pour des raisons socio-économiques, l'application de telles mesures apparaît très difficile dans la plupart des pays africains. La seule mesure réaliste de contrôle de la maladie dans ces pays est la vaccination massive et répétée. Les tentatives de contrôle de la PPCB à travers la vaccination obligatoire combinée à la quarantaine des animaux affectés, datent de la période coloniale. Il apparaît cependant que cette approche conventionnelle, n'a pas été un succès dans l'éradication de la maladie, du moment que son incidence continue à augmenter dans plusieurs pays du continent. Il semble que les campagnes actuelles de vaccination servent seulement, en tout cas pour le moment, à limiter la maladie clinique dans des proportions raisonnables plutôt que d'éradiquer l'infection. Par ailleurs la quarantaine est impossible à faire respecter pendant une longue période, vu le caractère extensif des élevages, le partage des pâturages et la transhumance, qui prédominent dans la plupart des pays africains. Par conséquent, la recherche de stratégies alternatives dans le contrôle de la maladie s'impose.

Les méthodes alternatives de contrôle de cette maladie, comme les traitements antibiotiques, ont toujours été déconseillées sur la base de stratégies d'éradication. En fait, il est communément admis que le traitement des animaux infectés avec des antibiotiques compromet la lutte contre la maladie en rendant un grand nombre d'animaux porteurs chroniques pouvant constituer une source de contagion (Orue and Mémery, 1961 ; FAO, 1967). Une telle considération du monde scientifique entrave l'éventuelle utilisation rationnelle des traitements antibiotiques comme option dans la lutte contre la PPCB.

En se basant sur la littérature scientifique, il apparaît cependant que cette thèse n'a pas été objectivement évaluée, et du reste, il existe d'anciennes publications affirmant le contraire(. Camara (1956, 1971; Hudson and Etheridge, 1965; Moret *et al*., 1949; 1951; Provost, 1974; Turner, 1960; Windsor and Masiga, 1977). De plus, bien qu'illégal l'usage des antibiotiques contre la PPCB par les éleveurs et même par les auxiliaires vétérinaires est une pratique courante dans les pays africains. Aussi, malgré son interdiction officielle, cette pratique illégale va certainement gagner de l'ampleur en raison de la privatisation des services de soins cliniques et de la disponibilité croissante des antibiotiques.

Pour faire le bilan de la situation et en tenant compte de tout ce qui précède, la FAO a souligné, dans les conclusions et recommandations d'une conférence électronique sur le contrôle de la PPCB, l'urgence de réactualiser objectivement la question de l'usage des traitements antibiotiques dans le but d'une résolution finale basée sur des connaissances scientifiques précises(FAO 2002).

Dans les pays africains notamment, y compris le Mali, l'oxytétracycline longue action (oxytétracycline LA) est l'antibiotique le plus couramment utilisé dans le traitement des animaux infectés de PPCB ou d'autres maladies infectieuses. L'objectif de la présente étude est d'évaluer, à travers des expérimentations animales, l'efficacité de traitement de la PPCB par cette molécule et de déterminer le risque de transmission de la maladie à partir d'animaux traités. Il est attendu qu'une meilleure connaissance de l'efficacité de ces agents thérapeutiques antimicrobiens notamment l'oxytétracycline dans le traitement de l'infection de la PPCB pourrait avoir des implications dans les stratégies de lutte contre la maladie

Materiel et Methodes

Animaux d'expérience

L'expérimentation a concerné 30 bovins dont 16 de type zébu cliniquement sains et 14 de type Ndama cliniquement malades de PPCB et traités avec l'oxytétracycline LA. Les 16 zébus étaient âgés de 3 à 4 ans et ont été obtenus à partir de plusieurs troupeaux des cercles de Bandiagara et de Koro (Mali), zone à faible prévalence de PPCB, et convoyés dans les étables du Laboratoire Central Vétérinaire (LCV), Bamako. Les documents et données des services nationaux de la santé animale14 ainsi que les informations recueillies auprès des éleveurs sur place établissent que les animaux de ces troupeaux n'ont jamais été exposés à la PPCB ni vaccinés contre la maladie durant les cinq dernières années. Les animaux sélectionnés ne présentaient aucun anticorps contre MmmSC par la réaction de fixation du complément (RFC) et le test de Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA). Ils étaient également indemnes de brucellose (test d'agglutination) et de tuberculose (test d'intradermoréaction). Ces animaux formant le groupe «Contact» ont été bagués avec des numéros de C1 à C16 et placés en quarantaine, dès leur

arrivée, pendant une durée d'un mois durant laquelle 2 prélèvements de sang ont été effectués pour confirmer leur statut sérologique. Les animaux ont également été traités contre diverses parasitoses et vaccinés contre la pasteurellose et le charbon symptomatique.

Quant aux animaux naturellement infectés par MmmSC et présentant des signes cliniques typiques de PPCB, ils ont été acquis à Sokela, village situé à 15 Km de l'arrondissement de Sanso (Cercle de Bougouni, Région de Sikasso), lors d'un foyer actif. Les bovins du troupeau étaient de race NDama. Les signes cliniques observés ont été la dyspnée, la toux, l'anorexie, l'abattement, le jetage nasal, la salivation et l'hyperthermie. Le foyer aurait nouvellement apparu dans la zone en octobre 2004 suite à l'introduction d'animaux transhumants venus de la région de Ségou, Mali. Le foyer a connu un réveil en janvier 2005 à Sokela. Sur un effectif de 50 bovins, il a été dénombré 38 animaux malades dont 17 cas de mortalité. L'évolution de la maladie dans le troupeau a été lente et a duré trois mois avec un pic en février lors du passage de l'équipe du LCV. La morbidité a connu une ascension marquée entre février et mars avec un pic en début février pour ensuite tomber brutalement en fin mars indiquant ainsi la fin du foyer. Quant à la mortalité, elle a évolué rapidement courant février pour ensuite décroître régulièrement début mars. La confirmation du foyer a été faite sur la base des analyses bactériologiques (isolement de MmmSC à partir du liquide pleural et des prélèvements de poumons). Quatorze bovins cliniquement malades ont été sélectionnés et traités sur place pendant 3 jours à l'oxytétracycline LA 20% suivant les indications du fabricant (1 injection au jour 1, puis au jour 3 à raison de 1ml/10 kgs de poids vif) avant d'être transportés dans les étables du LCV. Le critère de sélection de ces animaux a été le stade clinique de la maladie qui a été déterminé à partir de la percussion de la poitrine et l'épreuve de la course (camara, 1971) ainsi que les renseignements relatifs au début de l'apparition des premiers signes cliniques chez chaque animal, fournis par les éleveurs et le vétérinaire sanitaire de la localité. La RFC et le test de c-ELISA réalisés sur ces animaux se sont révélés fortement positifs pour une grande majorité d'entre eux. Ces animaux formant le groupe «Infecté» ont été bagués avec des numéros de 11 à 114 et transportés dans les étables du LCV. Toutes les dispositions légales pour le transport de ces animaux furent prises à cet effet.

Protocole expérimental

Les animaux «infectés et traités», dès leur arrivée, ont été maintenus en observation pendant un mois afin de permettre l'antibiotique d'agir. Après ce délai d'attente, ces animaux ont été placés en contact permanent et étroit avec les animaux sains «contacts», dans une étable isolée et sécurisée, pour une transmission naturelle de l'infection.

Pendant toute la durée de l'expérimentation, les animaux avaient accès à la cours de l'étable dans la journée avec un accès libre à la paille et à l'eau. La nuit, ils étaient enfermés dans un box (largeur: 8m, longueur: 10m, hauteur: 4,30m, présentant une fenêtre grillagée de 1,9m x 2,30m au nord et deux portes métalliques et grillagées de 2m x 2,38m au sud et à l'est permettant l'aération) destiné à favoriser le contact étroit entre les animaux. Tous les animaux étaient nourris avec de la paille, du tourteau de coton et des blocs salés ainsi qu'avec de l'herbe fraîche lorsque celle-ci était disponible.

Examens cliniques et post-mortem

Un examen clinique quotidien de tous les animaux a été pratiqué. Les signes cliniques comme l'abattement, l'anorexie, la toux, le jetage, les difficultés respiratoires et l'amaigrissement étaient particulièrement observés. Les mesures de température rectale et de fréquence respiratoire ont été enregistrées une fois tous les deux jours pendant toute la période d'expérimentation. Les animaux présentant des signes de détresse ont été immédiatement abattus pour abréger leur souffrance.

Une autopsie complète a été pratiquée sur tous les animaux abattus durant l'expérimentation ou sacrifiés en fin d'expérimentation. Les poumons et les ganglions ont été attentivement examinés pour la recherche de lésions macroscopiques de PPCB et les résultats enregistrés. En outre, d'autres organes tels que le cœur, les reins et le tube digestif étaient également examinés.

Collecte des échantillons

Des prélèvements de sang pour la récolte de sérum ont été faits séquentiellement sur l'ensemble des animaux avec un intervalle de 1-2 semaines sur une période de 10 mois à compter du jour de la mise en contact. Des lavages bronchoalvéolaires (LBA) ont été effectués au rythme d'un mois, de 2 mois et de 6 mois post-contact, et à l'abattage selon la méthode décrite par Niang et al. (2004) sur les animaux contacts durant la période de l'expérimentation et sur les animaux infectés seulement après mort ou abattage.

Des échantillons de tissus pulmonaires, de séquestres pulmonaires et de ganglions lymphatiques ont été prélevés sur les animaux morts ou abattus en cours ou en fin d'expérimentation.

Les échantillons collectés étaient placés immédiatement dans la glace, puis transportés au laboratoire. Le sang, après coagulation à la température ambiante, était centrifugé pour permettre la récolte du sérum, aliquoté et conservé à –20°C, jusqu'à l'analyse sérologique. Les échantillons de LBA étaient immédiatement traités pour l'isolement et l'identification de MmmSC. De même, les échantillons de tissus pulmonaires, de liquide pleural, de séquestres pulmonaires et de ganglions étaient ensemencés immédiatement après prélèvement.

Analyses de laboratoire

Pour l'isolement de MmmSC, des dilutions en série de chaque échantillon étaient faites dans le milieu de Gourlay et de Brain Heart Infusion (BHI). La confirmation de MmmSC était effectuée par le test d'immunodiffusion après deux passages sur milieu solide (CIRAD, 1985).

L'analyse sérologique spécifique de MmmSC a été effectuée par la RFC et le c-ELISA suivant les protocoles livrés avec les kits (respectivement CIRAD-EMVT et Institut Pourquier, Montpellier, France). La RFC a été t réalisée en dilutions successives à partir de 1:5 tandis que le c-ELISA a été exécuté en dilution unique de 1:10.

Pour l'analyse moléculaire, la PCR développée par Dedieu *et al.* (1994), utilisant une paire d'amorces spécifiques pour l'amplification d'un fragment de 275 bp de MmmSC a été uniquement faite sur les échantillons de LBA et des poumons.

Resultats

Données cliniques

Durant toute la période d'observation, aucun animal du groupe contact n'a présenté de signes cliniques caractéristiques de la PPCB (tableau 1).

Chez les animaux infectés et traités, les signes cliniques enregistrés, à leur arrivée, ont été surtout dominés par la toux sèche, la dyspnée, la prostration, le jetage et l'amaigrissement. Tous ces signes ont complètement disparu un à deux mois après le traitement à l'oxytétracycline LA chez presque tous les animaux (12/14) (tableau 2;) exceptés chez deux animaux (11 et 19) où on a assisté à la persistance de la toux. Parmi ces deux animaux rebelles un est mort (19) par la suite. Le diagnostic post-mortem effectué sur cet animal a révélé la présence de lésions chroniques caractérisées par la présence d'un gros séquestre (15cm de diamètre) dans le poumon gauche.

Données de l'autopsie

Aucune lésion caractéristique de la PPCB n'a été décelée à l'autopsie chez les animaux contacts (tableau 1). En revanche, tous les animaux infectés ont présenté des lésions chroniques caractérisées essentiellement par la présence d'adhérences pulmonaires et seulement 4 sur les 16 animaux concernées (11, 14, 19 et 113) ont présenté de séquestres pulmonaires visibles (tableau 2).

No Animal	Maladie clinique			Lésions	Isolement de MmmSC			PCR	
	Principaux signes cliniques enregistrés	Etat général durant l'expérimentation	Type de lésions	LBA	P	GP	SP	LBA	P
C1	RAS	Bon état	RAS	-	-	-	PA	-	-
C2	RAS	Idem	RAS	-	-	-	PA	-	-
C3	RAS	Idem	RAS	-	-	-	PA	-	-
C4	RAS	Idem	RAS	-	-	-	PA	-	-
C5	RAS	Idem	RAS	-	-	-	PA	-	-
C6	RAS	Idem	RAS	-	-	-	PA	-	-
C7	RAS	Idem	RAS	-	-	-	PA	-	-
C8	RAS	Idem	RAS	-	-	-	PA	-	-
C9	RAS	Idem	RAS	-	-	-	PA	-	-
C10	RAS	Idem	RAS	-	-	-	PA	-	-
C11	RAS	Idem	RAS	-	-	-	PA	-	-
C12	RAS	Idem	RAS	-	-	-	PA	-	-
C13	RAS	Idem	RAS	-	-	-	PA	-	-
C14	RAS	Idem	RAS	-	-	-	PA	-	-
C15	RAS	Idem	RAS	-	-	-	PA	-	-
C16	RAS	Idem	RAS	-	-	-	PA	-	-

LBA (lavage broncho-alvéolaire); P (poumon); GP (ganglion pulmonaire); SP (séquestre pulmonaire); RAS (rien à signaler); PA (Pas applicable); - (négatif).

Réponses sérologiques

Les résultats sérologiques ont montré une séroconversion avec des titres d'anticorps significatifs et persistants aussi bien en c-ELISA (Fig.1) qu'en RFC (résultats non présentés) chez tous les animaux infectés. Par contre, tous les animaux contacts ont gardé leur statut sérologique négatif durant toute la durée de l'expérimentation (Fig. 6).

Isolement de MmmSC

Comme indiqué dans les tableaux 1 et 2, les tentatives d'isolement de MmmSC à partir des LBA ainsi qu'à partir des poumons et des ganglions pulmonaires chez les animaux des deux groupes sont restées vaines. En revanche, MmmSC a été isolé des échantillons prélevés à partir de tous les séquestres issus des poumons des animaux infectés (I1, I4, I9 et I13) (tableau 1).

PCR spécifique de MmmSC

Les tentatives de mise en évidence de MmmSC par la PCR à partir des échantillons de BAL et des poumons collectés sur les animaux contacts sont restées vaines.

Discussions

La présente étude avait pour objectif d'évaluer la valeur thérapeutique de l'oxytétracycline LA dans le traitement de la PPCB et de déterminer le risque de transmission de la maladie chez les zébus par contact avec des animaux naturellement infectés et traités avec la molécule. Il est communément admis que le traitement antibiotique des animaux malades de PPCB est contre-indiqué, car on pense que son utilisation peut entraîner les animaux traités à devenir porteurs chroniques avec des séquestres pulmonaires pouvant constituer une source de propagation de la maladie. Cependant des points de vue contradictoires sur la question ont été soulevés lors de la Conférence Electronique de la FAO sur la PPCB(FAO, 2002). De ce fait, la nécessité de conduire de façon prioritaire des recherches sur le sujet a été souhaitée.

Globalement, les résultats obtenus lors de la présente étude démontrent que le traitement à l'oxytétracycline LA a guéri cliniquement la majorité des animaux infectés de la PPCB (12/14). Ces résultats indiquent par ailleurs que le traitement n'a pas permis la guérison bactériologique chez tous les animaux car il a été possible de réisoler MmmSC chez tous les animaux ayant présenté des séquestres pulmonaires (4/14). Toutefois, ces animaux infectés et traités n'ont pas transmis la maladie dans les conditions de station et cela malgré un contact étroit et prolongé avec des bovins susceptibles. Ceci dénote que le traitement à l'oxytetracycline LA, même s'il ne stérilise pas l'organisme, peut significativement réduire le portage et l'excrétion des mycoplasmes chez les animaux infectés et traités à un seuil où la contagiosité de la maladie est nulle. Cependant, nous reconnaissons

Tableau 2. Récapitulatif des signes cliniques, des lésions autopsiques, de l'isolement de MmmSC et de la PCR chez les animaux du groupe infecté naturellement de la PPCB et traité à l'oxytétracycline longue action

No Animal	Maladie clinique				Lésions	Isolement de MmmSC				PCR	
	Principaux signes cliniques enregistrés avant traitement			Suite de la maladie après traitement	Type de lésions	LBA	P	GP	SP	LBA	P
I1	Toux, abattement, dyspnée, écoulement nasal, prostration l'amaigrissement			Toux persistante	A, SP	-	-	-	+	-	-
I2	Idem			Guérison	A	-	-	-	PA	-	-
I3	Idem			Idem	A	-	-	-	PA	-	-
I4	Idem			Idem	A, SP	-	-	-	+	-	-
I5	Idem			Idem	A	-	-	-	PA	-	-
I6	Idem			Idem	A	-	-	-	PA	-	-
I7	Idem			Idem	A	-	-	-	PA	-	-
I8	Idem			Idem	A	-	-	-	PA	-	-
I9	Idem			Toux persistante, mort	A, SP	-	-	-	+	-	-
I10	Idem	Guérison	A	-	-	-	PA	-	-		
I11	Idem	Idem	A	-	-	-	PA	-	-		
I12	Idem	Idem	A	-	-	-	PA	-	-		
I13	Idem	Idem	A, SP	-	-	-	+	-	-		
I14	Idem	Idem	A	-	-	-	PA	-	-		

A (Adhérence pulmonaire); LBA (lavage broncho-alvéolaire); P (poumon); GP (ganglion pulmonaire); SP (séquestre pulmonaire); + (positif); PA (pas applicable);- (négatif)

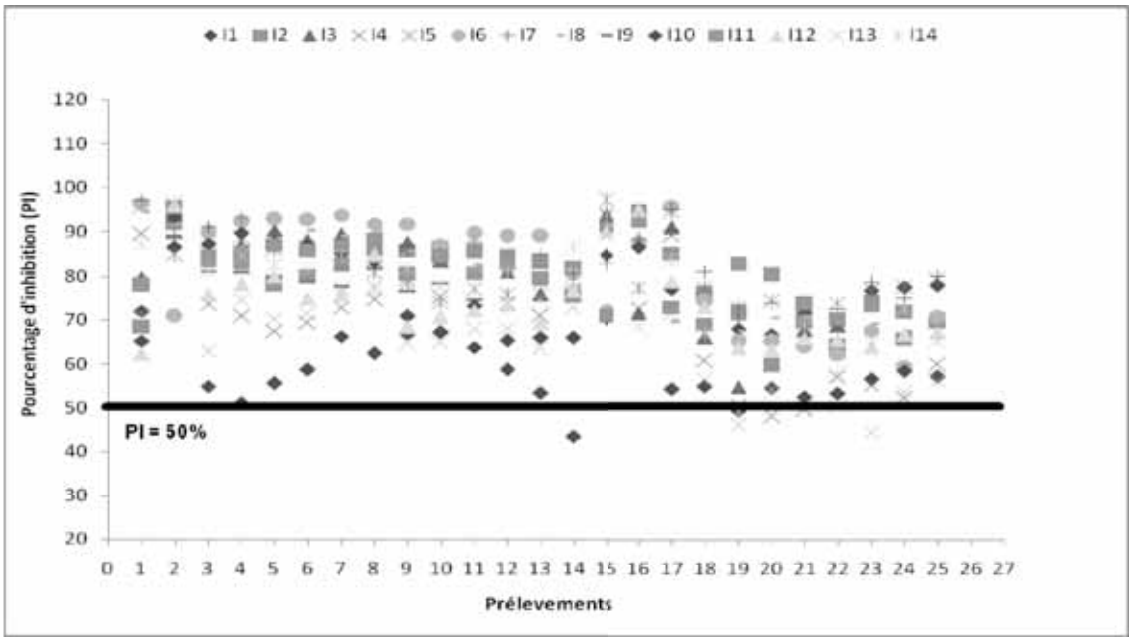

Figure 1. Statut sérologique des animaux du groupe naturellement infecté de la PPCB et traité à l'oxytétracycline longue action. Tous les animaux ont montré une séroconversion avec des titres d'anticorps élevés et persistants durant toute la durée de l'expérimentation.

les limites de cette expérience, car au départ, il n'y a pas été inclus un groupe témoin (animaux infectés et non traités mis en contact avec des animaux susceptibles) pour comparaisons et qui aurait permis de mesurer réellement l'impact du traitement sur la transmission de la maladie. Toutefois, à l'occasion de nos diverses séries d'expériences menées dans les mêmes conditions que la présente et ceci en mettant des bovins sains avec des bovins présentant des signes cliniques typiques de PPCB obtenus par intubation endobronchique, et non traités avec loxytetracycline, les résultats obtenus ont montré, en contraste avec ceux de la présente expérimentation, une bonne reproduction expérimentale de la maladie et ainsi que le réisolement des mycoplasmes en quantité massive dans tous les échantillons prélevés aussi bien chez les animaux donneurs que chez les animaux du groupe contact (résultats non présentés ici). Ceci réellement dénote que, dans la présente expérience, le fait que les animaux traités n'aient pas transmis la maladie pourrait être dû au traitement antibiotique qui a sans doute bien réduit l'excrétion des mycoplasmes et non pas le fait que ces animaux soient dans la forme chronique.

D'une manière générale, les données disponibles dans la littérature scientifique sur le rôle et l'effet exact des antibiotiques dans la pathogenèse de la PPCB et la formation des animaux porteurs chroniques sont presque inexistantes. Il existe certes une documentation ancienne sur quelques aspects généraux de l'antibiothérapie dans la PPCB, notamment l'efficacité de certains agents antimicrobiens dans le traitement de la maladie, mais rien n'est démontré en ce qui concerne leur rôle dans la formation des séquestres et dans la transmission de la maladie.

