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The Bulletin of Animal Health and Production in Africa publishes articles on original research relevant to animal health and production activities which may lead to the improvement of the livestock industry in Africa and better utilisation of her animal resources. The journal is published quarterly.

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Two copies of articles should be sent to the Editor, Organisation of African Unity/Interafrican Bureau for Animal Resources, P.O. Box 30786, Nairobi, Kenya.
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Introduction stating the purpose of the work.

Materials and Methods regular.

Results regular.

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Name of country, year of reference, followed by the name of the department or organisation, first page number.
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# BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

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LAMENESS AS A DISEASE OF INTENSIFICATION IN DAIRY CATTLE UNDER URBAN AND PERI-URBAN PRODUCTION SYSTEM IN THE ADDIS ABABA MILK SHED

FIKRE LOBAGO1, ALEMAYEHU LEMMA1, AZAGE TEGEGN2 and MARKOS TIBBO3

1 Faculty of Veterinary Medicine, Addis Ababa University (AAU), P.O. Box 34, Debre Zeit, Ethiopia
2 International Livestock Research Centre Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia
3 Sheno Agricultural Research Centre, P.O. Box 112, Debre Berhan, Ethiopia

LA BOITERIE, UNE MALADIE IMPORTANTE CHEZ LE BETAIL LAITIER SOUS LES SYSTEMES DE PRODUCTION URBAIN ET PERI-URBAIN A ADDIS-ABEBA

Résumé

Une enquête préliminaire a été faite entre octobre et décembre 1999 pour décrire et évaluer l’ampleur de la boiterie chez 964 bovins laitiers élevés dans des fermes urbaines (Addis-Ababa), périurbaines (Sebeta et Kaliti) et urbaines dans une petite ville (Debre Zeit). Sur la base de la taille des troupeaux, ces fermes étaient classées comme petites, moyennes et grandes, et on a recueilli des données sur l’état des fermes et sur les animaux à l’aide d’un questionnaire structuré. Un examen clinique était effectué sur les affections musculo-squelettiques et les animaux affectés étaient identifiés et décrits en termes de catégorie de l’animal, de type de boiterie et de la partie du corps affectée. Les affections telles que la taille excessive des sabots et l’hyperplasie interdigite cutanée qui ne provoquaient pas la boiterie étaient de 16,3%. Sur les 964 animaux examinés, 73 (7,6%) ont présenté des signes cliniques évidents de boiterie sur au moins l’une de leurs pattes. La boiterie était plus prévalente (P<0,01) chez les vaches que chez d’autres groupes, suivi par les taureaux producteurs, les veaux et les génisses. La patte de derrière et l’ongle latéral étaient plus souvent affectés (P<0,01) que la patte de devant et l’ongle médian respectivement. La plupart des cas de boiterie (68,5%) se produisaient soit uniquement au niveau de l’articulation, soit à d’autres parties de la patte. A l’exception des jarrets falciformes qui n’affectaient pas seulement un tissu, la proportion de lésions observées sur les pattes était plus forte (82,9%) dans le système de production intensif et chez le groupe d’animaux productifs (les vaches laitières) dans les troupeaux. Cette étude a montré que la boiterie est une maladie économiquement importante chez le bétail laitier dans les systèmes de production urbain et périurbain, et l’ampleur des affections s’accroît avec le niveau d’intensification. Il est recommandé d’entreprendre la recherche et le développement.

Mots-clés : Boiterie, production laitière, maladie importante, urbain, périurbain

Summary

An exploratory survey was undertaken to describe and quantify the magnitude of lameness in 964 dairy cattle between October and December 1999 under urban (Addis Ababa), peri-urban (Sebeta and Kaliti), and urban farms in a secondary town (Debre Zeit). Based on herd sizes, these farms were classified as small, medium, and large, and data were collected on farm conditions and individual animals using a structured questionnaire. Clinical examination was made on the musculoskeletal disorders and animals with disorders were identified and described in terms of category of animal, class of lameness and site of disorders. The disorders including hoof over-growth and interdigital skin hyperplasia that did cause lameness were 16.3%. Out of the total 964 animals examined, 73 animals (7.6%) clinically exhibited clear signs of lameness in at least one of their limbs. Lameness was more prevalent (P<0.01) in cows than other groups followed by breeding bulls, young stock, and heelers. The hind limb and the lateral claw were more frequently affected (P<0.01) than the front limb and the medial claw, respectively. Most of the lame cases (68.5%) had their sites of involvement either in the joint alone or joint and other areas of the limb. Excluding ‘Sickle’ and ‘cow-hocked’ conditions that did not involve only one tissue, the proportion of lesions that affected the limb structure was higher (82.9%) in the intensive production system and in the productive group of animals (milking cows) in the herds. This study indicated that lameness is an economically important disease among dairy animals in urban and peri-urban production. Research and development interventions are recommended.

Keywords: Lameness, dairy production, diseases of intensification, urban, peri-urban.

* Corresponding author: Current Address: PO Box 100344, Addis Ababa, Ethiopia; E-mail: m.habte-michael@cgiar.org
Introduction

In Ethiopia, where the demand for milk and milk products exceeds supply, the increase in human population, economic pressures and competition have put a great challenge to the traditional milk production systems. The unprecedented high rate of urbanization has also exacerbated the situation. As a result, the per capita milk consumption in Addis Ababa alone has dropped from 24 liters to less than 16 liters over the last two decades. In order to increase the milk supply to major urban centers, market-oriented intra-urban and peri-urban dairy production systems have recently emerged. The number of farms and the level of intensification is growing at a fast rate and these farms use improved genotypes, have large herd sizes, provide housing, use hired labour and artificial insemination and in some cases process milk.

In these dairy production systems, diseases of intensification and reproductive wastage are becoming important constraints and affect the productivity of animals and profitability of the business. The major diseases of intensification include mastitis, lameness, infertility, and reproductive wastage. The economic losses associated with these diseases vary from country to country. For example, in North America estimates of total milk production losses due to mastitis range from 5 to 25% while, in Ethiopia losses in milk production have been reported to be in the order of 15 to 20%. Lameness incurs the largest loss of income in dairy enterprises next to mastitis and infertility because of reduced milk production, increased veterinary costs and pre-mature culling of animals ranging from 2 to 10%. A recent study on intensive small-scale dairy farms in Debre Zeit town showed that out of 57 diseases diagnosed, the proportional prevalence of musculoskeletal disorders was found to be 14%.

The causes of lameness are multifactorial and its occurrence is associated with risk factors such as nutrition, climate, housing, breed, age, and production level of animals and as a sequel of other diseases. This study was designed to quantify the magnitude of the problem, to characterize the major types of musculoskeletal disorders, to identify the major risk factors associated with it and to assess its impact on productivity of dairy animals in urban and peri-urban production systems in the Addis Ababa milk shed.

Materials and Methods

Study area and animals

Thirty three dairy farms located in Addis Ababa (intra-urban), Sebeta and Kaliti (peri-urban) and Debre Zeit (secondary town) were used for the study. These farms were classified on herd size as small (1 to 5 animals), medium (6 to 50 animals) and large (>50 animals). Nine hundred and sixty-four crossbred animals were examined over a period of three months between October and December 1999.

Study design and data collection

The study was undertaken in two phases: the first phase involving clinical examination of all animals to generate categorical variables that helped to quantify and describe the magnitude of musculoskeletal disorders, and the second phase that involved only lame animals for a detailed study.

Data were collected on farms and individual animals using a checklist format adapted and modified