Les toutes premières études sur le traitement de la PPCB ont indiqué l'efficacité des tétracyclines, de la tylosine, de l'érythromycine et beaucoup d'autres anti-bactériens dans le traitement de la maladie(Camara, 1956 and 1971 ; Hudson and Etheridge, 1965 Moret *et al.,* 1949 and 1951 ; Turner 1960). Par ailleurs, l'efficacité de l'érythromycine, des tétracyclines, de la tiamuline, de la lincomycine et de la spiramicyne en traitement prolongé ou à doses fortes est soulignée aussi bien pour la PPCB que d'autres mycoplasmoses comme la pleuropneumonie contagieuse caprine, l'agalactie contagieuse et la pneumonie enzootique du porc (Bailey 1972 ; Foggie *et al*., 1971 ; Provost 1974 ; Provost, *et al*., 1987). Selon Huhn (1971), les tétracyclines sont capables, expérimentalement, de prévenir la transmission des mycoplasmes ou de supprimer la formation des lésions chez les porcins en cas de pneumonie enzootique, mais vu la posologie prolongée, le traitement n'est pas économique. De même, Goodwin (1979) et Keller (1980) signalent une efficacité de l'antibiothérapie associée à des traitements anti-inflammatoires dans le traitement contre la pneumonie enzootique du porc. En revanche, Stalheim (1976) a mis en doute l'efficacité de la plupart des antibiotiques courants dans le traitement des mycoplasmoses s'accompagnant d'arthrite chez les bovins.

Il semble ainsi que la perception selon laquelle l'antibiothérapie prédispose les animaux atteints de la PPCB à développer le statut de porteurs chroniques et par conséquent des disséminateurs dangereux de la maladie n'est pas bien fondée. On conçoit aisément que ces prises de position découlent des extrapolations faites

jadis à partir des traitements au Novarsénobenzol qui est un arsenical et non un antibiotique(Mornet 1954) bien qu'à ce niveau aussi, il n'existe pourtant pas dans la littérature scientifique accessible des expériences justifiant la transmission de la maladie à partir des animaux blanchis par le traitement au Novarsénobenzol. Certes dans un passé lointain, Orue et Mémery (1961), ont démontré le développement de lésions encapsulées riches en mycoplasmes chez les animaux traités au Novarsénobenzol, mais ils n'ont jamais prouvé que ces animaux puissent transmettre la maladie chez d'autres animaux réceptifs. Malgré cela, ces auteurs ont suggéré que ces animaux peuvent avoir des conséquences désastreuses dans l'épidémiologie de la maladie car pouvant excréter des mycoplasmes et par conséquent, cette thérapeutique et ainsi que tout traitement chimique apparemment efficace (sulfamides et antibiotiques), favorise donc, non seulement la persistance de la maladie, mais également sa dissémination. C'est justement sur la base de telles hypothèses non éprouvées que les épidémiologistes vétérinaires ont préconisé une interdiction pure et simple de l'utilisation des antibiotiques dans le traitement de la PPCB. Or il existe d'anciennes données scientifiques affirmant le contraire. Windsor et Masiga (1977) tentèrent sans succès, malgré un contact prolongé et une soumission à un régime intense de stress, de transmettre la maladie chez les animaux susceptibles à partir d'animaux naturellement guéris d'une infection artificielle avec MmmSC et ayant des séquestres pulmonaires. Ces auteurs n'ont pas également pu reproduire la maladie chez des animaux guéris mis en contact avec des animaux malades de la forme aiguë ou réinfectés par intubation avec une souche virulente de MmmSC.

Cet essai expérimental que nous venons de mener avec l'oxytétracycline LA a conduit à la guérison de la majorité des animaux traités à l'exception de deux bovins dont un meurt part la suite. Le critère de sélection des animaux malades sur le terrain a été déterminé à partir de la percussion de la poitrine et l'épreuve de la course(Camara,1971) et ainsi que les renseignements fournis par le vétérinaire sanitaire de la localité et les éleveurs relatifs au début de l'apparition de signes cliniques chez ces animaux. Ces paramètres ne peuvent attester avec certitude le stade clinique réel chez l'animal malade. En fait, cette information est difficile à déterminer dans les conditions du terrain puisqu'elle suppose de connaître la date exacte à laquelle l'animal a été malade, c'est-à-dire l'apparition des premiers signes cliniques. Donc, il ne peut être exclu que ces 2 animaux rebelles aient été dans la phase avancée de la maladie au moment du traitement. En effet, il est admissible que lorsque la PPCB a évolué pendant longtemps et a crée des lésions organisées, l'antibiothérapie ne peut plus apporter la guérison totale.

Nos résultats ont montré aussi que le traitement n'a pas permis la totale guérison bactériologique car il a été possible de réisoler MmmSC chez tous les animaux ayant présenté des séquestres pulmonaires. Dans cet ordre d'idées, Yaya et al., (2004) ont noté un effet positif sur l'état clinique des malades avec le même type d'antibiotique, mais la guérison bactériologique n'était pas totale. De même, Nicolas *et al*., (2006) ont signalé une baisse significative du nombre de cas cliniques et de mortalités chez des bovins naturellement infectés de la PPCB et traités par l'Advocine sur une période de six mois dans la région de Caprivi en Namibie où la mortalité et la morbidité se produisaient malgré la vaccination. Huebschle *et al*., (2006) étudiant l'effet de la Danofloxacin dans le traitement des bovins naturellement infectés de la PPCB et du rôle de ces animaux traités dans la diffusion de la maladie ont démontré que bien que les guérisons clinique et bactériologique n'aient pu être totalement obtenues, le traitement a significativement réduit la transmission de la maladie chez les animaux sains par contact contrairement chez le groupe des animaux malades non traités mis en contact avec les animaux sains. Ces résultats, bien que semblables aux nôtres, contrastent légèrement avec les conclusions auxquelles nous sommes parvenues. En effet, la différence tient du fait que dans notre expérimentation, bien que le traitement n'ait permis la totale guérison clinique (12/14) et bactériologique (reisolement de MmmSC à partir des séquestres pulmonaires), les animaux infectés traités n'ont pas du tout transmis la maladie et cela malgré un contact étroit et prolongé avec des bovins susceptibles. On peut supposer que le traitement a pu, à coup sûr, fortement réduire l'excrétion des mycoplasmes et ce qui expliquerait peut être qu'il n'ait pas eu de transmission de la maladie chez les animaux sains par contact. A notre avis, c'est cet aspect qui compte car l'important ici c'est de pouvoir limiter l'expansion du contage de la maladie. Un autre fait marquant de la présente étude a été le réisolement de MmmSC à partir des séquestres pulmonaires et cela plus de 10 mois après le traitement. Ceci, à notre connaissance, n'a été que très peu publié à ce jour. Certes l'isolement de mycoplasmes à partir des séquestres pulmonaires a été fréquemment démontré(Provost, *et al*., 1987 ; Windsor and Masiga 1977), mais de façon générale les durées pendant lesquelles ces isolements ont été faits soit n'ont pas été indiquées ou ont été de courte durée. Dans tous les cas, nos résultats paraissent être très importants au niveau épidémiologique car pouvant attribuer une durée au portage chronique dans les modèles de transmission. Ils peuvent être également importants d'un point de vue réglementaire pour les durées pendant lesquelles un pays ne peut pas être déclaré indemne après le dernier cas clinique.

Au regard de tout cela, nous estimons que ce qui est actuellement requis n'est pas la condamnation catégorique de l'usage des antibiotiques dans le traitement de la maladie, mais des efforts en vue d'optimiser la formulation et le régime des médicaments à utiliser dans le

cadre des critères établis et acceptés pour le contrôle de la maladie.

Conclusion

Au vu des résultats présentés ici, on peut donc dire que le traitement à l'oxytétracycline, quoique n'ayant pas permis la guérison clinique et bactériologique de la totalité des animaux traités a tout de même sans doute permis de réduire l'excrétion des mycoplasmes dans le milieu extérieur, ce qui expliquerait peut être qu'il n'ait pas eu de transmission de la maladie chez les animaux sains par contact dans nos conditions expérimentales. A notre avis, c'est cet aspect qui compte le plus car l'important ici c'est de pouvoir limiter l'expansion du contage de la maladie. Ces résultats peuvent avoir des implications importantes dans le contrôle de la PPCB en Afrique.

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EFFECT OF HEMIORCHIDECTOMY ON SPERMIOGRAM AND TESTICULAR CHARACTERISTICS OF WEST AFRICAN DWARF RAM

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EFFET DE L'HEMIORCHIDECTOMIE SUR LE SPERMOGRAMME ET LES CARACTÉRISTIQUES TESTICULAIRES DU BELIER NAIN D'AFRIQUE DE L'OUEST

Résumé

Huit béliers nains d'Afrique de l'Ouest en bonne santé d'âge variant entre 1,5 et 2,5 ans et pesant entre 13 et 18 kg, ont été répartis au hasard en groupes pour une orchidectomie des testicules droit et gauche. L'hemiorchidectomie a été réalisée après trois semaines de quarantaine et les caractéristiques des testicules excisés ont été examinés. Sur une période de sept semaines, les spermogrammes ont été par la suite enregistrés et étudiés ; après quoi les testicules intacts ont été enlevés et leur réactivité aux hemiorchidectomies étudiée. Le spermogramme de base obtenu à partir de tous les animaux avant hemiorchidectomie a servi de témoin, tandis qu'une comparaison a été faite entre les testicules enlevés pendant l'hemiorchidectomie et le testicule intact qui a été retiré par la suite. L'hypertrophie compensatrice a été observée chez tous les animaux après cette procédure dans les deux groupes. Toutefois, cette compensation n'a pas varié de manière significative en comparant les animaux soumis à l'orchidectomie testiculaire gauche avec ceux de orchidectomie testiculaire droite, ($p < 0,05$). La concentration du sperme, la circonférence du testicule, le poids des testicules, la largeur des testicules, le volume de sperme, la motilité des spermatozoïdes, le pourcentage des spermatozoïdes vivants et la longueur du testicule n'ont pas montré de différence significative ($p < 0,05$). Cette étude a montré que les béliers ayant subi l'hemiorchidectomie sont appropriés pour des fins de reproduction ; d'où la recommandation de leur utilisation en cas de perte accidentelle, d'atrophie irréversible ou de dégénérescence d'un testicule unique.

Mots clés: hemiorchidectomie, spermogramme, testicules, Béliers nains d'Afrique de l'Ouest

Abstract

Eight healthy west African dwarf rams aged between 1.5 and 2.5 years weighing between 13 and 18 kg were assigned randomly into groups for right and left testicular orchidectomy. The *Hemi-orchiidectomy* was performed after three weeks of quarantine and testicular characteristics of excised testes examined. Thereafter, for a period of seven weeks, *spermiograms* were studied and recorded after which intact testes were removed and responsiveness to *hemiorchidectomy* studied. Basal spermiogram, gotten from all the animals before *hemiorchidectomy* served as control while comparison were made between testes removed during hemiorchidectomy and the intact testis removed thereafter. Compensatory hypertrophy was observed in all the animals after the procedure in the two groups. However this compensation was not of significant variation comparing animals assigned to left testicular *orchidectomy* with those for right testicular *orchidectomy*, ($p<0.05$). Sperm concentration, testicular circumference, testicular weight, testicular width, sperm volume, motility, percentage-live *spermatozoa* and testicular length did not show any significant difference($p< 0.05$). This study showed that hemiorchidetomised rams are suitable for breeding purposes hence their recommendation for use in the event of accidental loss, irreversible atrophy or degeneration of a single testis.

Key words : *Hemiorchidectomy, Spermiogram, Testicular* Characteristics, West African Dwarf, Ram.

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Introduction

The sheep are among the most versatile of the species managed today in adaptability to diverse production environments and purveyors of various products throughout the world. (Noakes *et al.,* 2001). They are multipurpose animals that produce milk, meat, wool and hide,(Khan *et al.,* 2005), and play important role in the economy of the nations. The meat and milk obtained from these animals constitute the major source of animal protein for a greater part of the population. In Nigeria they come in handy for ceremonial purposes. (Ademosun ,1973). 20% of the world sheep population is located in the tropical and subtropical regions. A rough estimate of their number in the African humid tropics is over 20 million with about 80% found in Nigeria, the West African Dwarf being the predominant Breed.(Charray *et al.,*1992). This breed is trypanotolerant.(Josefina de cambella, 1980). Under traditional husbandry 2-10% of the lambs born are twins but this increases to 15-29% with appropriate supplementary feeding. (Charray *et al,*1992). The virility of the male (ram) is investigated among other means through spermiogram. Spermiogram shows the basic parameters of semen including volume, colour, concentration, spermatozoa morphology, and motility. (Noakes *et al.,* 2001). The evaluation of Locomotor system, scrotal sac, scrotal circumference, testicular mobility, the epididymis and prepuce form the full complement of breeding soundness evaluation in the ram.(Noakes *et al.,* 2001). In many species of animals, the removal of one testis (*hemiorchidectomy*) elicits compensatory hypertrophy of the remaining testis which has been characterized chiefly as an increase in the testicular weight. Some workers, (Broockfor *et al.,* 1983), recorded increase testicular weight after unilateral castration in the bull while others, (Brown *et al.*,1991), recorded similar results in prepubertal rams. A previous study on hemiorchidectomised buck revealed that testicular attributes showed a compensation by *hypertrophy* of the intact testis after *hemiorchiodectomy*. It concluded therefore that hemiorchidectomised West African Dwarf bucks could be used for breeding purposes (Oyeyemi *et al.,*1998).

This work was done to investigate the effect of hemiorchidectomy on the intact testis and semen quality in West African Dwarf Ram. There is dearth of information on this. The work is expected to make available useful information to livestock farmers, Agricultural Extension personnel, breeders and Artificial Insemination stations.

Materials and Method

Eight healthy sexually matured West African dwarf Rams were used.. Age was determined by dentition, (Blood *et al.,*2007), and it ranged between 1 and 2 years. Average weight was 15.4± 2.13kg. Animals were kept in a standard sheep pen and fed concentrate and roughage in the evening. They were released between 0700-1600hours to graze on Axonopus compressors (carpet grass), *Panicium* maximum (guinea grass) and *Pennisetum pueperium* (Elephant grass) in the morning. Water was given ad *libitum*. Twenty eight days of quarantine was observed during which they were deloused, deticked and dewormed. They were placed on *Penicillin* and *Streptomycin* at dosage rate of 20,000 I.U./kg and 10mg/kg body weight respectively. Animals in their intact state served as control. Semen was collected and analyzed for volume, color, percentage - live *spermatozoa*, sperm mass activity, motility, concentration, sperm count and semen pH. Thereafter animals were grouped into two. A group of four underwent left *hemiorchidectomy* (LH) while the other four were given right *hemiorchidectomy* (RH) treatment .Then weekly semen collection was done for seven weeks from the *hemiorchidectomised* animals. Semen collection was with the aid of electro ejaculator (Oyeyemi and Akusu,1998). Seminal volume was measured immediately after collection using a graduated plastic collecting tube. Semen colour was determined by visual assessment s while mass activity was scored by observing the wave motion under x40 magnification. Sperm motility was scored after observation of a drop of semen in 2.9% of sodium citrate on pre-warmed slide. The pH was determined using pH meter while percentage live spermatozoa was estimated from 200 sperm cells counted in a eosin-nigrosin stained smear (Oyeyemi *et al.*,2002). With the use of *Haemocytometrer*, sperm concentration was determined. *Hemiorchidectomy* was done following paramedian *intrascrotal* approach. The procedure was done for all the animals slated for left and right *hemiorchidectomy* and testicular parameters taken thereafter followed by weekly *semenology*. Testes were weighed using electrical weighing scale. Circumference, length and width were measured using flexible tape rule. Testicular volume was determined by water displacement by method. Data was subjected to one - way analysis of variance, mean, and differences were checked using Student's t - test. (Snedecor and Cochran, 1980)

Result

The mean Testicular weight, length and circumference at *hemiorchidectomy* (having removed the effect of body weight) were 4.77g, 0.35g, 0.40g and 0.7g as against 6.64g, 0.32g, 0.43g and 0.81g post *hemiorchidectomy* (Table 1). The differences in testicular weight and width were significant at $p<0.05$. Although the left *hemiorchidectomy* group had higher testicular characteristic values compared to right hemiorchidectomy, none was significant ($p<0.05$). (Table 1). Semen colour was slightly more creamy in the left and right *hemiorchidectomised* animal compared to control while mean seminal volume was not significantly different form pre heiorchidectomised semen. Mean sperm concentratiion post hemiorchi-

dectomy was higher compared to pre *hemiorchidectomy* ($p<0.05$) Post *hemiorchidectomy* mean sperm concentration was 6.33x109 cells/ml while pre *hemiorchidectomy* mean sperm concentration was 3.51x109 cells/ml. No significant difference was observed with mean percentage live and motility comparing pre and post hemiorchicdectomy values. Pre and post mean percentage live values were 83.1% and 88.6% respectively while pre and post motility were 82.1% and 84.3% respectively.

Discussion

Significant increase in the testicular weight post *hemiorchidetomy* conforms with the findings of some authors.(Oyeyemi et al.,1998). Two other authors (Oyeyemi and Akusu,1998) had opined that testicular weight increases from *prepubertal* age to puberty and maturity after which no significant increase will be noticed. Rams used in this work were sexually matured hence the testicular weight increase is attributable to compensatory *hypertrophy*. Significant increase ($p<0.05$) in testicular length reported by some workers, (Oyeyemi et al.,1998), working on West African Dwarf buck is at variance with the findings of this work. However both works agree on the insignificant difference between right and left castration. The spermiogram recorded in this study prior to hemiorchidectomy were similar to those recorded by some workers (Noakes et al., 2001). In consonance with another report, (Oyeyemi et al.,1998), post left and right castration spermiogram was not significantly higher in value than the pre hemiorchidectomy ($p<0.05$). Post hemiorchidectomy mean sperm concentration of 6.33x 10^9 cells/ml was above the 2.5-6.0 x 10^9 range recommended for breeding soundness in ram by some workers, (Noakes et al, 2001), thereby establishing the fact that hemiorchidectomised rams are viable for breeding .The study therefore recommends the use of *hemiorchidectomised* ram for breeding purpose. However, a period off time, after the hemoiorchidectomy is recommended for West African dwarf rams for *hypertrophy* to occur.

Table 1: means of testicular chracteristics of testes removed during and after *hemorchidectomy*

	TESTICULAR WEIGHT (g)	TESTICULAR WIDTH (cm)	TESTICULAR LENGTH (cm)	TESTICULAR CIRCUM.(cm)
MEAN (DURING)	4.77 1.68	0.25 0.05	0.40 0.09	0.70 0.12
MEAN (AFTER)	6.64 1.84	0.32 0.06	0.43 0.08	0.81 0.13
T-TEST	0.036	0.016	0.260	0.059
MEAN (RH /AFTER)	6.12 2.3	0.28 0.05	0.37 0.07	0.72 0.13
MEAN (LH/AFTER)	7.03 1.67	0.35 0.05	0.48 0.06	0.88 0.09
T-TEST	0.3	0.055	0.051	0.071

RH - Right hemiorchidectomy group
LH - Left hemiorchidectomy group

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CHARACTERIZATION OF A HETEROGENEOUS POPULATION OF RABBITS FOR PROLIFICACY, PRE-WEANING LITTER TRAITS AND KIT SURVIVAL

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CARACTERISATION D'UNE POPULATION HETEROGENE DE LAPINS POUR LA FECONDITE, LES CARACTERES DE LA LITIERE AU PRE- SEVRAGE ET LA SURVIE LAPEREAUX

RÉSUMÉ

Cette étude a été menée pour décrire les caractéristiques de fécondité et de la litière d'une population hétérogène de lapins du Nigéria. Les données portent sur des échantillons de 535 lapins avec 10 males et 48 femelles repartis en trois groupes de pairs afin de caractériser la prolificité, les traits de pré-sevrage de la litière et la survie des lapereaux dans les conditions de station. Dans cette population, la couleur du pelage comptait respectivement pour 61% de lapins blancs a taches noires, 26% de lapins noirs a taches blanches et 13% de lapins blancs et noirs mélangés, - ceci pour indiquer le degré d'hétérogénéité de la population. Parmi les femelles, la fréquence la plus élevée du nombre tétines de lapins était de celles ayant 10 tétines (soit 53%), alors que celles ayant 9 et 8 tétines étaient respectivement de 31 % et 16%, ce qui montre la nature hétérogène de la colonie de lapins. La taille des portées à la naissance (LSB) et le nombre des lapereaux vivants (NBA) a varié entre 1 et 10 et celui des survivants entre 1 et 7, respectivement. Les portées les plus fréquentes pour la classe des LSB était de 4 à 5 (soit 52%), tandis que les portées ayant ≤ 3 lapereaux et ≥ 6 lapereaux étaient respectivement de 19% et 29%.. Le poids moyen global des lapereaux lapins à la naissance était de 45,56 ± 9,82 g, tandis que 34% des lapereaux avaient un poids ≤ 40 g, ce qui a contribué au taux élevé de mortalité globale au pré-sevrage. Pour ce qui est de la taille de la portée au sevrage (TPS), les portées de 3 à 4 lapereaux ont été les plus fréquentes (47%), tandis que des portées de ≤ 2 bébés et ≥ 5 lapereaux ont constitué 23% et 30%, respectivement. Le taux de survie lapereaux lapins au pré-sevrage a été faible dans les portées de grande taille (57%), comparativement aux portées de taille moyenne (71%) et les portées de petite taille (77%). Les pertes avant sevrage ont été élevées au cours des deux premières semaines de vie et ont sensiblement diminué pendant la durée d'avant sevrage. Le gain de poids quotidien des jusqu'à 28 jours d'âge variait de 3 à 21 g, avec une moyenne globale de 7,47 ± 3,59 g.

Mots clés: Lapins, population hétérogène, caractérisation, traits de la litière, survie des lapereaux.

ABSTRACT

This study was conducted to describe prolificacy and litter characteristics of a Nigerian *heterogeneous* population of rabbits. Data on 535 kits sired by 10 bucks and 48 does across three parities were used to characterize this population of rabbits for prolificacy, pre-weaning litter traits and kit survival under on-station conditions. For this population, coat colour of does were 61%, 26% and 13% for all white with black patches, all black with white patches and a mixture of white and black colours, respectively, indicating the degree of heterogeneity in the population. Among the does, the highest frequency of teat number was 10 teats (53%), while those with 9 and 8 teats were 31% and 16%, respectively, reflecting the heterozygous nature of the colony. Litter size at birth (LSB) and number born alive (NBA) ranged between 1 and 10 and 1 and 7 kits, respectively. The most frequent litters for NBA class was 4 to 5 kits (52%), while litters with ≤ 3 kits and ≥ 6 kits were 19% and 29%, respectively. Overall mean for kits' weight at birth was 45.56 ± 9.82 g, while 34% of the kits had birth weights ≤ 40 g which contributed to the high overall pre-weaning mortality rate. For litter size at weaning (LSW), litters of 3 to 4 kits were the most frequent (47%), while litters of ≤2 kits and ≥5 kits constituted 23% and 30%, respectively. Pre-weaning kit survival rate was low in large-sized litters (57%), compared with medium- (71%) and small-sized litters (77%). Pre-weaning losses were high in the first two weeks of life and markedly reduced in the remaining pre-weaning period. Daily kit gain to 28 days of age ranged from 3 to 21 g, with an overall mean of 7.47 ±3.59 g.

***Key words*:** Rabbits, *Heterogeneous* population, characterization, Litter traits, Kit survival.

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Introduction

In many parts of sub-Saharan Africa (SSA), *heterogeneous* populations of rabbits represent the stock of animals used predominantly in backyard systems. These stocks are a product of planned and indiscriminate crossing among local and exotic breeds of rabbits that were introduced to many countries in SSA about 100 to 150 years ago (Lukefahr, 1998). These breeds of rabbits were imported at different times and in different waves via United States Agency for International Development, Heifer Project International or via Christian missionary activities in the humid zones of SSA (Price *et al*., 1982; Lukefahr and Cheeke, 1991a). The most common breeds of rabbits that were imported included New Zealand White, Californian, Chinchilla, Flemish Giant, among others (Lukefahr and Cheeke, 1991a). Lukefahr (2000) described the importation of sixteen breeds of rabbits from Denmark, Switzerland and the USA to Ghana, which were then inter-crossed with local stocks, giving rise to a highly heterogeneous population of rabbits, as part of a national rabbit programme.

Attention should be drawn to the full characterization of these heterogeneous stocks for the following reasons:

(a) they represent the stocks used predominantly by backyard units (Opoku and Lukefahr, 1990; Lebas et al., 1997), some investigators (Collin and Lebas, 1996) noted that three-quarters of all breeding does in SSA are found in traditional backyard systems;

(b) these stocks have shown some degree of adaptation to sub-optimal conditions (poor feeding, housing and healthcare) under backyard and smallholder systems largely, on account of their heterogeneity (Lukefahr, 2000);

(c) appropriate exploitation of these stocks could be a viable option in the provision of suitable genotypes to backyard rabbit units in SSA, since reports have indicated that newly imported breeds showed poor adaptation, especially under field conditions (Opoku and Lukefahr, 1990);

(d) using *heterogeneous* stocks of rabbits could help to avoid the logistics (e.g. money and time) involved in the importation of new breeds into the developing world, as well as the risk of disease transmission (e.g. *pasteurellosis*) along with imported stocks (Lukefahr, 2000). It will also help to avoid a dependency situation where rabbit genetic stocks and other inputs for rabbit production are derived from external sources (Lukefahr, 2004). According to this author, this contributes to the development of sustainable small scale rabbit production systems in poverty alleviation programmes.

There is a need to characterize *heterogeneous* rabbit populations, taking into account established models and guidelines of Khalil (1993). Thus, the main objective of the present study was to give some description for litter characteristics, for rabbit populations in Southwestern Nigeria, with emphasis on prolificacy, pre-weaning kit survival rate and daily kit gainunder on-station conditions.

Materials and Methods

Rabbit population and management

This study, which is a part of a large scale project to characterize and evaluate the performance of a Nigerian *heterogeneous* population of rabbits, was conducted at the Rabbit Unit of the Teaching & Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria, between April 2006 and March 2007. Ten bucks and 48 does of 8 to 10 months of age and weighing between 1.5 and 2.5 kg were used in the establishment of the rabbit colony. These animals were identified by means of ear tattoo and cage tags. All the rabbits were housed individually in row cages of dimension 76 × 62 × 42 cm, placed 90 cm from the floor. The cages were made of wood with galvanized wire mesh at the sides and bottom. In each cage, there were two earthen pots for feed and water. The cages were located inside buildings that were designed with wood and wire mesh at the sides to ensure adequate ventilation and comfort.

Matings were routinely done in the morning before the weather gets hot. Each doe was taken to the cage of the buck and after mating, returned to her cage. Pregnancy tests were conducted on day 14 post-mating. Does that were found not gravid were re-mated immediately. For all pregnant does, kindling boxes were placed in their cages on day 25 post-mating. At kindling, the nest boxes were checked daily and total and live litter sizes and weight at kindling were recorded. The litters were monitored daily for mortality. The size and weight of each litter was recorded weekly until weaning at day 28 post-kindling.

Feeding

The rabbits were fed a palm kernel-based diet with the following proximate per cent composition: dry matter 90.95; ash 7.40; crude fibre 5.36; ether extract 7.08; crude protein 22.10 and nitrogen free extract 49.01; gross energy (Kcal/Kg) 4.58. (Note: Equals signs could be deleted in the preceding sentence) Ingredients used to formulate the diet as described by the manufacturer (Joam Farm Ventures®, Ile-Ife, Nigeria) included maize (34%), wheat offal (32%), soya (5%), palm kernel cake (23.8%), oyster (2%), bone meal (2%), methionine (0.1%), lysine (0.1%), pre-mix (0.25%), salt (0.25%), and fish meal (0.5%).

Data collected and Statistical analysis

Data recorded for each doe included: (1) doe coat colour (three categories of all white with black patches, all black with white patches at the ears, nose, tail and

feet, and a mixture of black and white); (2) doe teat number (three classes of 8, 9 and 10 teats); (3) litter size recorded at birth (LSB) and number born alive (NBA) recorded within 24 hours of kindling and live litter sizes recorded weekly thereafter at days 7, 14, 21 and 28 post-kindling, (4) LSB classes defined as low for litters with ≤ 3 kits, medium for 4 to 5 kits and high for 6 kits or more; (5) litter and kit weights at kindling and at days 7, 14, 21 and 28 post-kindling. From these variables, daily kit gain (g) and kit survival rate from kindling to day 28 post-kindling were computed. Detailed methodology is as described in Oseni and Ajayi (2010).

Data were summarized using Microsoft Excel, while data analysis employed Univariate and Frequency Procedures of SAS (2004).

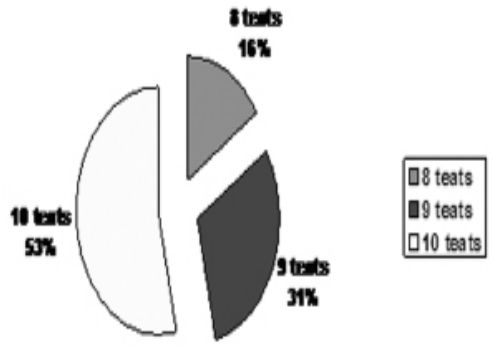

Figure 1. Distribution of does by teat number

Results

Figure 1 shows the distribution of does in different teat number classes of 8, 9 and 10 teats. The highest frequency class was does with 10 teats (53%), followed by does with 9 teats (31%), while the least category were does having 8 teats (16 %). The distributions of does by coat colour classes are shown in Figure 2. Over two-thirds of the does in the colony were all white with black patches, while 26% had all black with white patches and the remainder (13%) were a mixture of black and white colours.

Table 1a shows the overall means of litter sizes from kindling through weaning at day 28 post-kindling. Means for litter size at birth (LSB), number born alive (NBA) and litter sizes at days 14 and 28 were 4.66 ± 1.51, 4.49 ± 1.47, 3.96 ± 1.41 and 3.62 ± 1.50, respectively. For all the does and across all parties, the range in LSB were 1 to 10 kits, while ranges in NBA were 1 to 7, and litter sizes at days 14 and 28 post-kindling were 1 to 7 and 1 to 6 kits per litter respectively. The most frequent LSB, NBA and litter sizes at days 14 and 28 were 5, 5, 4, and 4 kits respectively for this *heterogeneous* population of rabbits. Frequency distribution of NBA in different classes of LSB (Figure 3) reveals that the highest frequency (52%) were litters with 4 to 5 kits, while litters with ≤ 3 kits and ≥5 kits had frequencies of 19% and 29%, respectively. The trend for litter size at weaning (LSW, Figure 4) indicates that majority of the litters had between three and four weaners, when compared to litters of less than 2 kits and greater than or equal to 5 kits that were 23% and 30%, respectively.

Table 1. Overall means for litter size, survival rate (%), kits' daily gain, litter and kits' weights (g) from kindling through weaning.

Trait	n	Mean	Minimum	Maximum
a. Litter sizes				
Litter size at kindling	116	4.66±1.51	1	10
Number born alive	112	4.49±1.47	1	7
Litter Size at day 14	102	3.96±1.41	1	7
Litter Size at day 28	99	3.62±1.50	1	6
b. Kit and litter weights (g)				
Kit weight at kindling	114	45.56±9.82	25	82
Kit weight at day 14	101	150.94±61.18	75	440
Kit weight at day 28	99	254.95±106.17	120	675
Litter weight at kindling	112	204.12±66.10	50	400
Litter weight at day 14	102	542.98±149.80	150	895
Litter weight at day 28	99	840.04±291.54	120	1350
Daily kit gain to 14 d	101	7.51±3.97	3	28
Daily kit gain to 28 d	99	7.47±3.59	3	21

Overall means and standard deviations for kit and litter weights at kindling through weaning at day 28 post-kindling are shown in Table 1b, while Figure 5 shows the frequency distribution of kit birth weight by classes, defined as kits with low, medium and high birth weight. Kit weight at kindling ranged from 25 g to 82 g with an overall mean of 45.56 ± 9.82 g. The frequency distribution of

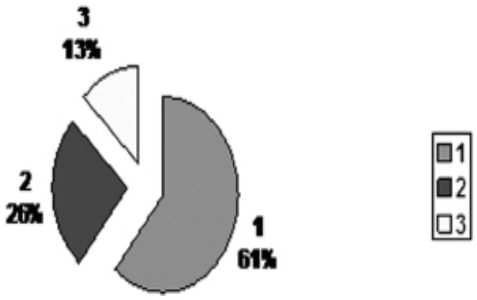

Figure 2. Distribution of does by the coat colours
[1 = all white with black patches; 2 = all black with white patches and 3 = a mixture of black and white colours]

kits' weight at kindling (Figure 5) showed that one-third of all the kits had birth weights of ≤ 40 g, while kits with weights ranging between 40 and 50g, and greater than 50 g were 44% and 22% respectively.

Means for daily kid gain (DKG) from kindling to days 14 and 28 (Table 1) were 7.51 ± 3.97g and 7.47 ± 3.59g. Of interest however, is the wide range of 3g to 21 g for ADG between kindling and weaning at day 28.

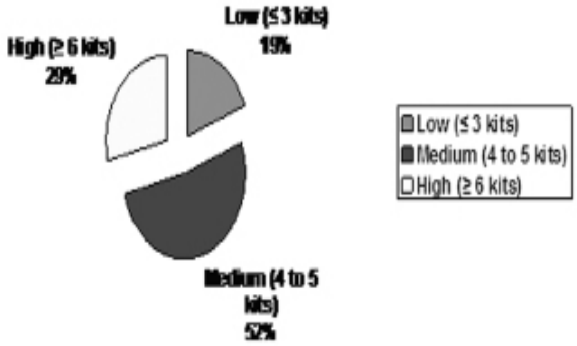

Figure 3. Frequency distribution of number of kits born alive by the number of kits per litter.

Table 2 shows pre-weaning kit survival (KSR) as affected by the litter size at birth classes, defined as low litter sizes (≤3 kits), medium (4 to 5 kits) and large litter sizes (≥ 6 kits). For these categories, total kit survival to 14 and 28 days post-kindling were taken as a proportion of total kits kindled in each of the classes. Results show that at day 28 post-kindling, 77%, 71% and 57% of all kits

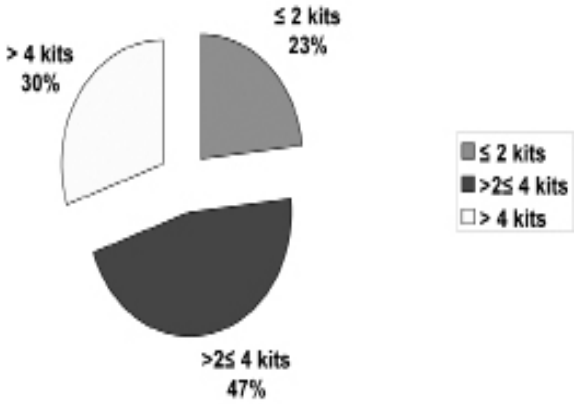

Figure 4. Distribution of litter size at weaning for a *heterogeneous* population of rabbits

kindled survived in the low, medium and high litter sizes at birth classes respectively. Figure 6 shows the trend for survival rate during the pre-weaning phase. Most of the pre-weaning losses were within the first two to three weeks of life, while losses between days 21 and 28 post-kindling were minimal.

Discussion

High frequency of does with 10 teats could reflect some degree of *heterozygosity* in the population of rabbits evaluated. According to Lukefahr *et al*., (1980), high teat number is associated with *heterozygousity* in crossbred populations. These authors noted that crossbred does had significantly large number of teats when compared to their purebred parents. The fact that the population of rabbits under evaluation arose from breeds imported at different times could also have contributed to the heterozygous nature of this population.

As shown in Figure 2, the rabbit population showed a mixture of coat colours indicating a high degree of heterogeneity, on account of their multiple breeds of origin . Such diversity in coat colours is characteristic of heterogeneous stocks of rabbits. Local rabbit populations displaying astounding *phenotypic* variability in coat colours have been reported from Togo (Lukefahr, 1998) and Cameroon (Lukefahr and Cheeke, 1991).

In terms of prolificacy, the range of LSB was 1 to 10, while the most frequent LSB class was 4 to 5 kids. In comparison, Ayyat and Marai (1998) in Egypt, reported that the most frequent LSB was for litters with 5 to 7 kits (47.5%), followed by litters with greater than 8 kits (28.4%) and litters with less than or equal to 4 kits (24.1%). Thus, the range of LSB and NBA observed in this study is slightly lower and may indicate a generally reduced prolificacy among this heterogeneous population of rabbits. Overall, the means for LSB and LSW noted in this study are within the ranges cited in literature reports from sub-

Table 2. Pre-weaning kit survival as affected by the litter size at birth category.

Range Born	Category	Total kits at 14 d	Total kits % Survival at 14 d	Total kits at 28 d	% Survival at 14 d	at 28 d
1 to 3 kits	Low	54	46	85	43	77
4 to 5 kits	Medium	270	218	81	194	71
≥ 6 kits	High	211	138	65	121	57
All	All	535	402	75	358	67

Saharan Africa (Ehiobu *et al*., 1997; Onifade *et al*., 1999). For litter size at weaning, almost half of the does weaned 3 to 4 kits per litters. The trend could be attributed largely to the low prolificacy and/or high pre-weaning mortality, as evidenced by the high frequency of kits born with birth weight less than or equal to 40 g.

Such a high proportion of kits with birth weights of ≤ 40 g could have contributed to the overall high pre-weaning mortality rate. According to Poigner *et al*., (2000), high mortality rates are associated with low birth weight. This perhaps explains the high overall mortality rates recorded.

Most of the pre-weaning losses were however, within the first two to three weeks of life, while losses between days 21 and 28 post-kindling were minimal. An increase in pre-weaning mortality occurred in litters with large sizes and nearly most of the pre-weaning mortality occurs in the first two weeks of life (Rashwan and Marai, 2000), a period when the kits rely exclusively on the mother's milk. At about 21 days of age, the kits start to nibble and feed on concentrates and forages provided for the dam in addition to suckling, which further enhances kit survival.

Overall, the means for daily kit gain (DKG) from kindling through weaning was about circa 7.5 g. Of interest however, is the wide range of 3 to 21 g recorded for kits derived from different litter sizes. This is largely due to differences in the number of kits per litter, which in a companion paper (Oseni and Ajayi, 2010) affected DKG and kit survival rate. These authors demonstrated that kits in small litters recorded significantly high DKG when compared to kits in medium and large litters. Other investigators (Ayyat and Marai, 1998) reported that DKG between kindling and weaning at d 28 were 13.6 ± 0.08, 14.4 ± 0.10 and 14.9 ± 0.15 for NBA classes that were ≤4, 5 to 7 and ≥8 kits, respectively.

These results indicated a higher kit survival rate in low litter sizes. In contrast, there was higher kit mortality in larger litters. As a solution, intra-litter homogenization has been suggested as a management strategy to boost kit survival in large litters (Poigner et al., 2000). According to these authors, the method involves decreasing weight differences within litters, or kits of low birth weight are transferred to smaller litters.

Conclusion

The rabbit population under evaluation showed astounding *phenotypic* variability in coat colours and a high frequency of does with 10 teats, indicating some degree of *heterozygosity* and *heterogeneity*. One-third of all the kits recorded low birth weight and this contributed to high pre-weaning mortality rate. Pre-weaning kit survival rate varied by the size of the litter, with higher survival rate recorded for low litter sizes.

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THE EVALUATION OF ACTIVATED DIETARY CHARCOAL FROM CANARIUM SCHWEINFURTHII ENGL. SEED AND MAIZE COB AS TOXIN BINDER IN BROILER CHICKENS

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EVALUATION DU CHARBON ALIMENTAIRE ACTIVÉ À PARTIR DES SEMENCES DE CANARIUM SCHWEINFURTHII ENGL. ET DES ÉPIS DE MAÏS EN TANT QU'ÉLÉMENTS DE COMBINAISON DE TOXINE DANS LES POULETS.

Résumé

Cent vingt poussins males âgés de 3 semaines élevés en cage dans un dispositif complètement aléatoire ont été utilisés pour évaluer l'effet du charbon des noyaux de *Canarium schweinfurthii* (charbon A), de rafles de maïs (charbon B) ou de leur association (M) dans la proportion 1/1 comme capteur d'aflatoxine B1 dans l'aliment de poulet de chair. A cet effet, les poussins ont reçu soit un aliment contenant du tourteau d'arachide de bonne qualité (C-) soit un aliment (C+) contenant du tourteau d'arachide infesté d'Aspergillus flavus (22,02ppb/kg d'aflatoxine B1), soit l'aliment C+ supplémenté avec 0,2 ou 0,4% de charbon activé de noyaux de Canarium (A0,2 et A0,4), de rafles de maïs (B0,2 et B0,4) ou de l'association des charbons A et B (M0,2 et M0,4). La consommation alimentaire a été significativement ($P<0,05$) plus élevée pour tous les poussins recevant l'un ou l'autre charbon ou leur association (4166 à 4679g) comparée à celle des poussins du groupe C+ (4075g). Au taux d'inclusion de 0,2%, la consommation alimentaire la plus élevée a été enregistrée chez les poulets soumis à la ration.

M0,2 (4679g) comparée aux rations C-, C+, A0,2, B0,2. En général, le poids a baissé avec l'augmentation du taux d'inclusion du charbon dans l'aliment. Les sujets qui ont respectivement consommé l'aliment A0,2, A0,4, et M0,2 ont enregistré le poids vif et le gain de poids les plus élevés ($P<0,05$) comparés à tous les autres groupes de poulets. Le gain de poids le plus élevé a été enregistré avec 0,2% du mélange de charbon A et B comparé à 0,4%. Cependant, l'inclusion de 0,2% du charbon B et 0,4% du mélange de charbon A et B dans l'aliment a induit une augmentation significative ($P<0,05$) de l'indice de consommation. Les poulets des traitements C+ et M0,4 ont enregistré le plus faible poids et la plus petite densité de l'intestin comparés aux poulets de tous les autres groupes. L'inclusion des charbons A, B et M dans l'aliment a induit une diminution significative ($P<0,05$) du poids relatif du foie comparé à C+. Le rendement carcasse, les poids relatifs du cœur et de la graisse abdominale n'ont pas été affectés par les rations. Le poids relatif du pancréas le plus élevé a été enregistré chez les poulets du contrôle négatif C- (0,20g) comparé à tous les autres groupes de poulets (0,13 – 0,18g). Il a été conclu que l'inclusion de 0,2% du mélange (1/1) de charbon de rafles de maïs et des noyaux de Canarium ou de 0,4% de charbon de rafles de maïs dans un aliment contaminé peut permettre de fixer l'aflatoxine B1 et d'améliorer les performances de croissance des poulets de chair.

Mots clés: Aflatoxine B1, *Canarium schweinfurthii,* Capteur de toxine, Charbon végétaux, Poulets de chair, Rafles de maïs.

Abstract

One hundred and twenty 3-week old male broiler chickens were used to evaluate the effects of dietary charcoal from *Canarium schweinfurthii* (charcoal A) and maize cob (charcoal B) on aflatoxin B1 toxicosis in broiler chickens. The individually caged birds were randomly allotted to 8 groups of 15 birds and fed in a completely randomised design a diet with either fresh groundnut meal (C-), groundnut meal infested with 22.02 ppb of aflatoxin B1 (C+) or diet C+ supplemented with either 0.2 or 0.4% of charcoal A (A0.2 and A0.4 respectively), charcoal B (B0.2 and B0.4 respectively) or a 1/1 mixture of A and B (M0.2 and M0.4 respectively). The results indicated that the inclusion of charcoal either individually or as a mixture significantly ($P<0.05$) improved feed intake (4166.66 to 4679.16g) as compared with that of birds fed diet C+ (4075.00g). At 0.2% inclusion, the highest feed intake was recorded with the birds fed M0.2 (4679.16g) as compared with A0.2, B0.2, C- and C+. In general, there was a drop in weight with increasing level of charcoal in the diet. Birds fed diets A0.2, A0.4 and M0.2 had a significantly ($P<0.05$) higher live body weight and body weight gain when compared with all the other groups. The highest weight gain was recorded with birds fed the 1/1 mixture of charcoal A and B at 0.2% inclusion in diet as compared with 0.4% inclusion. However, the inclusion of 0.2% charcoal B and 0.4% of mixture in the diet significantly ($P<0.05$) increased feed conversion ratio. Birds from treatments C+ and M0.4 had smaller intestine weight and intestine density as compared with the birds from all other treatments. Carcass yield, relative weight of heart and abdominal fat were not significantly affected ($P>0.05$) by the treatments. Both charcoal A and B significantly ($P<0.05$) yielded smaller liver as compared with C+. The highest pancreas weight was recorded in birds fed C- (0.20g) as compared with the birds fed any other diet (0.13 – 0.18g). It was concluded that up to 0.4% of maize cob charcoal and 0.2% of a 1/1 mixture of charcoal from Canarium schweinfurthii seed and maize cob could be used as feed additive to absorb aflatoxin B1 and promote growth performance of broiler chickens.

Key words: Aflatoxin B1, Broiler chickens, *Canarium schweinfurthii,* Maize cob, Plant charcoal, Toxin biner.

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Introduction

Aflatoxins are *hepatotoxic mycotoxins* produced in grains (corn, peanut, and cotton-seed), and feedstuffs contaminated by the molds *Aspergillus flavus* or *Aspergillus parasiticus* (*Lindmann et al*., 1993; *Nahm*, 1995; *Kubena et al*., 1997; *Chen*, 2003; *Linden*, 2006). *Aflatoxin* B1 is the most potent and prevalent of the 18 known naturally-occuring aflatoxins (*Antonio et al*., 1996; *Ramos et al*., 1996; *Placinta et al*., 1999; *Mabbett*, 2004). Animals can develop acute or chronic poisoning from eating the contaminated grain or feedstuff (*Anthony,* 1997; Mabbett, 2005; *Raju et al*., 2006). Aflatoxins are destroyed at high pH (Mabbett, 2004), and can be removed by treating dried corn and other grains with *ammonia* (*Mabbett*, 2005). This procedure is however, not practical and is certainly not applicable particularly on groundnut cake which is the most available and cheaper source of protein in African countries. Groundnut meal is an excellent medium for microbial growth, with the risk of fungal growth increasing rapidly with rising moisture content. This fungal growth mostly produced aflatoxins which are widespread contaminant of tropical commodities especially groundnut and maize (Mabbett, 2004). Although chronic aflatoxicosis may respond to withdrawal of the contaminated ration and replacement with well-balanced diet, a fast-acting treatment is needed for the acute condition to save affected animals.

Activated charcoal has been shown to be a tenacious absorbent of a wide variety of toxic agents (Ramos *et al*., 1996; Mabbett, 2005; Ruttanavut *et al*., 2009), and thus seemed a likely candidate for the study of aflatoxin decontamination. A report that aflatoxin B1 is strongly absorbed in vitro (Ramos *et al*., 1996) stated that intestinal absorption of aflatoxin might be decreased by activated charcoal. The objective of the present study was to evaluate the individual and mixture of charcoals from *Canarium* seed and maize cob as therapy of *aflatoxicosis* in broiler chickens.

Material and Methods

Animal

The experimental birds were from a flock of Hybro male broiler chicks brooded to 21 days of age in a deep litter system at a density of 20 birds\m^2. Birds were given vaccines in drinking water against Newcastle disease and Infectious Bronchitis on the 8th day with booster doses on the 23rd day of age, and against Gumboro disease on the 10th day of age. Coccidiosis prevention was done using Vetacox® for 3 consecutive days per week from the 2nd to the 5th week of age. Birds were administered commercial antistress (Aliseryl®) in drinking water during the first 3 days upon arrival, after each vaccination, weighing sessions and transfer from brooding to finishing cages.

Experimental diets

Aflatoxin B1 was produced on groundnut meal substrate. Five kilograms of peanut meal were sucked in 4 litters of water in a 10-litter bucket overnight. The bucket was autoclaved at 125°C for 15 min, cooled and inoculated with *Aspergillus flavus*. Then, 1 litter of sterile water was added and the bucket was incubated at 20°C for 10 days. After incubation, the moulty groundnut meal was steamed at 100°C for 1 h to kill the spores, followed by drying in hot air oven overnight at 60°C. The dried groundnut culture was ground to fine powder and analysed for its aflatoxin B1 content using the Enzyme Linked Immuno-Sorbent Assay (ELISA) with Immuno enzymatic kits (Transia Plate Aflatoxin B1, PT 53 2004-Rev.4). After fermentation, the peanut culture obtained containing 116.50 ppb of aflatoxin B1 was mixed with mycotoxin free ingredients to a final concentration of 22.02 ppb of aflatoxin B1 and served as positive the control (C+).

Activated charcoal was obtained from *Canarium schweinfurthii* seed (charcoal A) and maize cob (charcoal B) collected in villages around the University Experimental Farm and sieved to pass a 1 mm mesh. Two basic diets were formulated (Table 1) to contain either fresh groundnut meal contaminated with less than 0.5 ppb of aflatoxin B1 (C-) or groundnut contaminated with 22.02 ppb of aflatoxin B1 (C+). The six additional diets were made by supplementing diet C+ with 0.2 or 0.4 of charcoal A (A0.2 and A0.4), charcoal B (B0.2 and B0.4) or a 1/1 mixture of charcoal A and B (M0.2 and M0.4).

Experimental design

A total of 120 male *Hybro* birds 21 days of age with an average weight of 678g were individually caged at a density of 0.12m^2/bird and fed ad *libitum*. Each of the 8 experimental diets including the controls (C-, C+, A0.2, A0.4 B0.2, B0.4 M0.2 and M0.4) was fed to fifteen birds (experimental units), chosen at random in a completely randomized design with 8 treatments replicated 15 times.

At 49 days of age, 5 birds per treatment were randomly selected for carcass evaluation. The birds were fasted for 24 hours weighed and slaughtered as indicated by *Jourdain* (1980). The weight of ready to cook carcass, abdominal fat, liver, heart, pancreas, gizzards, head, legs and the weight, length and circumference of the intestine were measured. For the intestine, the cut was done from the start of the duodenal loop to the end of the *cloaca*. The density of the intestines was calculated as the ratio of the weight/length of the intestine.

Statistical analysis

All data were subjected to analysis of variance procedures as described by *Vilain* (1999) and in case of statistical difference, the means were compared using the Duncan's Multiple Range test. The SPSS computer software package was used for all statistical analysis.

Table 1: Composition of experimental diets

Ingredients (%)	Diets	
	C-	C+
Maize	65.7	65.7
Good peanut meal	20	/
Mould contaminated peanut meal	/	20
Soybean meal (48%)	4	4
Fish meal (60%)	4	4
Bone meal	1.3	1.3
Premix (5%)1	5	5
Total	100	100
Calculated chemical composition		
Crude protein (CP)	20.77	20.77
Calcium	1.04	1.04
Non-phytate phosphorus	0.63	0.63
Lysine	1.02	1.02
Methionine	0.42	0.42
Metabolizable Energy (Kcal/kg)	3001.95	3001.95

[1]*Premix* 5%: CP=40%, *Lysine*= 3.3%, *Methionine*=2.40%, Ca=8%, P=2.05%, *Metabolizable Energy*=2078kcal/kg

Results

The means of feed intake, Final body weight, body weight gain, feed conversion ratio are shown in Table 2. The data indicated that the inclusion of charcoal A and B significantly (P>0.05) improved feed intake of birds compare to birds fed diet containing 22.02ppb of *aflatoxin* B1 without charcoal (C+). The highest feed intake was recorded in the treatment with 0.2% inclusion of combined charcoal A and B. There was no significant difference (P>0.05) in feed intake of birds fed diets C-, A0.2, A0.4, B0.2, B0.4 and M0.4. There was a slight drop in feed intake with increasing level of charcoal. At 0.2%, the highest feed intake was recorded with the birds fed the 1/1 mixture of charcoal A and B as compared to individual charcoal A or B, C- and C+. Feed intake was not significantly affected (P>0.05) with the inclusion of 0.4% of any of the charcoal or their combination as compared to C-. When compared with C+, the inclusion of 0.40% of individual or combined charcoal A and B significantly (P<0.05) increase feed intake.

Birds fed diets A0.20, A0.40 and M0.20 had a significantly higher live body weight and body weight gain than those fed C-, C+, B0.20, B0.40 or M0.40 diets. There was a drop in weight gain with increasing level of charcoal. However, the highest weight gain was recorded with birds fed 0.2% combined charcoal A and B. The inclusion of 0.2% of charcoal B and 0.4% of mixed charcoal A and B in the diet significantly (P<0.05) increased feed conversion ratio.

Data related to digestive organ development are shown in Table 3. Intestine circumference and relative weight of gizzard of birds in all treatments were not significantly (P>0.05) affected by the inclusion of charcoal either individually or in combination. Birds from treatment M0.2 had longer intestine as compared with those from all other treatments. Birds from treatments C+ and M0.4 had lighter intestine and smaller intestine density as compared with those from treatments C-, A0.2, A0.4, B0.4 and M0.2.

The dressing carcass, relative weight of heart and abdominal fat were not significantly affected (P>0.05) by any of all treatments (Table 4). C+ significantly (P<0.05) increased the liver weight while both charcoal A and B maintained its proportion unchanged as compared with C-. The heaviest pancreas was recorded in birds fed C- (0,20g) as compared to birds fed all other diets (0.13 – 0.18g). Apart from birds fed 0.4% charcoal B, all other birds had smaller head and legs as compared with C- and C+.

Discussion

The main purpose of this study was to investigate whether the inclusion of individual or combined activated charcoal from *Canarium* seed and maize cob in diets contaminated with aflatoxin B1 could improve the growth performance of broiler chickens. Feed intake and body weight gain were significantly improved in birds fed charcoal suggesting inactivation of *aflatoxin*B1. Charcoal may bind *aflatoxin* B1 within the intestinal tract, preventing its absorption (*Ramos et al*., 1996), and may aid in the binding of *endoxins* produced by *pathogenic microflora* in the intestine (*Dalvi* and *Ademoyero*, 1984; *Bertina*, 1989; *Komkrich*, 2004). The depression in feed intake recorded in chickens fed diet contaminated with 22.02 ppb of *aflatoxin* B1 without charcoal could be associated with gastrointestinal injury caused by this toxin. *Robens* and *Richard* (1992) suggested that severe oral lesions in animals, as caused by mycotoxins, impair their ability to eat, thus resulting in reduced weight gains. When wood charcoal was added to diets containing *aflatoxins* or T-2 toxin, reductions in feed intake and body weight gain of chickens were better (Dalvi and Mc Gowan, 1984). The result of the present study is in agreement with the study of *Dalvi* and *Mc Gowan* (1984) on broilers fed 10 ppm of *aflatoxin* B1 and 0.1% activated charcoal given in the drinking water as an antidote. This treatment resulted in trend of improvement in feed consumption (10%) and weight gain (28%) over birds receiving 10 *ppm* aflatoxin B1 alone. Similar results were obtained with chickens dosed with 10 ppm of *aflatoxin* B1 and 200 *ppm* of activated charcoal by *Dalvi* and *Ademoyero* (1984). The highest feed intake was recorded with 0.2% charcoal fed individually or in

Table 2: Performance of broiler chickens fed diets with *aflatoxin* B1 contaminated groundnut meal and graded levels of individual or combined activated charcoal from *Canarium* seed (A) or maize cob (B)

Parameters	Treatments							
	C-	C+	A 0.20	A 0.40	B 0.20	B 0.40	M 0.20	M 0.40
Feed consumption (g)	4391.66 ±457.62b	4075.00 ±296.64c	4387.50 ±308.92b	4333.33 ±297.76b	4166.66 ±350.23bc	4466.66 ±278.68b	679.16 ±146.98a 679.16	4375.00 ±361.24b
Initial weight (g)	678.33 ±48.23a	679.16 ±45.87a	678.33 ±34.44a	678.33 ±35.30a	678.66 ±8.75a	679.16 ±14.28a	679.16 ±49.23a	679.16 ±41.88a
Final weight (g)	2387.50 ±114.83b	±45.87a 2275.00	2462.50 ±275.13a	2408.33 ±78.52a	2256.66 ±148.71c	2366.66 ±135.70b	2491.66 ±119.02a	2290.00 ±167.48c
Total weight gain (g)	1709.16 ±114.51a	1595.83 ±84.28b	1770.00 ±264.93a	1730.00 ±91.81a	1540.00 ±205.86b	1687.50 ±136.33a	1812.50 ±139.66c	1610.83 ±200.63b
Feed conversion ratio	2.57 ±0.31a	2.55 ±0.13a	2.51 ±0.39a	2.50 ±0.14a	2.74 ±0.42b	2.48 ±0.32a	2.58 ±0.19a	2.72 ±0.12b

a, b, c: Means with different letters in the same row are significantly different (P<0.05)

combination and there was a slight drop in feed intake as the charcoal level increased. This result is not in agreement with *Ruttanavut et al.* (2009) who reported that weight gain and feed efficiency of Aigamo ducks tended to be improved with increasing dietary bamboo charcoal powder.

In the present study, treatment with activated *Canarium* seed or maize cob charcoal increased intestine weight and density as compared to diet contaminated with *aflatoxin* B1 without charcoal (C+). This increased of the weight/length ratio which is considered as an indicator of the intestinal villi size of the *mucosa* layer (*Abdel-Fattah*, 2008), could be associated with the improvement of intestinal *mucosa* thickness. The increased in the intestine weight with charcoal treatments is similar to that observed by *Gunal et al.*(2006) and *Ruttanavut et al.* (2009). It has been suggested that high intestine density increased surface area capable of greater absorption of available nutrients (Ruttanavut *et al*., 2009) thus leading to an increased body weight gain.

The carcass yield, relative weight of heart and abdominal fat were not significantly affected by inclusion of charcoal in the diet. The result of this study is corroborates those by *Emadi* and *Kermanshahi* (2006). Relative weight of pancreas decreased with increasing level of charcoal in the diets as compared to C- treatment. This result is in agreement with the study by *Emadi* and *Kermanshahi* (2006). The decrease of the liver weight in birds fed charcoal A, charcoal B or association of charcoal A and B is in agreement with previous reports on the effect of 200 mg/kg of dietary activated charcoal on birds fed diet containing 6mg/kg of aflatoxin B1 (*Ademoyero* and *Dalvi,*

Table 3: Digestive organ development of broiler chickens fed diets with *aflatoxin* B1 contaminated groundnut meal and graded levels of individual or combined activated charcoal from *Canarium* seed (A) or maize cob (B)

Parameters	Treatments							
	C-	C+	A 0.20	A 0.40	B 0.20	B 0.40	M 0.20	M 0.40
Intestine length (cm)	197.33 ±14.29a	194.00 ±11.00a	187.66 ±17.78b	202.33 ±11.01a	197.33 ±6.50a	210.33 ±20.55a	240.33 ±16.62c	204.33 ±24.00a
Intestine circumference (mm)	23.66 ±3.21a	23.33 ±2.08a	22.66 ±2.08a	24.33 ±2.30a	21.66 ±1.15a	23.33 ±2.08a	25.33 ±3.05a	25.33 ±4.04a
Relative intestine weight (% of LW)	93.33 ±15.27b	80.00 ±5.00c	106.06 ±15.27a	93.33 ±11.54b	96.66 ±5.77a	111.66 ±12.58a	98.33 ±10.40b	71.66 ±10.40c
Intestine density (weight/Length)	0.46 ±0.04b	0.40 ±0.00c	0.56 ±0.05a	0.45 ±0.04b	0.48 ±0.01b	0.52 ±0.02ab	0.40 ±0.01c	0.34 ±0.01d
Gizzard (% of LW)	1.46 ±0.38a	1.54 ±0.19a	1.51 ±011a	1.47 ±0.13a	1.56 ±0.11a	1.45 ±0.25a	1.37 ±0.13a	1.45 ±0.05a

a, b, c: Means with different letters in the same row are significantly different (P<0.05)

Table 4: Carcass yield and relative weight of organs (%) of broilers fed diets with *aflatoxin* B1 contaminated groundnut meal and graded levels of individual or combined activated charcoal from *Canarium* seed (A) or maize cob (B)

Parameters	Treatments							
	C-	C+	A 0.20	A 0.40	B 0.20	B 0.40	M 0.20	M 0.40
Carcass yield (%)	76.46 ±1.95a	76.85 ±1.03a	74.65 ±2.29a	75.49 ±0.38a	76.43 ±0.55a	76.32 ±1.72a	75.57 ±2.16a	74.93 ±0.10a
Head	3.27 ±0.69a	3.34 ±0.32a	2.77 ±0.38b	2.77 ±0.54b	2.93 ±0.54b	3.26 ±0.15a	2.62 ±0.63b	2.24 ±0.30b
Legs	5.34 ±0.62a	5.30 ±0.12a	4.77 ±0.59b	4.48 ±0.18b	4.97 ±1.16b	5.20 ±0.13a	4.75 ±0.75b	3.62 ±0.79c
Liver	1.69 ±0.08a	2.95 ±0.05b	1.67 ±0.05a	1.67± 0.18a	1.60 ±0.22a	1.69 ±0.26a	1.73 ±0.42a	1.63 ±0.09a
Heart	0.54 ±0.11a	0.47 ±0.06a	0.58 ±0.05a	0.53 ±0.02a	0.48 ±0.10a	0.48 ±0.04a	0.55 ±0.14a	0.55 ±0.06a
Pancreas	0.20 ±0.01a	0.17 ±0.01b	0.13 ±0.01b	0.18 ±0.03b	0.16 ±0.04b	0.14 ±0.00b	0.16 ±0.01b	0.16 ±0.01b
Abdominal fat	1.09 ±0.56a	1.79 ±0.39a	1.79 ±0.39a	1.98 ±0.53a	1.86 ±0.59a	1.61 ±0.17a	1.66 ±0.53a	1.71 ±1.08a

a, b: Means with different letters in the same row are significantly different (P<0.05)

1983). The results of this study agreed with the finding of Ramos *et al*. (1996) that charcoal protects the liver from *aflatoxin* B1 by reducing or eliminating *histopathological lesions* in the liver.

Conclusion

It can be concluded that up to 0.4% of maize cob charcoal and 0.2% of a 1/1 mixture of charcoal of *Canarium schweinfurthii* seed and maize cob could be used as natural feed additive to absorb *aflatoxin* B1 from the feed and promote performance of broiler chickens.

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EFFECTS OF TRIPSACUM LAXUM AND LEUCAENA LEUCOCEPHALA SUPPLEMENTARY FEEDING ON GROWTH OF WAD SHEEP AND GOATS GRAZING NATURAL PASTURE

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EFFETS DE L'ALIMENTATION COMPLEMENTAIRE AU TRIPSACUM LAXUM ET LEUCAENA LEUCOCEPHALA SUR LA CROISSANCE DES MOUTONS ET CHÈVRES S'ALIMENTANT AUX PATURAGES NATURELS

Résumé

Une étude des effets de l'alimentation complémentaire auTripsacum laxum et Leucaena leucocephala sur la croissance des moutons et des chèvres nains d'Afrique de l'Ouest (WAD) s'alimentant sur les pâturages naturels dans la région du Nord-Ouest du Cameroun, a été réalisée à l'IRAD Mankon sur 42 moutons et chèvres (dont 21 moutons et 21 chèvres) en saison sèche (de Novembre 2008 à Février 2009). L'expérience consistait à réaliser 3 traitements:
(a) - aux feuilles de Leucaena apportées comme complément au taux de 254,3 ± 0,00 gdm / h / j ;(b) - aux feuilles de Tripsacum introduites en tant que complément au taux de 314,3 ± 0,00 gdm / h / j et ; (c) - en absence de suppléments (pour le groupe témoin). Les trois traitements ont été administrés au hasard sur 21 animaux de chaque espèce (soit 7 animaux par traitement). Tous les animaux s'alimentaient sur des pâturages naturels comme régime de base. La supplémentation a réduit la mortalité et augmenté de façon significative les gains de poids quotidiens les moutons et les chèvres recevant du Leucaena (32,5 ± 0,33 et 42,2 0.46g/h/d respectivement, P <0,05) et pour les ovins et caprins nourris au supplément de Tripsacum (20,5 ± 0,69 et 61,4 ± 0,64 g / h / j, respectivement, P <0,05) en comparaison aux brebis et chèvres de contrôle (± -6,0 ± 0,71 et 26,5 0.70g/h/d respectivement). Les résultats in vivo en matière sèche ont montré que pour la dégradabilité des protéines, les feuilles de Leucaena et Tripsacum sont très dégradables (72,0% chez les ovins et 74,5%; chez les caprins ; - 62,7% chez les ovins et 64,4% chez les caprins respectivement). Cette étude a révélé que la productivité des petits ruminants s'alimentant sur des pâturages naturels peut être grandement améliorée durant la saison sèche, si l'alimentation de ceux-ci est complétée par des fourrages de Tripsacum laxum et Leucaena leucocephala.

Mots clés: chèvre naine d'Afrique de l'Ouest, mouton de Djallonké, Tripsacum laxum, Leucaena leucocephala, gain de poids, suppléments.

Abstract

A study of the effects of *Tripsacum laxum* and *Leucaena leucocephala* supplementary feeding on growth of West African Dwarf (WAD) sheep and goats grazing on natural pasture in the North West Region of Cameroon, was conducted at IRAD Mankon on 42 WAD sheep and goats (21 sheep and 21 goats) in the dry season (November 2008 to February 2009). The experiment consisted of 3 treatments: (a) *Leucaena* leaves fed as supplement at the rate of 254.3±0.00 gDM/h/d, (b) *Tripsacum* fed as supplement at the rate of 314..3±0.00 gDM/h/d and (c) no supplementation (control group). The three treatments were allocated at random to the 21 animals of each species (7 animals per treatment). All the animals grazed on natural pasture as basal diet. Animals were dewormed prior to commencement of the study and weighed twice a month. Supplementation reduced mortality and significantly increased daily weight gains in the Leucaena supplemented sheep and goats (32.5±0.33 and 42.2±0.46g/h/d respectively, P<0.05) and the *Tripsacum* supplemented sheep and goats (20.5±0.69 and 61.4±0.64g/h/d respectively, P<0.05) compared to the control sheep and goats (-6.0±0.71 and 26.5±0.70g/h/d respectively). *In vivo* dry matter and protein degradability results showed that *Leucaena* and *Tripsacum* leaves are highly degradable (72.0% in sheep and 74.5% in goats; 62.7% in sheep and 64.4% in goats, respectively). This study revealed that productivity of small ruminants grazing on natural pasture can be greatly improved in the dry season if their feeding is supplemented with Tripsacum laxum and Leucaena leucocephala forages.

Key words: West African Dwarf goat, West African Dwarf sheep, *Tripsacum laxum*, *Leucaena leucocephala,* weight gain, supplementation.

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Introduction

The intensification of crop agriculture leading to a reduction in the grazing land and increasing feed scarcity, has resulted in reductions of both the number and productivity of small ruminants in Cameroon. The alternation of the wet and dry seasons in the rain zones of West and Central Africa has a great influence on the availability, productivity and quality of natural pastures *(Fai and Fomunyam*, 2000; *Njwe and Olubajo,* 1985). In the wet season, the grasses grow rapidly, and although their quality may be moderate early in the season, they mature quickly, with resulting decline in quality by the end of the season. Rapid maturation significantly influences the nutritive value and efficiency of herbage utilization by animals.

In the dry season, pasture dry matter production and the nutritive value of the herbage decline drastically to the extent that animals experience a reduction in protein, energy and mineral intake (Njwe and Olubajo, 1985). This leads to manifestations such as loss of weight, cessation of growth and decline in milk production, poor body condition and high mortality. This has greatly affected small ruminant production in Cameroon, especially in the grass fields or savanna in the dry season. Determined efforts have to be made to improve on their nutrition during this period of feed scarcity because feeding is an essential aspect of small ruminant raising and is the highest expense of any meat/ milk sheep and goat operation. Profitable meat (sheep and goat) production can only be achieved by optimizing the use of high quality forage and browse and the strategic use of concentrate feeds. This can be achieved by developing a year round forage programme, allowing for as much grazing as possible throughout the year. To do this, investigations on new feed resources as supplements for small ruminant feeding need to be carried out, especially on those forages which are drought resistant and can easily be cultivated in marginal lands which do not support the growth of crops consumed by man.

The main objective of this study was to determine the effects *Tripsacum laxum* and *Leucaena leucocephala* supplementary feeding on the performance of West African Dwarf (WAD) sheep and goats grazing natural pasture as basal diet

Materials and Methods

Study site

This study was carried out at the *Institute for Agricultural Research and Development* (IRAD) Mankon station. The station is situated at 16km from Bamenda and has an annual rainfall of 2,209mm, mean monthly minimum temperature of 14.7 (11.7-16.7) ^{0}C and maximum temperature of 28.7 (25.2-36.2) ^{0}C (Fai and Fomunyam, 2000). Situated at an altitude of 1200m above sea level, it covers a land surface of 250ha on rolling, undulating and flat savanna hills, lying on the general *Bambili, Bafut, Mankon, Mbengwi* and *Bali plain.*

Housing

The open-sided animal house that contained the pens and the experimental animals had walls made of concrete blocks, with the upper part made of strong wire mesh. The roof was of aluminium sheets. The floors of the pens were made of wooden slats raised about one metre above ground level. The spaces between the slats were about 1.25cm. The pens were cleaned daily.

Experimental design

The study consisted of three treatment groups for each species (sheep and goats), with mean initial animal weights (Kg±SEM) of 14.5±1.82, 14.6±1.62 & 14.8±1.84 for sheep, and 12.0±1.16, 11.5±0.91 & 11.7±1.11 for goats on Leucaena, Tripsacum and control respectively. The 21 animals of each species were distributed between the 3 groups (7 animals per group; 3 males and 4 females of ages ≤ 12 months) in a randomized design and factorial arrangement, with two animal species and two forage supplements as factors. The treatments comprised:

- Grazing natural pasture + Guatemala grass (*Tripsacum laxum*) as supplement.
- Grazing natural pasture + *Leucaena leucocephala* as supplement.
- Grazing natural pasture only (control).

Prior to commencement of the feeding trial, there was an adjustment period of two weeks during which all the animals grazed together on pastures in paddocks, consisting predominantly of grass species (*Brachiaria ruziziensis* and *Hyperenia rufer*) and minor forage species (*Pennisetum purpureum, Desmodium intortum and Stylosanthes sp*), which served as the basal diet. During this time, the animals were treated against ecto- and endo–parasites using Deteki (acaricide) and *Albendazole* (*anthelmintic*) respectively.

After the 14 days adjustment period, the animals were allowed to graze together in rotation at one week intervals between two fenced paddocks containing natural pasture (as described above) for 8 hours (from 9 am to 5 pm) each day. They were penned at night from 5 pm to 9 am the following day in six different pens, each 396 cm long, 247.5 cm wide and 165 cm high, according to their experimental groups, during which the forage supplements were fed.

Forage supplementation

One year regrowth of *Guatemala* grass (*Tripsacum laxum*) was harvested each morning at 10 am when dew had dried up, tied and fed to the 2 groups on *Tripsacum* supplementation (8kg per experimental group per day). On the other hand, fresh leaves of browse (*Leucaena leucocephala*) were harvested about 30minutes to feeding

Table 1: Chemical composition of *Leucaena* and *Tripsacum forages* (%DM) at the beginning and at the heart of the dry season

Parameters	Leucaena leucocephala		Tripsacum laxum	
	November	January	November	January
Dry matter (DM) %	43.00	49.00	25.00	30.00
OM (%DM)	95.61	96.56	91.71	93.21
CP (%DM)	16.68	14.24	8.62	7.13
EE (%DM)	2.24	2.54	1.35	4.33
NFE (%DM)	65.03	68.35	50.69	50.93
CF (%DM)	11.66	11.43	31.05	74.33
NDF (%DM)	60.99	63.69	78.83	43.77
ADF (%DM	26.61	21.62	43.98	10.08
ADL (%DM)	15.38	12.26	11.92	30.56
Hemicellulose (%DM)	34.38	42.07	34.85	30.56
Cellulose (%DM)	11.23	9.35	32.05	33.68
Metabolisable energy (MJ/KgDM)	6.21	6.73	7.34	7.94
Ash (%DM)	4.38	3.43	8.29	6.78

Table 2: *Leucaena* and *Tripsacum* dry matter record during the 90 days study period.

Date	Forage sample	Weight of tray only (g)	Weight of tray and sample before drying (g)	Weight of tray and sample after drying (g)	% Dry matter
31/11/2008	Tripsacum	585	685	610	25
	Leucaena	609	709	652	43
30/12/2008	Tripsacum	585	685	611	26
	Leucaena	611	711	653	42
30/01/2008	Tripsacum	650	750	678	28
	Leucaena	585	685	629	44
28/02/2009	Tripsacum	650	750	680	30
	Leucaena	585	685	634	49

time, and fed to the 2 groups on Leucaena supplementation (4 kg per experimental group per day). The animals were fed these forage supplements from 5 pm – 9 am, after which the left over was collected and weighed daily. The quantities of forage offered were intended to supply a mean value of 23.2 gDM digestible nitrogen/h/day (average digestible nitrogen requirement by a small ruminant gaining about 50g live weight per day). The difference between amount offered and left over was taken as the quantity consumed. The control group did not have access to forage supplementation, but all the experimental animals had free access to water and mineral licks (45% bone ash, 35% table salt, 9% cement, 10% limestone and 1% premix). Forage dry matter (DM) intake was recorded daily and each animal weighed (Salter, 25 kg x 100g) twice a month. The feeding experiment ran for 3 months (from November 2008 to January 2009).

Analysis of browse and grass supplements

Proximate analysis of the forage supplements for Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fibre (CF), Neutral Detergent Fibre (NDF), Neutral Detergent Lignin (NDL), Acid Detergent Fibre (ADF), Cellulose, Hemicellulose, Ether Extract (EE), Nitrogen Free Extract (NFE), Ash and Metabolizable Energy (ME) was done at the start of the study and again later in the dry season. These analyses were carried out at the Nutrition and Biochemistry Laboratory of IRAD *Mankon* specialized station, and the Animal Nutrition Laboratory of the University of Dschang, following standard methods (AOAC, 1970,1980)

Digestibility trial

A digestibility test was carried out on each forage supple-

Table 3: Results of a digestibillty trial in which three sheep and three goats were fed on *Leucaena leucocephala forage*

a). Results of analysis of *Leucaena leucocephala* and *faeces* (g/kg DM):

		Organic matter	Crude protein	Ether extract	Crude fibre	N-free extractives
Leucaena leucocephala		965.6	142.4	25.4	114.3	683.5
Faeces	Sheep	884.6	157.9	59.2	210.1	457.8
	Goat	909.3	164.8	77.7	203.5	463.3

b). From these figures the quantities (kg) of dry matter and its components which were consumed, excreted and, by difference, digested were calculated as follows:

		Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extractives
Consumed	Sheep	0.808	0.781	0.115	0.021	0.092	0.553
	Goat	0.627	0.606	0.089	0.016	0.072	0.429
Excreted	Sheep	0.224	0.198	0.035	0.013	0.047	0.102
	Goat	0.152	0.138	0.025	0.012	0.031	0.070
Digested	Sheep	0.584	0.583	0.080	0.008	0.045	0.451
	Goat	0.475	0.468	0.064	0.004	0.041	0.359

c). The digestibility coefficients were calculated by expressing the weights digested as proportion of the weights consumed:

	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N-free extractives
Sheep	0.723	0.746	0.696	0.381	0.489	0.816
Goat	0.758	0.772	0.719	0.250	0.569	0.837

d). Finally, the composition of *Leucaena leucocephala* was calculated in terms of digestible nutrients, with the following results (g/kg dry matter):

	Digestible Organic matter	Digestible Crude protein	Digestible Ether extract	Digestible Crude fibre	Digestible N-free extractives
Sheep	720	99	9	56	558
Goat	745	102	6	65	572

Table 4: Results of a digestibillty trial in which three sheep and three goats were fed on *Tripsacum laxum* forage.
a). Results of analysis of *Tripsacum laxum* and *faeces* (g/kg DM):

Treatment	No of Animals	Initial wt (kg)	Final wt (kg)	Daily feed offered (gDM/h/d)	Daily Feed intake (gDM/h/d)	Daily wt gain (g/h/d)	Daily feed intake (g/kgw0.75
A	7	14.5±1.82a	17.3±1.97a	254.3±0.00a	217.1±0.09a	32.5±0.33a	14.9±0.00a
B	7	14.6±1.62a	16.4±1.82ab	314.3±0.00b	213.5±0.09a	20.5±0.69ab	14.6±0.00a
C	7	14.8±1.84a	14.2±1.47b	/	-6.0±0.71b	/	/

Means within the same column bearing different letters (a and b) are significantly different (P<0.05).
A = *Leucaena leucocephala* supplemented group
B = *Tripsacum laxum* supplemented group
C = Control group

ment using three mature male animals of each species. The animals were tied *faecal* collection bags (napkins) with nylon linings and maintained in 12 individual metabolism cages for seven days adaptation period, followed by seven days experimental period. Mean quantities of DM Guatemala grass (1587g/h/d and 1570 g/h/d) and browse (Leucaena) (1387 g/h/d and 1131 g/h/d) were respectively offered to six sheep and six goats (3 sheep and 3 goats per forage type). These animals were given water and mineral licks ad libitum during both the ad-

aptation and experimental periods. The quantity of DM forage consumed daily and daily DM faecal outputs were recorded for the seven days experimental period. Faecal samples were collected, weighed when still fresh, and immediately transferred to the laboratory for dry matter determination. In the laboratory, these were subjected to a temperature of 105^{0}C for 24 hours in an oven, after which they were weighed. Forage DM consumed was determined by subtracting quantity of forage left over from that offered and converting the fresh weight value to dry matter value by calculation. The faecal output as well as left over forage for each day's feeding were collected on the following day. The animals were weighed at the beginning and at the end of the experiment. Proximate analysis was done on the forage consumed and faeces voided. The Digestible DM, OM, CP, CF, EE and NFE were calculated.

Statistical analysis

The body weight gain and DM feed intake of the animals were analysed using the *Friedman's test* and one way analysis of variance, and significant means separated using *Duncan's* multiple range test (Steel and Torrie, 1980). The Student's t-test was used to determine the significance of difference between two treatment means, and paired comparisons between means was done using the *Wilcoxon's* signed rank sum test (*Nana*, 2008). These analyses were done with the SPSS statistical package version 12.0. Significance was set at $P < 0.05$.

Table 5 Feed intake and weight gain (± SEM) of West African Dwarf Goats grazed on natural pastures and fed *Leucaena leucocephala* or *Tripsacum laxum* as supplement.

Treatment	No of Animals	Initial wt (kg)	Final wt (kg)	Daily feed offered (gDM/h/d)	Daily Feed intake (gDM/h/d)	Daily wt gain (g/h/d)	Daily feed intake (g/kgw0.75
A	7	12.0±1.16a	15.8±1.12a	254.3±0.00a	119.8±0.09a	42.2±0.46a	10.0±0.00a
B	7	11.5±0.91a	17.0±1.13a	314.3±0.00b	139.3±0.07b	61.4±0.64a	12.1±0.00b
C	7	11.7±1.11a	13.9±1.49a	/	26.5±0.70a	/	/

Means within the same column bearing different letters (a and b) are significantly different (P<0.05).
A=*Leucaena leucocephala* supplemented group
B=*Tripsacum laxum* supplemented group
C= Control group

Results

Chemical composition of supplements

The chemical composition of forage supplements used in the study is presented in Tables 1 and 2. Results showed that *Leucaena leucocephala* presented a higher dry matter and organic matter content than *Tripsacum laxum,* which increased during the period of study (November 2008 to January 2009) as the dry season progressed. Crude protein in *Leucaena* was double that of *Tripsacum*, during the period of study, but the general trend was a gradual decrease of this nutrient in both forages with increasing intensity of the dry season. Results further indicated a higher nitrogen free extract, crude fibre and acid detergent lignin values in *Leucaena* than in *Tripsacum* during the study period. On the other hand, *Tripsacum* had a higher value of neutral detergent fibre, cellulose, metabolizable energy and ash contents. Generally, the chemical composition of these two forages was not significantly affected as the dry season progressed.

Quantitative analysis of supplements and digestibility

The average quantity of dry matter intake of *Leucaena* was 0.808kg and 0.627kg per head per day for sheep and goats respectively, and the average quantity of dry matter excreted in faeces was 0.224kg and 0.152kg, per head per day for sheep and goats respectively. Similarly, the sheep and goats consumed an average of 0.836kg and 0.579kg dry matter respectively per head per day of *Tripsacum* forage, and excreted 0.268kg and 0.177kg dry matter per head per day in faeces.

The results of digestibility trial and chemical analysis in g/kg dry matter are shown in Tables 3 and 4. Dry matter and protein degradability results showed that Leucaena digestibility coefficients and total digestible nutrients were higher in both sheep and goats than *Tripsacum* digestibility coefficients and total digestible nutrients. Goats showed higher apparent digestibility values (74.5% for *Leucaena* and 64.4% for *Tripsacum*) than sheep (with digestibility values of 72.0% for *Leucaena* and 62.7% for *Tripsacum*), even though sheep consumed more dry matter forage than goats (P<0.001).

Feed intake and weight gain

The results of forage supplement intake and weight gain are shown in Tables 5 and 6. Animals consumed more of *Tripsacum* than they did of *Leucaena* supplement, and dry matter intake was significantly higher in sheep than in goats. In the sheep, the general trend was an increase in live weight in the *Tripsacum* and *Leucaena* supplemented

groups throughout the experimental period (Table 5, Fig. 1). The highest mean body weights were recorded on the 90th day (17.3±1.97kg) and 45th day (16.8±2.05kg) of the experimental period for *Leucaena* and *Tripsacum* supplemented groups respectively, and these were significantly higher than the mean initial weights (P<0.05). The control sheep lost weight, but the change from the mean initial weight as time progressed was not statistically significant (P>0.05).

Goats fed *Tripsacum* and *Leucaena* supplements gained significantly more weight than sheep (P<0.001), (Table 6, Fig. 2). The highest mean body weights in goats were recorded on the 90th day (15.8±1.12kg), 90th day (17.0±1.38kg), and 75th day (14.2±1.59kg) of the experimental period for *Leucaena, Tripsacum* and control groups respectively, and these were significantly higher than the mean initial weights (P<0.001).

As per comparison between treatments, sheep of the control group lost weight and had significantly lower mean weight than the *Leucaena* group between the 75th and 90th day (P<0.05). Even though *Tripsacum* supplemented sheep gained weight, this was not significantly different from the control and the *Leucaena* groups (Table 5 and Fig. 1). On the other hand, goats on the different feeding regimes gained weight. However, the differences in weight gain between the three treatments were not statistically significant throughout the experiment (Table 6 and Fig. 2). Goats of the control and the *Tripsacum* groups gained significantly higher weights (2.18±0.71kg and 5.1±0.64kg respectively) than sheep on the same feeding regimes (-0.65±0.71kg and 1.8±0.69kg respectively) (P<0.05). There was a significant difference in live weight gain between sheep and goats fed Luecaena supplement, with goats gaining more weight during the experimental period (3.53±0.46kg) than sheep (2.74±0.33kg) (P<0.05). In general, supplemented sheep and goat groups gained more weight than their control counterparts. Thus, *Tripsacum* supported faster growth than *Leucaena* (P<0.001) in goats, but showed no significant difference in sheep (P>0.05).

Mortality

During the experiment, three deaths were recorded: two sheep and one goat. Post-mortem examination on these deceased animals revealed that two (one sheep and one goat of the control groups) had died of parasitic gastro-enteritis, and one sheep of the *Tripsacum* supplemented group had suffered from *anaemia.*

Discussion

The overall dry matter and protein contents of *Leucaena* were high during the period of study (November 2008 - January 2009). Similar observations had been reported with *Leucaena and Calliandra* (Jones, 1994; *Mecha* and *Adegbola*, 1980; *Norton* and *Ahn*, 1997). However, the chemical composition of *Leucaena foliage* was different from that reported by earlier workers (*Gupta et al.,* 1986; Jones, 1979). They reported a crude protein range of 27.4 to 32.3% dry matter. The crude protein values of 16.68 and 14.24% dry matter recorded in this study for the dry season months of November and January respectively were lower than those reported by *Pamo et al.* (2006) during the rainy and dry seasons in Cameroon. The variability of these forages can be attributed to the effect of tropical weather conditions, soil type and variability among varieties. The high cellulose and hemicellulose contents in *Tripsacum* and *Leucaena* forages form significant sources of energy for the ruminants. The results obtained in this study show that the various food components and the energy value of these forages are not greatly affected by the intensity of the dry season.

Dry matter, organic matter and protein degradability

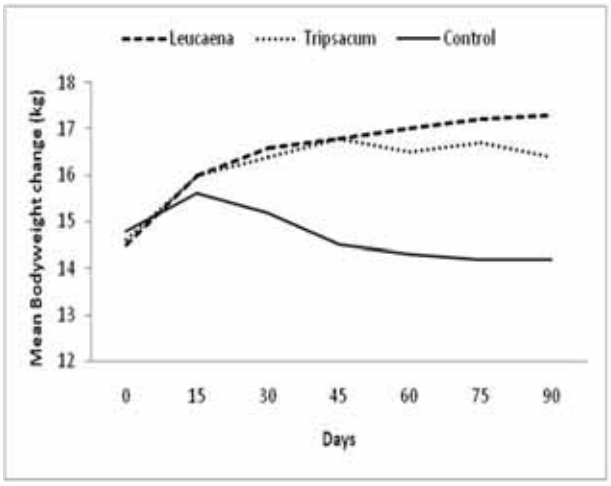

Figure 1. Bodyweight change of sheep on various feeding regimes

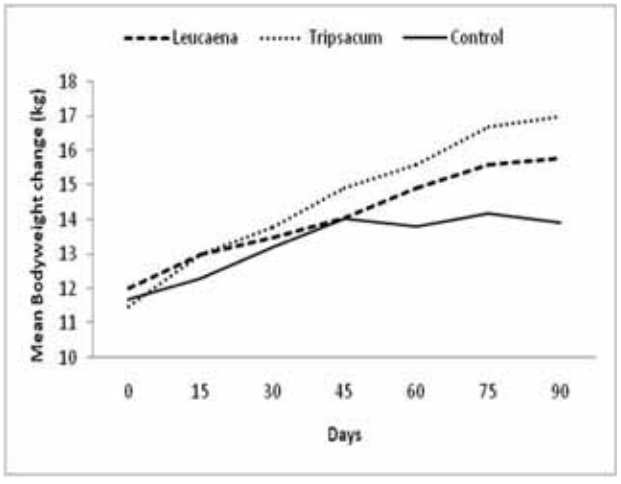

Figure 2. Growth rate of goats on various feeding regimes

results for *Leucaena* and *Tripsacum* indicate that goats are more efficient converters of forage material into animal products than sheep. This affirms the conclusion by *Louca et al.* (1982) that goats derive their advantage from an ability to consume vegetation types not normally eaten by other ruminants, and from a higher digestive efficiency for poor quality roughages. However, *Van Soest* (1982) attributed the fact that goats digest feed better than sheep and cattle to the goat's efficient selection from the material offered.

Supplemented sheep and goats as well as the control goat group gained weight throughout the study. The loss in body weight observed in sheep of the control group at the heart of the dry season could be due to their inefficiency to digest poor quality pastures. The higher digestive efficiency of goats compared to sheep explains why the control goats were still able to gain weight during the dry season. This qualifies the goat as a very hardy animal. The comparatively higher weight gain per day in goats fed *Tripsacum* supplement than in those on *Leucaena* supplement could be due to the fact that *Tripsacum* was more succulent than *Leucaena,* and supplied more energy. It would appear that digestible crude protein intake was significantly higher in *Leucaena leucocephala* supplemented goats than in those fed *Tripsacum laxum* as a result of the high protein content in the foliage, but might not have been well utilized due to the presence of mimosine, an anti-nutritional factor, that is toxic when consumed in high amounts. The weight gain (26.5, 42.2 and 61.5g/d) recorded in the control, *Leucaena* and *Tripsacum* supplemented groups of goats respectively in this study falls within the range of 30 to 50g/d reported by Adebowale and Ademosun (1981) in goats fed *ad libitum* concentrate feed. Data obtained on East African Dwarf goats (*Wilson,* 1958) showed maximum weight gains of 85 to 100g/d, but much higher weight gains of West African Dwarf goats (400g/d) were reported by *Akinsoyinu et al.*(1975, 1976) and *Oyenuga and Akinsoyinu* (1976). However, these values appear unrealistic because West African Dwarf goats would reach their mature weights within a few months. There were, however, improvements in the performance of the supplemented animals in this study. The low mortalities recorded might have been due to supplementation. Supplementation with *Leucaena* and *Tripsacum* increases the total nutrient intake and digestibility in the gut of ruminants, especially when forages are poor in quality.

Conclusion

Tropical forages are inadequate to sustain sheep and goat growth in the dry season with only mineral supplements. Supplementation with *Tripsacum* and *Leucaena* in the present study led to an increase in weight gain and survival rate in sheep and goats during the dry season. However, the study suggests that small ruminant farmers need to show more concern for supplementary feeding with these forages, especially for sheep in the dry season, since sheep are not efficient digesters of poor quality feeds like goats. *Leucaena* and *Tripsacum* become increasingly important components of diet as the dry season progresses. There may be an advantage for including several browse and grass species to provide diversity of diet, particularly where mimosine toxicity from *Leucaena* can be important. Cut and carry browse and grass feeding represents a highly flexible, relatively simple, improved feeding strategy which can be implemented with minimal capital or management inputs. With all these put in practice, the great experience of the traditional sheep and goat farmer is likely to be the best starting point.

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Impact

When sheep and goats were fed *Guatemala* grass (*Tripsacum laxum*) and legume browse (*Leucaena leucocephala*) to supplement their intake from natural pasture during the dry season, they tended to grain more weight and survived better than those feeding only on natural pasture. Goats gained more weight than sheep, probably because of their greater efficiency to digest poor quality pastures. The results of this study suggest that cut and carry browse and grass feeding represents an improved feeding strategy, which can be implemented with minimal capital or management inputs, to improve on the growth of sheep and goats grazing natural pasture during the dry season.

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ETUDES COMPAREES DE LA CROISSANCE DES POULES LOCALES (Gallus gallus) ET D'UNE SOUCHE LABEL AU CAMEROUN

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COMPARATIVE GROWTH PERFORMANCES OF LOCAL CHICKEN (Gallus gallus) ECOTYPES AND A COMMERCIAL LABEL IN CAMEROON

Abstracts

The growth of local chickens collected from the Western Highlands and the Forest zones of Cameroon was evaluated under intensive management alongside with a commercial label. The aim was to record the potentials of local chickens for developing appropriate genetic improvement strategies. Each genetic type was reared separately from hatching to week 52 of age and was subjected to the same health care, management and feeding. Results showed that normal commercial males (DW*N) were 48.75%, 49.55% and 41.98% heavier at 16th week than their counterparts from Centre, south and North-West/West (NO/OU) ecotypes. Feed conversion ratios between 12 and 16 weeks of age were 3.16 for DW*N and varied from 3.92 to 4.16 for local ecotypes. Normal (DW*N) and dwarf (DW*N) local females weighed 1550g and 1260g, respectively and were heavier that the heaviest local hen (889g) from the NO/OU. Feed conversion ratios were 4.62 (DW*N and DW*DW), 4.94 (Centre), 4.31 (NO/OU) and 4.35 (South). At 18 weeks of age, normal females 'DW*N' (1792 g) were heavier than their dwarf 'DW*DW' counterparts (1453 g) while hens from NO/OU (964 g), Centre (960 g) and South (1047 g) were overall lighter. Mortality rate was highest in the growing phase; but less than 8% from 18 to 52 weeks of birds' age. It was concluded that commercial labels could potentially be used for improving local chicken growing performances through crossbreeding.

Key words: Cameroon, local chicken, commercial label, growth, skeletal measurements, mortality

Résumé

Les performances de croissance des populations de poules locales des hauts plateaux (Régions de l'Ouest et du Nord-Ouest) et des forêts ont été évaluées en station en présence des souches commerciales de type label. L'objet de l'étude a été d'avoir une meilleure connaissance des potentialités de ces deux types génétiques afin de développer des stratégies ultérieures de leur amélioration génétique. Chaque type génétique était élevé séparément de l'éclosion jusqu'à la 52ème semaine et était soumis aux mêmes soins de santé, de management et d'alimentation. Les principaux résultats montrent que les mâles labels normaux (DW*N) sont 48,75%, 49,55% et 41,98% plus lourds à 16 semaines d'âge que leurs homologues locaux respectifs du Centre, du Sud et du Nord-Ouest/Ouest (NO/OU). L'indice de consommation entre 12 et 16 semaines d'âge est de 3,16 pour le DW*N et varie de 3,92 à 4,16 chez les écotypes locaux. Les femelles normales 'DW*N' (1550g) et naines 'DW*DW' (1260 g) sont plus lourdes que la femelle locale la plus lourde (889g) du Nord-Ouest/Ouest. Les indices de consommation sont de 4,62 (DW*N et DW*DWW), de 4,94 (Centre), de 4,31 (NO/OU) et de 4,35 (Sud). Chez les femelles adultes à 18 semaines, la DW*N (1792 g) a un poids corporel supérieur à celui de la DW*DWW (1453 g) tandis que les femelles du NO/OU (964 g), du Centre (960 g) et du Sud (1047 g) sont plus légères dans leur ensemble. La mortalité en station a été élevée chez les jeunes mais inférieure à 8% de 18 à 52 semaines d'âge. Il est conclu que le label se présente comme le type le plus indiqué pour améliorer les performances pondérales et squelettiques des poules locales dans une stratégie utilisant le croisement.

Mots clés : Cameroun, poule locale, poulet label, croissance, mesures squelettiques, mortalité.

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Introduction

La poule locale représente près de 70% du cheptel avicole national (Fotsa et al., 2007b) estimé à plus de 35 millions de sujets (INS-Cameroun). Son élevage constitue un moyen pour résoudre les problèmes liés à la socio économie et rituelle des paysans dotés de peu de moyens. Cette importance numérique ne contribue cependant que pour près de 10 % au produit intérieur brut et produit 1,8 Kg de viande de poulets et 20 œufs par habitant consommés annuellement (Poné Kamdem, 1998). En outre, le système extensif dans lequel les poules locales sont élevées, pour la plupart, est caractérisé par une croissance lente des sujets, l'absence d'un environnement logistique, alimentaire et sanitaire adéquats suivi d'une conduite approximative d'élevage (Sarkar et Bell, 2006 ; Fotsa et al., 2007a,c). En conditions améliorées d'élevage, les animaux pourraient mieux exprimer leur potentiel de production. L'objet de cette étude est d'évaluer les performances de croissance des poules locales en présence d'un label témoin en vue de renforcer leur capacité productive ultérieure. Plus spécifiquement, il s'agira de la connaissance de ses performances d'incubation, de croissance, des mesures squelettiques et de la mortalité.

Materiels et Methodes

Caractéristiques de la zone d'étude

Zone des hauts plateaux de l'Ouest

La zone des hauts plateaux de l'Ouest couvre les Régions de l'Ouest et du Nord-Ouest du Cameroun. Elle est située à une altitude moyenne de 1240 m avec une température moyenne annuelle de 19°C pour une hygrométrie supérieure à 80%. Les précipitations annuelles moyennes sont de 2000 mm. La zone représente 6% du territoire national et rassemble près de 25% de la population totale. La population est à plus de 80% constituée des agriculteurs et éleveurs. Près de 80% des exploitations agricoles possèdent de la volaille et de petits ruminants en élevages traditionnels familiaux. Il existe dans cette zone environ 27 000 éleveurs de moutons, 89 000 éleveurs de chèvres, 77 000 porciculteurs et 220 000 éleveurs de volailles.

Zone forestière humide à régime pluviométrique bimodal

La zone forestière humide bimodale s'étend sur la majeure partie du plateau sud-camerounais entre 500 et 1000 m d'altitude. La température moyenne annuelle est de 25°C pour une hygrométrie variant de 99,6% à 100%. La zone reçoit en moyenne 2000 mm d'eau annuellement. Sur une superficie totale de 47,3% du territoire national, elle couvre les Régions du Centre, du Sud et de l'Est. Les systèmes d'élevage les plus répandus sont les élevages traditionnels avicoles, ovins, caprins, et porcins avec cependant, les élevages modernes qui s'intensifient autour de la ville de Yaoundé.

Matériel animal

Le matériel animal local a été issu de la souche parentale mise en place à partir d'œufs fécondés collectés des zones rurales et incubés à la station expérimentale de l'Institut de Recherche Agricole pour le Développement de Mankon (IRAD) du Cameroun. Élevé en claustration jusqu'à la maturité, ce troupeau a été mis en reproduction par population de chaque région (écotype) suivant un rapport mâle/femelle de 1 coq accouplé à 7 ou 8 poules en évitant autant que possible les accouplements entre les sujets apparentés.

Les sujets de souche commerciale de type label ou témoins quant à eux utilisés dans cette étude ont été issus d'un croisement entre parents déjà croisés eux-mêmes. Les témoins obtenus ont été comparables à un génotype de 3ème génération, qui a pu conserver une partie de la vigueur hybride liée au croisement, mais qui a été susceptible d'exprimer une plus grande variation phénotypique que les parents de deuxième génération F2.

Le troupeau parental a été constitué de quatre types génétiques (trois écotypes locaux et d'un label) suivants : 230 sujets du Centre, 321 du Sud, 167 du Nord-Ouest et de l'Ouest, 500 sujets labels. Pour chaque écotype, trois cycles de ramassage d'œufs de 14 jours chacun ont été collectés et conservés à 18°C. Les œufs ont été incubés les 15èmes jours. Des éclosions successives ont été obtenues correspondant ainsi aux lots 1, lot 2 et lot 3.

Suivi des oiseaux

A l'éclosion, tous les poussins ont été identifiés par famille de père ou parquet pedigree et par génotype puis bagués à l'aile gauche et pesés individuellement. Ils ont été élevés au sol sur litière de copeaux de bois pendant six semaines dans un même bâtiment. Chaque écotype a été placé dans un cercle de garde de 4 m² sous deux éleveuses comportant deux lampes à incandescence de 100 W chacune supplées par un réchaud à pétrole en cas de coupure d'électricité. L'élevage en phase jeune et adulte a été fait sans complément lumineux dans la mesure où la durée de l'éclairage naturel a été de 12 heures. Les sujets locaux et le témoin ont été élevés en sexes mélangés jusqu'à 12 semaines, âge auquel les mâles de chaque écotype ont été séparés des femelles.

Pendant la période d'élevage, les températures minima et maxima relevées ont été respectivement de 20°C et 26°C alors que les valeurs minima et maxima de l'hygrométrie relative ont été respectivement de 62% et de 82%.

Soins donnés aux oiseaux

Tous les sujets ont été vaccinés à l'éclosion contre les différentes maladies virales (pseudo peste aviaire, Gumboro, Marek, variole, bronchite infectieuse) puis soumis

à un programme vaccinal complet et de traitements contre des maladies bactériennes si nécessaire. Avant le transfert de reproductrices en cages individuelles, elles ont été soumises à deux traitements antiparasitaires à intervalle de 10 jours.

En fonction du stade de développement, le programme alimentaire a été le suivant :

- de l'éclosion à la 8ème semaine, les deux sexes ont été nourris à un aliment démarrage 'Broiler mash 1'acheté dans le commerce contenant 21,9 % de protéines brutes (PB), 5,6 % de matières grasses (MG), 3,6 % de cellulose brute (CB) et 6,7 % de minéraux;

- de la 9ème à la 12ème semaine et de la 17ème à la 52ème semaine chez les mâles, un aliment 'Grower mash' acheté dans le commerce a été distribué contenant 19,5 % de PB, 6 % de MG, 12 % d'Humidité, 4.7 % de CB et 6 % de Minéraux. Le même aliment a été distribué chez les femelles de la 12ème à la 20 ème semaine;
- de la 12ème à la 16ème semaine, un aliment finition 'Broiler mash 2' acheté dans le commerce a été distribué chez les mâles ayant les caractéristiques suivantes : 19,0 % de PB, 6,97 % de MG, 40,41 % de sucre+amidon, 3,6 % de CB, 0,45 % de méthionine, 0,7 % de phosphore, 1 % de lysine, 12500 U.I/kg de vitamine A, 2500 U.I/kg de vitamine D3, 30 mg/kg de vitamine E et 10 mg/kg de sulfate de cuivre ;
- de la 20ème semaine à la 52ème semaine de ponte, un aliment 'ponte' acheté dans le commerce a été distribué chez les poules en ponte ayant les caractéristiques ci-après : 16 % de PB ; 7,0 % de MG ; 36 % de sucre+amidon ; 4 % de CB.

Constitution des types génétiques

En phase adulte, deux phénotypes ont été observés chez les témoins femelles. Ces deux phénotypes ont été issus de la ségrégation du gène du nanisme DW*N au locus DW chez les parentaux témoins qui a donnée une moitié de femelles normales (notées DW*N) et une moitié de femelles naines (notées DW*DW). Tous les mâles ont été DW*N. A partir de ces observations, le regroupement de toutes les femelles DW*N et DW*DW dès l'éclosion a été possible puisque les poussins ont été identifiés individuellement à cet âge.

Ainsi chez les mâles, on a dénombré 4 types génétiques, 3 types génétiques ou écotypes du Cameroun (Centre, Sud, Nord-Ouest/Ouest [NO/OU]) et un type Label et chez les femelles, 5 types génétiques dont 3 écotypes Camerounais comme chez les mâles et 2 témoins (femelles normales DW*N et femelles naines DW*DW).

Collecte et calculs des données

Performances d'incubation

- Le taux d'infertilité : (nombre d'œufs clairs / nombre total incubé) x 100
- Le taux d'éclosion :(nombre d'œufs éclos / nombre total incubé) x 100
- Mortalités embryonnaires : (nombre d'œufs fertiles non éclos / nombre total incubé) x 100
- Poids individuels à l'éclosion
- Longueur et diamètre du tarse à l'éclosion.

Performances des jeunes et des adultes

Poids corporel

Chez les mâles : les poussins ont été pesés individuellement toutes les quatre semaines jusqu'à la 12ème semaine puis aux 16, 18, 32, 36 et 52 semaines.

Chez les femelles : les pesées individuelles ont été faites toutes les quatre semaines de l'éclosion à la 12ème semaine, âge auquel les femelles ont été séparées des mâles puis aux 16 18, 32, 36 et 52èmes semaines.

Mesures anatomiques ou mensurations corporelles. Les mensurations corporelles sur les surfaces non emplumées (longueur des tarso-métatarses, diamètre du tarse, longueur des barbillons, hauteur de la crête) et le pourtour thoracique ont été prises sur tous les sujets à la 36ème semaine d'âge.

Consommation alimentaire et efficacité alimentaire

Chez les mâles et chez les femelles : la quantité d'aliment consommée par écotype a été mesurée globalement de 12 à 16 semaines et par conséquent l'efficacité alimentaire calculée de façon globale aussi.

Mortalités

Les mortalités ont été collectées quotidiennement jusqu'à la 16ème semaine chez les deux sexes. A partir de cet âge, la mortalité n'a été prélevée que chez les femelles jusqu'à la 52ème semaine. Chez les mâles, on n'en a pas tenu compte à partir de la 16ème semaine car des prélèvements ont été faits pour l'étude de la carcasse, le test de dégustation et pour la vente.

Analyse statistiques des données

L'analyse de la variance de tous les caractères a été effectuée en utilisant le Type Génétique (TG) et Lot (L) comme facteurs de variation à l'aide de la procédure GLM du logiciel SAS (SAS, 2001). Les moyennes estimées par la méthode des moindres carrés ont été comparées par le test de Student selon l'option PDIFF de la procédure GLM. Les taux de mortalités ont été comparés par le test de Khi-deux (Snedecor et Cochran, 1989).

Resultats

Performances d'incubation

Les tests de signification montrent (Tableau 1) que tous les paramètres d'incubation sont significativement (P ≤ 0, 0001) influencés par les facteurs 'type génétique et lot'. Le témoin est significativement supérieur (P ≤ 0,0001) à tous les écotypes locaux étudiés pour le taux d'éclosion et de fertilité d'une part, mais comparables à l'écotype du Centre pour le taux de mortalité embryonnaire d'autre part. Par ailleurs, il est relevé une grande variation entre les écotypes locaux pour tous les caractères étudiés, l'écotype du Sud ayant un meilleur taux d'éclosion comparé à celui du NO/OU et du Centre alors que la mortalité embryonnaire la plus élevée est observée au NO/OU suivie de celle du Sud.

Performances de croissance chez les jeunes

Poids corporel et efficacité alimentaire chez les mâles

Le tableau 2 montre que les principaux facteurs étudiés (type génétique et lot) ont significativement (P ≤ 0,0001) influencé toutes les variables étudiées. Les témoins sont plus lourds (P ≤ 0,0001) que tous les écotypes étudiés entre 0 et 16 semaines. Entre les écotypes locaux, celui du NO/OU est systématiquement plus lourd que ceux du Centre et du Sud à partir de 4 semaines d'âge. A 16 semaines, les écarts de poids corporel entre le témoin (DW*N) et les écotypes locaux (Centre, du Sud et du NO/OU) rapportés au poids du témoin DW*N sont respectivement de 48,75 %, 49,55 % et 41,98 %.

L'indice de consommation entre 12 et 16 semaines d'âge a été nettement meilleur (P ≤ 0,0001) pour le témoin DW*N (3,16 kg d'aliment/kg de gain de poids) par apport à celui des écotypes locaux variant de 3,92 à 4,16.

Poids corporel chez les femelles

Toutes les variables étudiées sont significativement (P ≤ 0,0001) influencées par le type génétique et le lot. Il ressort du même tableau que les femelles naines (DW*DW) sont comparables (P ≥ 0,05) aux femelles normales (DW*N) de l'éclosion à la 4ème semaine mais significativement différentes (P ≤ 0,05) à partir de la 8ème semaine. Cependant, les deux témoins DW*N et DW*DW sont plus lourds (P ≤ 0,0001) que tous les écotypes étudiés pendant toute la période d'étude

Tableau 1. Test de signification, moyennes des moindres carrés des performances d'incubation par type génétique

Performances d'incubation	Type génétique (TG)				Test de Signification		
	Centre	Sud	NO/OU	DW*N	TG	Lot (L)	TG *L
Total d'œufs incubés	694	733	509	878	-	-	-
Éclosion (%)	35,69c	43,58b	34,52c	53,26a	***	***	NS
Mortalité embryonnaire (%)	38,96c	42,48b	51,73a	35,01c	***	***	NS
Infertilité (%)	25,35a	13,94b	13,75b	11,73b	***	***	NS

1 : les variables éclosion, mortalité embryonnaire et infertilité ont été exprimées en pourcentage d'œufs incubés
a,b,c: Sur la même ligne, les valeurs portant les mêmes lettres ne diffèrent pas significativement (P ≥ 0,05) ;
***: p ≤ 0,001; **:P ≤ 0,01 ; NS : P ≥ 0,05

alors que des variations significatives (P ≤ 0,05) de poids sont observées entre tous les écotypes. Il apparaît aussi que les écotypes du NO/OU sont significativement plus lourds que ceux du Centre et du Sud alors que les écotypes du Centre et du Sud ont des poids corporels comparables. Par ailleurs, l'écart de croissance entre les témoins et les femelles locales se situe dans des proportions comparables à celles observées chez les mâles. Cet écart de poids corporel rapporté au poids de la DW*N est de 18,7 % entre la DW*N et la DW*DW. Rapporté au poids de la DW*DW, cet écart de poids est de 29,4 % entre la DW*DW et la femelle locale la plus lourde (NO/OU).

Performances pondérales chez les adultes

Le tableau 3 montre que le poids corporel chez les mâles est significativement (P ≤ 0,0001) influencé par le facteur 'Type Génétique' de 18 à 52 semaines d'âge et par le facteur 'Lot'. Il apparaît que l'écart de poids entre le témoin DW*N et les écotypes locaux et rapporté au poids de la DW*N a été de 57,61%. En revanche, parmi ces derniers, ceux du NO/OU ont été légèrement plus lourds que leurs homologues du Centre et du Sud quel que soit l'âge, même si entre 32 et 36 semaines les différences n'ont pas été significatives (p ≥ 0,05).

Chez les femelles, le facteur 'type génétique' a significativement influencé (P ≤ 0,0001) le poids corporel quelque soit l'âge. Il ressort en outre que les performances du témoin DW*N ont été significativement supérieures (P ≤ 0,0001) à celles du témoin DW*DW alors que les deux sont significativement plus lourdes que tous les écotypes représentés. Entre les écotypes, les poules du NO/OU ont été plus lourdes que celles du Centre et du Sud à 18 et à 32 semaines avec une différence non significative (P ≥ 0,05) entre 36 et 52 semaines. L'écart de poids entre les témoins DW*N et DW*DW rapporté au poids de

Tableau 2. Test de signification, moyennes des moindres carrés des poids à âges types et indice de consommation par type génétique chez les mâles et les femelles

Variable	Types génétiques (TG)					Test de signification		
	Centre	Sud	NO/OU	DW*N	DW*DW	TG	Lot (L)	TG*L
				Mâles				
Poids (g) à	N=65	N=54	N=43	N=126				
1jour	27,06^{b}	26,82^{b}	26,27^{b}	37,82^{a}		***	***	NS
4 Semaines	164,69^{c}	178,19^{c}	212,34^{b}	398,17^{a}		***	***	***
8 Semaines	426,43^{c}	425,20^{c}	510,75^{b}	1006,48^{a}		***	***	***
12 Semaines	511,40^{c}	513,74^{c}	622,04^{b}	1127,22^{a}		***	***	***
16 Semaines	973,44^{c}	958,23^{c}	1102,02^{b}	1899,43^{a}		***	***	***
I.C[1].	4,19	3,92	3,95	3,16		-	-	-
				Femelles				
Poids (g) à	N=55	N=63	N=60	N=91	N=80			
1jour	28^{b}	26,37^{c}	26,92^{c}	37,02^{a}	35,54^{a}	***	***	NS
4 Semaines	159,96^{c}	168,82^{bc}	198,19^{b}	334,96^{a}	325,53^{a}	***	***	***
8 Semaines	395,85^{d}	384,07^{cd}	479,45^{c}	873,45^{a}	776,93^{b}	***	***	***
12 Semaines	485,18^{d}	466,67^{d}	580,06^{c}	1000,18^{a}	900,99^{b}	***	***	
16 Semaines	781,75^{d}	797,37^{d}	889,47^{c}	1550,37^{a}	1259,97^{b}	***	***	***
I.C[1].	4,94	4,35	4,31	4,62	-	-	-	-

NO/OU : Nord-Ouest/Ouest ; DW*N : label normal ; DW*DW : Label nain
1 : I.C. : Indice de Consommation calculé globalement par écotype et exprimé en kg d'aliment / kg de gain de poids
a,b,c: Sur la même ligne, les valeurs portant les mêmes lettres ne diffèrent pas significativement (P≥0,05) ;
***: P ≤ 0,0001 ;
N S: (P ≥ 0,05).

DW*N est passé de 18,90% à 18 semaines à 28,73% à 52 semaines d'âge. A 18 semaines, les DW*DW ont un écart de poids corporel de 27,96% avec les femelles locales les plus lourdes (NO/OU) et de 33,9% avec les femelles les plus légères (Sud). A 52 semaines, l'écart de poids corporel de la DW*DW par rapport aux poules du NO/OU et du Sud ou du Centre est respectivement de 29,49% et de 33,48%.

Mesures squelettiques

Chez les mâles, les mensurations corporelles (Tableau 4) sont significativement influencées (P ≤ 0,0001) par les types génétiques quelque soit l'âge. Cependant, les écotypes locaux diffèrent entre eux pour le diamètre du tarse à l'éclosion avec une valeur plus faible pour les sujets du Centre. Les écotypes locaux diffèrent entre eux pour la longueur du pilon et la profondeur thoracique. Les valeurs pour ces deux caractères ont été plus élevées pour l'écotype du NO/OU à 36 semaines, alors que l'écotype du Centre a présenté des valeurs les plus faibles par rapport à celles du NO/OU pour la longueur et le diamètre du tarse et la profondeur thoracique. L'écotype du Sud a montré des valeurs intermédiaires entre celles du Centre et du NO/OU pour les mêmes caractères.

Les écarts de mensurations entre les écotypes locaux et le DW*N rapportées aux valeurs de mensurations de la DW*N se sont confirmés aussi pour la hauteur de la crête et la longueur du barbillon mais, les oiseaux des zones des Hauts plateaux de l'Ouest et de la forêt dense humide ne diffèrent pas significativement (P ≥ 0,05) entre eux pour ces caractères.

Chez les femelles, la différence entre la femelle normale DW*N et la femelle naine DW*DW et entre la DW*N et les écotypes locaux a été hautement significative (P ≤ 0,0001) pour tous les paramètres squelettiques étudiés (Tableau 5). L'écart entre la DW*N et la DW*DW et rapporté aux valeurs de la DW*N pour les mensurations corporelles a varié de 5,6 % pour la profondeur thoracique à 20,6% pour la longueur du tarse. L'écart de la DW*N par rapport à l'écotype du NO/OU, le plus lourd, a été de 22,53 %, 14,17 % et 6,07 % respectivement pour le diamètre tarse, la profondeur thoracique et la longueur du tarse. Par ailleurs, les poules locales ont obtenu des tarses significativement (P ≤ 0,0001) plus longs que ceux des poules naines DW*DW. Les écarts (calculés par rapport aux valeurs les plus élevées) pour ce caractère ont été de 10,81 %, 9,68 % et 15,52% respectivement entre la naine DW*DW et l'écotype du Centre, du Sud et du NO/OU. Les mesures des appendices (crête et barbillon) ont présenté des écarts variant de 1,86 % à 9,72 % entre la DW*N et la DW*DW mais ces écarts ont été plus importants entre la DW*N et chacun des écotypes locaux.

Tableau 3. Test de signification, moyennes des moindres carrés des poids à âges types des femelles et des mâles de 18 à 52 semaines d'âge selon les types génétiques au Cameroun

Variable	Types génétiques (TG)					Test de signification		
	Centre	Sud	NO/OU	DW*N	DW*DW	TG	Lot (L)	TG*L
				Mâles				
Poids (g) à	N=60	N=51	N=51	N=31				
18 semaines	1136,42c	1175,69bc	1262,78b	2203,39a	-	***	***	***
32 semaines	1564,61b	1523,08b	1689,34b	2758,59a	-	***	***	***
36 semaines	1635,50b	1647,83b	1788,21b	3495,17a	-	***	NS	***
52 semaines	1707,92c	1757,40c	1941,83b	3800,77a	-	***	NS	NS
				Femelles				
Poids (g) à	N=53	N=61	N=52	N=63	N=78			
18 semaines	963,58d	1046,59c	1791,52a	1452,86b		***	***	***
32 semaines	1305,58d	1293,82d	1421,85c	2246,02a	1823,76b	***	***	***
36 semaines	1348,13c	1335,05c	1428,91c	2622,06a	1989,02b	***	***	***
52 semaines	1411,81c	1409,01c	1493,57c	2972,20a	2118,29b	***	***	***

a, b, c, d: Sur la même ligne, les valeurs portant les mêmes lettres ne diffèrent pas significativement ($P \geq 0,05$)
***: $p \leq 0,0001$

Mortalités

Les résultats consignés dans le tableau 6 n'ont concerné que la phase jeune en sexe mélangé et chez les femelles en phase adulte. Les données sur la mortalité n'ont pas été considérées chez les mâles à partir de la 16ème semaine car des prélèvements ont été faits pour le test de dégustation et pour la vente. Il ressort que le taux de mortalité a été hautement significatif ($P \leq 0,0001$) de 0 à 16 semaines et s'est situé à 49,23%. Le taux global de mortalité a varié de 64,17 % pour l'écotype du Sud à 39,52 % et 41,00 % pour l'écotype du NO/OU et du témoin (DW*N).

Discussion

Les performances d'incubations ont été globalement plus faibles par rapport aux valeurs rapportées dans la littérature pour l'élevage en milieu contrôlé (Fotsa, 1985) et en milieu paysan caractérisé le plus souvent par la divagation (Fotsa et al., 2010). Cette infériorité des paramètres évalués serait expliquée par des coupures d'électricité pouvant durer jusqu'à 10 heures au cours de l'incubation et particulièrement du 19ème au 21 ème jour. Dans les conditions normales, et au vu des valeurs observées, les taux de fertilité seraient bien meilleurs et se situeraient entre 75 % pour le Centre et 88 % pour le témoin avec des valeurs intermédiaires de 86 % pour chacun des autres écotypes (Sud et Nord-Ouest/Ouest). Ces valeurs se situeraient dans la marge respective de 67,6 %-94 % pour le taux de fertilité et de 72,8 % à 87,6 % pour l'éclosabilité trouvées à Ploufragan chez la race Fayoumi ou dans le croisement impliquant la race Fayoumi (Abdellatif, 1984).

Pour le poids corporel, l'étude fait remarquer que les labels n'ont atteint leur poids commercial de 2 kg qu'après 16 semaines, contre 12 semaines en France, son pays d'origine. Les performances pondérales des poulets locaux et du label de cette étude sont inférieures à celles obtenues dans une étude similaire entre les sujets locaux et la Dahlem Red au Cameroun (Mafeni *et al*., 1997). De cet auteur, il ressort que le poulet local et celui de la Dahlem Red ont des poids respectifs de 29,42 g, 171,25 g et 880,62 g pour les sujets locaux et de 48,36 g, 244,84 g et 1228,15 g pour les coqs Dahlem Red correspondant respectivement aux performances à l'éclosion, à 4 et à 12 semaines pour chaque type génétique. On constate donc un retard important de croissance du témoin par rapport à celle de son milieu d'origine qui serait dû à l'inadaptation aux conditions environnementales. Pour le poids corporel et l'efficacité alimentaire chez les mâles, les résultats observés dans cette étude rejoignent la conclusion des travaux antérieurs (Mafeni *et al*., 1997 ; Fotsa et Manjeli, 2001) montrant que les performances des poulets locaux ont été inférieures à celles du témoin élevé dans les conditions de milieu identique. Cependant, la différence de poids adulte entre les coqs de la Région du Centre et ceux de la Région du Sud rapportée lors de l'enquête dans la zone des forêts n'a plus été observée, on peut donc suggérer que cette différence a été plutôt due aux conditions d'élevage qu'à l'écotype. De la présente étude menée en milieu contrôlé où tous les sujets bénéficient des mêmes traitements, la différence significative observée entre les écotypes de la zone de forêt dense humide à pluviométrie bimodale et celui de la zone des Hauts Plateaux de l'Ouest semble montrer que les populations de ces deux zones diffèrent génétiquement. Chez les femelles, les poids à 4 et à 8 semaines observées chez la race locale africaine, Fayoumi (Abdellatif, 1984), avec respectivement 171 g et 469 g ont

Tableau 4. Test de signification et moyennes des moindres carrés des mensurations corporelles des mâles à l'éclosion et à 36 semaines par type génétique au Cameroun

Variable	Types Génétiques (TG)				Test de signification		
	Centre	Sud	NO/OU	DW*N	TG	Lot (L)	TG *L
Mesures corporelles à l'éclosion	N=65	N=54	N=43	N=126			
Longueur du tarse (cm)	2,431b	2,452b	2,493b	2,727a	***	***	***
Diamètre du tarse (cm)	0,293c	0,305b	0,299bc	0,352a	***	***	NS
Mesures corporelles à 36 semaines	N=36	N=39	N=39	N=63			
Longueur du tarse (cm)	10,86c	10,90c	11,87b	12,93a	***	NS	NS
Diamètre du tarse (cm)	1,28c	1,32bc	1,38b	1,82a	***	NS	NS
Profondeur thoracique (cm)	11,85c	12,05bc	12,37b	13,91a	***	**	*
Hauteur de la crête (cm)	4,44b	4,55b	4,47b	5,46a	***	*	NS
Longueur du barbillon (cm)	4,16b	4,60b	4,19b	7,08a	***	NS	NS

N : effectifs d'animaux : NO/OU : Nord-Ouest/Ouest ; DW*N : Label normal.
[a,b,c]: Sur la même ligne, toutes les valeurs portant les mêmes lettres ne diffèrent pas significativement (P≥0,05)
***: p ≤ 0,0001; **: P ≤ 0,001; *: P ≤ 0,05; NS: P ≥ 0,05

été légèrement inférieurs à ceux de l'écotype du NO/OU (198 g et 479 g) à âge égal. Cependant, les performances de croissance des oiseaux élevés au Cameroun ont été globalement inférieures à celles de la race Fayoumi et rapportées chez les oiseaux âgés de 8 semaines issus de la race Fayoumi croisée aux races sélectionnées (Abdellatif, 1984 ; Benabdeljelil et Mérat, 1995). Dans la présente étude, un retard de croissance pourrait être expliqué par l'épisode de choléra aviaire dont avaient souffert nos oiseaux de la 4ème à la 10ème semaine.

Les poids des poules locales en station ont été nettement supérieurs à ceux obtenus en milieu paysan dans les hauts plateaux de l'Ouest du Cameroun (Kéambou, 2006). Par ailleurs, la différence de poids corporel entre le DW*N et les locaux rejoint les conclusions émises au Malawi (Kadigi *et al*., 1998) et au Cameroun (Fotsa et Manjeli, 2001 ; Fotsa *et al*., 2007b) sur la supériorité des performances de croissance des souches sélectionnées par rapport aux populations locales. Mais l'écart de performance entre les oiseaux des Hauts Plateaux de l'Ouest et ceux de la zone des forêts suggère que les deux écotypes auraient des aptitudes génétiques différentes, dues soit à une sélection sur la croissance ou bien à l'existence de croisements incontrôlés entre les volailles du Nord-Ouest et des souches sélectionnées ; cette hypothèse pourrait être élucidée par une étude du polymorphisme moléculaire.

Les valeurs trouvées pour les mesures corporelles ou anatomiques dans cette étude ont été supérieures à celles déjà rapportées dans les Hauts Plateaux de l'Ouest pour la longueur du tarse en station (Fotsa et Poné, 2001) et aux mesures de la longueur du barbillon et du diamètre du tarse en milieu paysan (Kéambou, 2006). Par ailleurs, il a été rapporté des valeurs de diamètre de tarse plus élevées (Kéambou *et al*., 2007) que celles observées dans la présente étude chez les mâles (1,55 cm vs 1,38 cm) en élevage extensif. La profondeur thoracique des écotypes du Nord-Ouest/Ouest a montré des valeurs inférieures à celles trouvées antérieurement (16,6 cm vs 12,37 cm) (Fotsa et Poné, 2001 ; Fotsa *et al*., 2009), mais cet écart important s'expliquerait probablement par la méthode de mesure qui a été différente.

De façon générale, les écotypes locaux ont été plus bas sur patte et ont été de petite conformation comparés au témoin DW*N, qui par ailleurs a des appendices plus développés pour une meilleure déperdition de la chaleur. Cette disposition aiderait ces oiseaux à mieux s'adapter aux conditions climatiques de la zone tropicale.

Les performances des poules locales obtenues dans cette étude ont été inférieures à celles des poules croisées Fayoumi x Leghorn aussi bien à 18 semaines qu'à 50 semaines, âge final (Benabdeljelil et Mérat, 1995). Ce retard s'inscrit dans la suite de celui accusé par les écotypes Camerounais pendant la phase jeune. Cependant, les poids des oiseaux témoins commerciaux (pondeuse ISA) et du croisement (Fayoumi x Leghorn) rapporté dans la littérature (Benabdeljelil et Mérat, 1995) ont été intermédiaires entre ceux observés dans notre étude pour les DW*N et DW*DW ; ces différences de poids sont dues aux origines et spécificités génétiques distinctes des oiseaux utilisés dans les diverses études citées.

Par rapport à d'autres races locales comme la Gauloise noire (N'dri, 2006), des valeurs comparables ont été observées entre la DW*N et la Gauloise Noire pour la longueur du barbillon (3,61 cm Gauloise noire vs 3,60 cm label DW*N) et une valeur supérieure de la race locale pour la longueur du tarse (10,62 cm Gauloise noire vs 9,88 cm label normale). Notons que ces caractères décrits pour la poule Gauloise noire (N'dri, 2006) ont des valeurs de loin supérieures à celles de chaque écotype local observées dans cette étude et aux valeurs rapportées pour la poule locale du Nord-Ouest au Cameroun (Fotsa et Poné, 2001). Il s'agit notamment des caractères longueur du tarse, longueur du barbillon et

Tableau 5. Test de signification, moyennes des moindres carrés des mensurations corporelles à 1 jour et à 36 semaines chez les femelles selon les types génétiques au Cameroun.

Variable	Types génétiques (TG)					Test de signification		
	Centre	Sud	NO/OU	DW*N	DW*DW	TG	Lot (L)	TG*L
Mesures corporelles à 1 jour	N=55	N=63	N=60	N=80	N=91			
Longueur du tarse (cm)	2,43c	2,39d	2,49b	2,69a	2,75a	***	***	***
Diamètre du tarse (cm)	0,30b	0,30b	0,31b	0,35a	0,36a	***	***	NS
Mesures corporelles à 36 semaines	N=53	N=61	N=52	N=63	N=78			
Longueur du tarse (cm)	8,79c	8,68c	9,28b	9,88a	7,84d	***	***	***
Diamètre du tarse (cm)	1,04d	1,06d	1,10c	1,42a	1,31b	***	***	NS
Profondeur thoracique (cm)	11,00c	10,99c	11,08c	12,91a	12,19b	***	***	NS
Hauteur de la crête (cm)	1,79c	2,06b	2,08b	3,22a	3,16a	***	NS	***
Longueur du barbillon (cm)	1,85d	2,07c	2,21c	3,60a	3,25b	***	NS	***

N : Effectifs animaux ; NO/OU : Nord-Ouest/Ouest ; DW*N : label normal ; DW*DW : label nain
a, b, c, d: Sur la même ligne, les valeurs portant les mêmes lettres ne diffèrent pas significativement (P ≥ 0,05).
***: p ≥ 0,0001; NS: P ≥ 0,05.

Tableau 6. Taux de mortalité chez les jeunes de 0 à 16 semaines et chez les femelles de 18 à 52 semaines par type génétique de poules locales et du témoin (label) au Cameroun.

Types génétiques	Mortalité chez les jeunes (0-16 semaines)		Mortalité chez les femelles (18 à 52 semaines)	
	Effectif à l'éclosion	Taux par rapport à l'éclosion	Effectif à 16 semaines	Taux par rapport 18 semaines
Centre	230	52,17	56	3,57
Sud	321	64,17	62	1,61
NO/OU	167	39,52	62	6,45
Témoin	500	41,00	171	7,02
Test Khi-deux			***	NS

*** : P ≤ 0,0001:
NS : P ≥ 0,05.

hauteur de la crête. De cette étude, il a été évident que les écotypes locaux de poules, comparés à la DW*DW, ont été de petit format pour les caractères autres que la longueur du tarse.

La mortalité particulièrement élevée pendant la phase jeune a été due au choléra aviaire qui a affecté l'élevage de la 4ème à la 10ème semaine. Ainsi, au nombre de maladies décimant les populations de poules locales au Cameroun citées dans la littérature (Agbédé *et al.*, 1990 ; Ngou Ngoupayou, 1990 ; Ekue *et al*., 2002), une attention devra être portée sur le choléra aviaire pour les animaux bénéficiant d'une protection sanitaire contre les maladies virales.

Conclusion

A la suite du contrôle de performances de poules locales en comparaison avec le témoin commercial de type label en milieu contrôlé, il ressort pour les performances d'incubation que le label est significativement supérieur à tous les écotypes locaux pour le taux d'éclosion et de fertilité, mais comparable à l'écotype du Centre pour le taux de mortalité embryonnaire. Le constat analogue est observé aussi bien au stade jeune qu'adulte entre le label et les locaux pour le poids corporel. Cependant, il existe une variabilité entre les écotypes locaux avec les poids plus élevés pour celui du Nord-Ouest/Ouest par rapport à ceux des écotypes du Centre et du Sud de l'éclosion à la 32ème semaine d'âge. En outre, il est observé que le label a une conformation significativement supérieure à celle des écotypes locaux pris dans leur ensemble avec des écarts de conformation entre celui du Nord-Ouest et ceux du Centre et du Sud pour la longueur et le diamètre du tarse. Les mortalités ont été plus lourdes pour les écotypes du Centre et du Sud comparées à celles de l'écotype du Nord-Ouest/Ouest et du label au stade jeune. Il peut être conclut que les performances globales de croissance en station, comparées à celles couramment rencontrées en milieu paysan, ont été meilleures. Malgré les bonnes performances de croissance des labels, elles sont inférieures à la référence commerciale du pays d'origine ; probable-

ment, en raison de l'effet de la perte de combinaison épistatique dans un croisement de troisième génération et des problèmes sanitaires qui ont entraînés un fort retard de croissance de tous les types génétiques. Cependant, ce label se trouve être le type génétique adapté pour un croisement avec les locaux pour les caractères poids corporel et conformation.

Impact

Les travaux antérieurs réalisés sur les poules locales montrent qu'elles sont à croissance lente et de performances de ponte de loin inférieures à celles des animaux de type standard. Cependant, leurs caractéristiques telles que le plumage de coloris varié, les propriétés organoleptiques aussi bien de la viande que des œufs font à ce que leur élevage représente une identité socioéconomique et rituelle des populations. Pour aider ces dernières à tirer le maximum de profit en élevant leurs animaux locaux, il est nécessaire de tester des souches améliorées aux caractéristiques semblables. Ceci permettra d'envisager des stratégies ultérieures d'amélioration génétique des souches locales qui auront des performances et les caractéristiques recherchées et souhaitées. C'est dans cette optique que cette étude a été menée.

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SHORT COMMUNICATION

Brucella abortus antibodies in the sera of indigenous and exotic avian species in Nigeria

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Brucellosis is an endemic disease in Nigeria and evidence of infection has been confirmed in cattle (Ajogi, 1997; Cadmus *et al*. 2006, 2009), sheep, goats (Cadmus et al. 2006; Falade *et al*. 1975) and humans (Cadmus *et al*. 2006; Ocholi *et al*. 1993). The only few reports of serological evidence of avian brucellosis available in Nigeria (Abdu *et al*. 1984; Bale and Nuru, 1982; Junaidu *et al*. 2006) were from the northern part of the country. Fulani herdsmen keep chickens alongside their cattle and Brucella may be contained in discharges, faecal droppings and aborted materials of infected cattle. These may contaminate food material that free range chickens are exposed to and it is therefore possible to become infected. As previously reported, chickens feeding on cattle droppings as may be the case at the cattle post are likely to pick these bacteria when searching for food. They may succumb to infection with a possible subsequent reduction in egg production (Adesiyun and Abdu, 1984). Since chickens are kept for their nutritional and economic importance, chicken rearers, handlers and consumers could therefore be at risk of contracting this disease

This study was undertaken based on the hypothesis that the indigenous and exotic avian species in north-central and southwestern Nigeria contain demonstrable *Brucella abortus* antibodies.

We sought to determine the seropositivity of smooth *Brucella* (*abortus, melitensis, suis*) in the serum samples of indigenous and exotic avian species in north-central and southwestern Nigeria with a view to assessing the *epidemiological* role of these species in the spread of *brucellosis*.

Two sites including Abuja (north-central) and Ibadan (southwestern) Nigeria were chosen for the study. They were chosen due to the lack of documented report of avian *brucellosis* as well as the existence of mixed rearing of cattle and poultry species in these areas.

Blood samples were collected from 415 chickens including 367 local chickens, 42 guinea fowls, four ducks and two turkeys through the brachial vein in the wing into vacutainer tubes without *anticoagulant* (Table 1). Out of these, 275 were from Abuja while 140 were from Ibadan (Table 2). In all, 203 and 212 male and female birds respectively were screened. Sera were separated by centrifugation and aliquoted into 5 ml sterile plastic vials, stored at -20°C until they were assayed. All the sera were screened for agglutinin using the Rose Bengal test (RBT) as described (Alton et al. 1988).

Data were analysed using the Chi-square test at 0.05 level of significance for P value.

Out of the 415 birds screened, 3.61% (15/415) had demonstrable antibodies to Brucella comprising 3.64% (10/275) from Abuja and 3.57% (5/140) from Ibadan, between which there was no significant difference (x 2 = 0.059, P >0.05) (Tables 1 and 2). Seroprevalencerates of 3.82% (local chickens), 2.38% (guinea fowls) and 0.00% (ducks and turkeys) respectively were recorded for the various breeds screened (x2 = 0.037, P >0.05). A 3:2 male to female seroprevalence ratio was obtained showing no significant difference (x2 = 0.374, P >0.05) (Table 1).

As a result of lack of data on the seroprevalence of avian *brucellosis* in Abuja and Ibadan, there was no yardstick to ascertain the public health importance of this disease as it relates to the poultry industry. However, our findings show that almost one out of every 25 poultry screened could pose health risks to their handlers and therefore serve as risk factors for the transmission of brucellosis to other animals including cattle. The 3.61% seroprevalence rate obtained (Table 1) is higher when compared with the 2.8% recorded in the northwestern part of the country (Junaidu *et al.,* 2006) and 0.9% in Botswana (Mushi *et al*., 2008). Our current report, however; points at the possibility of an increasing trend in the disease since brucellosis is endemic in the cattle population in Ibadan (Cadmus *et al.,* 2006). Hence, a similar trend may

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Table 1. Breed and sex distribution of *brucellosis* in *avian* species

Chicken types	No sampled	Sex		No positive (%)		Overall positive (%)
		Male	Female	Male	Female	
Local Chicken	367	188	179	9 (4.79)	5 (2.79)	14 (3.82)
Guinea fowl	42	14	28	0 (0.00)	1 (3.57)	1 (2.38)
Duck	4	1	3	0 (0.00)	0 (0.00)	0 (0.00)
Turkey	2	0	2	0 (0.00)	0 (0.00)	0 (0.00)
	415	203	212	9 (4.43)	6 (2.83)	15 (3.61)

be expected in the poultry reared closely with cattle and other infected animals.

There was no significant difference (c2 = 0.059, P >0.05) between the *seroprevalence* rates obtained from Abuja (3.64%) and Ibadan (3.57%). This might be explained by the fact that the two areas are both urban centres where similar animal husbandry practices exist. *Seroprevalence* rates of 3.82% (local chickens), 2.38% (guinea fowls) and 0.00% (ducks and turkeys) respectively were recorded for the various breeds screened (c2 = 0.037, P >0.05). This also shows no significant difference since all these animals were being managed on free range system and by definition, free range chickens roam freely scavenging for food and water in their immediate environment and beyond, leading a semi-wild existence having to fend for themselves. Although government abattoirs are located in Abuja and Ibadan, slaughter of animals still occurs to various degrees at private homes; and in the absence of adequate disposal facilities for the resulting entrails and blood. *Brucella* contaminated materials including water could be ingested by these free ranging birds. As a result, they may be more predisposed to the *bacterium* through ingestion of infected materials. The zero prevalence observed in ducks and turkeys may not be unconnected with their scanty population in the areas surveyed and hence their limited exposure to *Brucella*.

Although more male (4.43%) than female (2.83%) were infected, there was no significant difference between the gender (c2= 0.374, P >0.05). This was expected since they both had equal chances of consuming *Brucella* contaminated materials while scavenging.

Despite the results obtained, there were some limitations associated with this study. First, only RBT which is a qualitative screening technique was used. Although RBT is a useful diagnostic technique for bovine brucellosis, it has been reported not to be useful in human (Falade, 1974) and caprine (Falade 1978; Falade *et al*. 1975) *brucellosis*. As reiterated by Cadmus *et al*., (2008), serological te chniques vary considerably in their ability to detect antibodies of a particular immunoglobulin class and RBT may not be as sensitive as some other tests in the diagnosis of *brucellosis* in the avian species (Chukwu and Boniface, 1988). Hence, the findings of this study might have under-reported (or over-reported due to the recognized *serological* cross reactions observed in other animals) the true picture of the seropositvity of this disease in the birds studied. Second, since infected animals may or may not produce all antibody isotypes in detectable quantities, the capacity of *serological* tests to reliably detect brucellosis depends on the presence of detectable antibodies at the time of examination; and as a result, some infected animals will inevitably elude detection (FAO, 2004). Third, the numbers of birds screened were very little in comparison with their population in the study area. Lastly, the only true method of confirming disease is by isolation of Brucella from infected birds, we were unable to carry out this work at the University where this serology was performed.

Despite the above limitations, this study has confirmed the *serological* evidence of *Brucella* in the avian species screened in the local settings of Nigeria under study. Given the fact that most poultry species in these areas are on free range together with other animals such as cattle, sheep and goat, brucellosis could readily be spread to other animals from the infected ones. Since there is no suitable vaccine for birds, the demonstrable antibodies obtained in these birds could be due to natural infection as observed by previous workers (Chukwu and Boniface, 1988; Junaidu *et al*., 2006; Mushi *et al*., 2008).

In view of the possible interspecies transmission of Brucella organisms, it is recommended that cattle and chickens should not be reared together. Furthermore, public awareness campaign to poultry owners and handlers should be embarked upon to highlight the danger of contracting *Brucella* from the poultry species.

Acknowledgements

We appreciate the Veterinary Laboratory Agency (VLA), UK for supplying the *Brucella antigens* used for this study.

Impact

This study has highlighted the assertion that mixed rearing of cattle and poultry as practised by most *Fulanis* could predispose poultry to bovine *brucellosis*. It further underscores the fact that poultry species should be included in the *epidemiology* and control of *bovine brucellosis* in Nigeria since they constitute a plausible source

Table 2. Breed and sex distribution of *brucellosis* in *avian* species sampled in Ibadan and Abuja

	Ibadan					Abuja				
Chicken types	No sampled	Sex				No sampled	Sex			
		Male	Male +ve (%)	Female	Female +ve (%)		Male	Male +ve (%)	Female	Female +ve (%)
Local Chicken	92	51	3 (5.88)	41	1 (2.44)	275	137	6 (4.38)	138	4 (2.90)
Guinea fowl	42	14	0 (0.00)	28	1 (3.57)	-	-	-	-	-
Duck	4	1	0 (0.00)	3	0 (0.00)	-	-	-	-	-
Turkey	2	0	0 (0.00)	2	0 (0.00)	-	-	-	-	-
Total	140	66	3 (4.55)	74	2 (2.70		137	6 (4.38)	138	4 (2.90)

of infection as demonstrated in this study. Since most households in the country keep poultry either at backyard or on a large scale, the risk of contracting brucellosis infection therefore becomes possible. Finally, mixed rearing of poultry with cattle and close contact between these species and man should be controlled.

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SHORT COMMUNICATION

Poultry Management Errors among Farmers in Maiduguri Metropolitan Council and Jere Local Government Area, Maiduguri, Nigeria.

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Poultry industry has been identified as one of the most important economic sectors in Nigeria due to numerous contributions made in terms of employment opportunities, supply of daily protein requirements of the people, manure production, and overall sustainable development (Akpa *et al*, 2007). To enhance further growth and maximise these advantages, a better understanding of management errors or errors that hinders production efficiency needs to be ascertained and possible measures aimed at curtailing it should be evolved. Drought and livestock diseases greatly reduced the population of both large and small ruminants and this has made poultry to play a vital role as a source of animal protein for both rural and urban dwellers (Ramin *et al,* 1984). The potential for increasing the protein intake of Nigerian is higher with the poultry industry when compared to other livestock farming due to its rapid turnover and the low take off capital (Nwosu, 1989).

Poultry production being a specialised and technically demanding industry especially in the control and prevention of disease outbreaks, there is therefore a need for a clear cut understanding of standard management practices by the farmer.

The contribution of poultry to the national economy cannot be over emphasized because it has improved the employment opportunity for the unemployed masses and has improved the animal production in the country. The industry fell short of its promise of self sufficiency in consumption of 35mg/head per day of animal protein by providing 5mg/head per day which is below the Food and Agriculture Organisation (FAO) recommendation.

In the past 10 years several large scale poultry operators have been forced out of the business due to various problems ranging from shortage and high cost of drugs, lack of veterinary services, poor quality of equipment and other inputs. Poultry production is believed to be a very good source of income which directly or indirectly reduces poverty level among poultry farmers and the nation in general, apart from selling poultry by-products by farmers, it equally serves as a source of protein to them, to achieve these benefits the farmers require a sound knowledge of standard management procedures to minimize mortality and other forms of losses from the enterprise. This study presents the results of investigation into some poultry management errors by farmers in two local government areas of Borno State in Nigeria.

The study was carried out in Maiduguri and Jere areas of Borno State. Forty (40) copies of open ended questionnaire were administered. The questionnaire consists of two parts, (A and B). Part A consists of the background information of the farmer and the profile of the farm while part B examines the management practices employed on the farm. The target population were the commercial and backyard poultry farmers including those with rural chickens. The responses of the farmers were then collated and analysed to ascertain the management styles in practice and these were compared with the standard poultry management system.

The tool used in analysing the results of this work includes descriptive analysis such as summary table, mean, range and percentages.

Out of the forty (40) questionnaires distributed, 32(80%) were completed and returned by the farmers. The study revealed the followings, on the basis of the ratio of feeders used, 3 (25%) out of the 12 respondents from MMC used less than 4 feeders per 100 birds while 4(33.3%) and 5(41.7%) of the respondents used between 4-8 and 10-15 feeders per 100 birds respectively. In Jere, 3(27.3%) out of the 11 respondents used less than 4 feeders per 100 birds while 5(45.4%) and 3(27.3%) uses between 4-8 and 10-15 feeders per 100 birds respectively (Table 1). The results of the ratio of drinkers to 100 birds showed that 4(33.3%) out of the 12 respondents in MMC used less than 4 drinkers per 100 birds while 5(41.7%) and 3(25%) were using 4-8 and 10-15 drinkers per 100 birds respectively (Table 1). The result from Jere indicated that 3(27.3%) out of 11respondents used less than 4 drinkers and 4 respondents each uses 4-8 and 10-15 drinkers per 100 birds (36.4%) as shown in Table 1

In MMC 5(45.5%) out of the 11 respondents fed the birds ad-libitum while 6 (54.5%) of the farmers gave feed 2-3 times per day. Also in Jere 7(38.9%) out of the 18 farmers gave feed ad-libitum while 11(61.1%) gave the feed 2-3 times per day (Table 1).

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Table 1: Results of various factors (feeders, drinkers, feeding regimen, types roofing and bedding) investigated in MMC and Jere LGA in Borno State, Nigeria

Factors	Local Government Area (LGA)	
No of feeders /100 birds	MMC	Jere
≤ 4	3	3
4-8	4	5
10-15	5	3
No of drinkers / 100 birds		
≤ 4	4	3
4-8	5	4
10-15	3	4
Feeding regimen		
ad libitum	5(45.5%)	7(38.9%)
2-3 times /day	6 (54.5%)	11(61.1%)
Types of roofing		
Zinc	10(90.9%)	15(88.2%)
Asbestos	1(9.1%)	2(11.8%)
Types of bedding		
Sawdust	1(11.1%)	1(6.7%)
Barred floor	8(88.9%)	14(93.3%)

The outcome of the study on the materials used for the roofing and flooring of the poultry houses is as presented (Table 1), in MMC 10 (90.9%) out of the 11 farmers used zinc while 1(9.1%) used asbestos for roofing of their poultry houses. As regards flooring/beddings of the pen, 1(11.11%) and 8 (88.9%) out of the 9 respondents used sawdust and barred floor respectively, however, in Jere 15(88.2%) and 2(11.8%) out of the 17 respondents used sawdust and barred floor respectively.

The outcome of this study revealed that some management errors were being committed by poultry farmers in the two Local Government areas studied namely Maiduguri Metropolitan Council and Jere Local Government Area, Maiduguri, Borno State.

On the basis of number of feeders used per 100 birds, less than half of the farmers that is 5(41.7%) from MMC used the recommended ratio of feeders to birds while in Jere only 3(27.3%) of the respondents used the recommended feeder to bird ratio. In the case of ratio of drinkers to birds, majority of the respondents, that is, 8(66.7 %) of the respondents from MMC and 8(72.7%) from Jere used the correct ratio of drinkers to birds. The implication of this is that the birds will be scrambling for feed resulting in stress which can result into poultry vices such as cannibalism, feather pecking, egg eating, and toe pecking and eventual serious economic losses.

The importance of water cannot be under estimated because water makes up a large proportion of the body of the chickens (that is 55% to 75%), therefore, adequate provision of water is essential for maximum productivity and wellbeing. A bird consumes 1.5 to 2times as much water as it does feed (Kellems and Church, 2002), hence deviations in adequate provision of water will affect broiler performance more than its occurrence in feed contents (Abbas *et al*., 2008). If the animal body water content is not maintained or deprived, an animal will die more rapidly compared to being deprived of food (McDonald *et al*., 2002).

When the feeding regimens used were studied, 5(45.5%) and 7(38.9%) out of the respondents from MMC and Jere used the recommended feeding regimen of ad-libitum respectively. It has been shown that the net return per broiler is to a great extent dependent on the amount of the feed used to produce one kg of broiler and energy-protein ratio of diet (Sharnam et al., 2008). Feeding the birds with quality feed ad-libitum enables the broilers and cockerels to gain more weight or attain the market weight at a relatively short period of time while the layers will maintain good and uninterrupted egg laying especially if the feed contain crude protein below 21-24% and energy level of 2700-3100KcalME/Kg (Rajni *et al*., 1998).

On the basis of roofing materials used by the farmers, majority of the farmers in both MMC and Jere, that is 10(90.9%) and 15(88.2%) respectively used zinc to roof their houses which is contrary to the recommended use of asbestos which is a poor conductor of heat, or a combination of asbestos and corrugated iron sheet that reduces heat in the poultry houses (Ayvlor and Hellins, 1986). Only 1(9.1%) and 2(11.8%) of the respondents from MMC and Jere respectively used asbestos as recommended.

The result on the types of bedding of the poultry houses showed that only 1(11.1%) and 1(6.7%) of the respondents from MMC and Jere used sawdust in contrary to the recommended chopped wood shavings. Chopped wood shavings is preferable compared to sawdust (which predisposes the birds to respiratory problems, feeding on the sawdust by birds mistaking it for feeds which can lead to series of infections) while 8(88.9%) from MMC and 14 (93.3%) from Jere used barred floor (which can harbour ectoparasites in the crevices) due to its absorbent or cushion nature and therefore recommended when chicken welfare is taken into consideration, more so, in broiler rearing system bedding is a crucial factor affecting birds' comforts, welfare, health and production efficiency (Manal *et al*., 2008).

Data obtained from this study showed evidences of poultry management errors among farmers in Maiduguri Metropolitan Council and Jere. The errors includes feeding regimen as can be seen that less than 50% of the respondents from MMC and Jere utilised the correct feeding regimen of ad-libitum, roofing materials used in the study areas were equally not the recommended corrugated iron sheet and asbestos which reduces heat in the poultry house. The use of sawdust and barred floor

by majority of the farmers without chopped wood shavings equally constituted an error.

Impact

Data obtained from this study showed evidences of poultry management errors among farmers in the studied areas. The errors includes feeding regimen, types of roofing materials used in the construction of poultry houses which was not the recommended corrugated iron sheet and asbestos which reduces heat in the poultry house. The use of sawdust and barred floor by majority of the farmers equally constituted a management error.

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SHORT COMMUNICATION

Comparative evaluation of *anticoagulatory* activity of *ethylenediamine* tetra-acetic acid (EDTA) and heparin for *haematological* analysis

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Sample collection for *haematological* analysis requires use of *anticoagulant*, the two commonly used in *trypanosomiasis* research being ethylenediamine *tetra-acetic* acid and *heparin*. Since these are known to have different modes of action (Lewis, 2001), it is important to ascertain whether they can be used alternatively. Further, Swiss White mouse has been used in research involving disease pathogenesis and trials of new drugs (Kibugu *et al*., 2009, Thuita *et al*., 2008). Since handling methods are important for accurate *haematological* results, appropriate blood sampling and processing techniques need to be employed. The small body size of the mouse is probably a limitation to blood sample collection. The aim of the present study was to evaluate the *anticoagulatory* efficacy of ethylenediamine tetra-acetic acid and heparin for electronic cell counting, and develop a suitable blood handling procedure for laboratory mice.

Protocols and procedures used in this study were approved by the KARI-TRC Institutional Animal Care and Use Committee and blood collection conformed to guidelines described earlier (UCSF, 2003). Nine adult male Swiss White mice from KARI-TRC colony were maintained on mice pellets and water ad libitum at a temperature of 21-25°C. These were acclimatized for seven days before the experiment commenced. Woodchippings were provided as bedding material. Blood collection procedures were carried out in the morning (between 8 and 10 am) when temperature is presumably low (Valeri *et al*., 1995). A pair of approximately 50 µl of tail blood was collected from each of the 9 adult mice into capillary tubes coated with either *heparin* or *ethylenediamine tetra-acetic* acid, and used to investigate the anticoagulation efficacy of the two reagents. Ten per cent *disodium* salt solution (Lewis, 2001) was used to prepare *ethylenediamine tetra-acetic* acid-coated capillary tubes by rinsing plain capillary tubes in the *ethylenediamine tetra-acetic* acid solution and drying them at room temperature overnight. Heparinised capillary tubes were obtained commercially. The sample from each capillary tube was transferred using a micro-pipette to a 100-µl vial, gently mixed on a roller mixer and analysed for full haemogram using Coulter Counter (Beckman Coulter® AC-T diff™). The performance of the two *anticoagulants* was analysed by the paired sample t-test procedure to determine the differences between them on SAS statistical package (SAS Institute Inc., Cary NC, USA, 1999-2001). The sample size used in this study was considered adequate since a paired t-test was used for statistical analysis and the data were collected under highly controlled laboratory conditions.

Table 1 shows the *anticoagulatory* performance of *ethylenediamine tetra-acetic* acid and heparin expressed as 95 % confidence intervals of mice *haematological* values, and probability significance differences between the means. There was a highly significant ($p<0.0001$) difference between the means of ethylenediamine tetra-acetic acid and heparin platelet counts with the values of ethylenediamine tetra-acetic acid-treated samples being about 3.5 fold those of the heparin-treated samples. However, there was no significant difference ($p>0.05$) between the two anticoagulants in terms of the other parameters.

The two anticoagulants differed on platelet count with ethylenediamine tetra-acetic acid giving higher platelet counts than heparin. While ethylenediamine tetra-acetic acid removes calcium molecules (an essential requirement for coagulation) in the blood by chelating effect, heparin neutralizes thrombin through inhibition of interaction of several clotting factors in the presence of a plasma co-factor, antithrombin III (Lewis, 2001). Ethylenediamine tetra-acetic acid may cause swelling and disintegration of platelets leading to artificially high platelet count since the fragments are large enough to be counted as normal platelets. Other possible causes of false high platelet counts include severe microcytosis and dust-contaminated diluent (Baker, 2001). The latter was avoided in our study through use of clean materials while microcytosis is ruled out since the mice used were not infected. This paper describes a method for collection of blood samples from Swiss White mice for automated haematological analysis. Previously, sample collection from mice has been hampered by low circulating blood volume and formation of clots in the collected blood

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Table 1. Mouse-specific *haematological* values expressed as 95 % confidence intervals obtained using the two *anticoagulants* and probability significance differences between the means

Parameter	Ethylenediamine tetra-acetic acid values	Heparin values	p-values
Red cell counts (x106 / µl blood)	8.75 – 12.51	10.41 - 12.13	0.4727
Haematocrit (%)	3715 – 53.90	43.02 – 52.60	0.5859
Haemoglobin level (g/dL)	10.98 – 15.71	12.46 – 15.85	0.5279
Mean corpuscular volume (fl)	40.04 – 45.83	39.27 – 45.59	0.0888
Mean corpuscular haemoglobin (pg)	11.43 – 14.01	11.50 – 13.88	0.7924
Mean corpuscular haemoglobin concentration (g/dL)	28.26 – 30.76	28.57 – 30.57	0.8053
Total white cell count (x103 / µl blood)	9.29 – 28.68	13.48 – 27.37	0.6398
Platelet count (x103 / µl blood)	923.15 – 1409.85	96.35 – 562.15	0.0001

samples (UCSF, 2003, Baker, 2001). This was a major pit-fall in haematological investigations in trypanosomiasis research since the mouse is the cheapest and most available animal model. The collection of blood through anticoagulant-coated tubes reduced chances of coagulation. Maintenance of normothermia to ensure optimal functioning of platelets and clotting proteins (Valeri *et al.,* 1995) further enhanced this effect. We concluded that ethylenediamine tetra-acetic acid and heparin had a disparity in platelet count values, and therefore it is necessary to specify the anticoagulant used when giving haematological results. In field conditions, where heparinised capillary tubes are unavailable, ethylenediamine tetra-acetic acid preparation described in this communication is a good alternative. Further, the blood collection and preparation procedure described here is suitable for haematological analysis of mice samples.

Impact

The *anticoagulatory* efficacy of *ethylenediamine tetra-acetic* acid and *heparin* for automated blood analysis was evaluated, and a blood handling procedure for laboratory mice developed. It was established that the two reagents can be used alternatively during blood analysis in animal disease diagnosis. In field conditions, where heparinised capillary tubes might be unavailable, ethyl*enediamine tetra-acetic* acid preparation described in this communication is a good alternative. Further, the blood collection and preparation procedure described here is suitable for analysis of blood samples of Swiss White mouse, one of the most commonly used animal models in biomedical research. This will enhance research output in disease *pathogenesis* and trials of new drugs, improving animal and human health care.

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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 2010

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.

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- Audience. Manuscripts submitted must be of broad interest to animal health and production professionals in general, they must capture and holds readers' attention.
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- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.

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Authors are invited to submit electronically their manuscripts via attachment only at 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).

Authors submitting articles to the BAHPA must follow the guidelines in this document. Submissions that deviate from these guidelines will be returned to the corresponding authors for changes and compliance. Your attention to and compliance with the terms and conditions described in the Authors Guidelines document is greatly appreciated! Adherence will increase the likelihood that your submission will be favorably reviewed, and will make the work of everyone involved – you, your reviewers, and your editors – easier.

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Editorial: articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.
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News and announcements: BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information. Please allow 3 months for the listing to be published.

Submission Guidelines
All manuscripts submitted to BAHPA should include the following features:

1. On page one of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, place where the work was carried out, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion 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.
4. The Abstract should not be longer than 300 words giving a synopsis of the findings presented and the conclusion(s) reached. Up to six keywords should be provided. The abstract should contain the objectives, brief description of materials and methods, highlights of significant results, conclusions and recommendations.
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6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Identify the statistical tests used to analyze the data. Indicate the prospectively determined P value that was taken to indicate a significant difference. Cite only textbook and published article references to support your choices of tests. Identify any statistics software used.
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Sequence of Preparation

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Examples of References

- Journal Articles: Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. Journal of American Science, 1(2): 14-21.
- Books: Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.
- Chapter in a Book: Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- Reports: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- Conference Proceedings: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- Thesis: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- Web links: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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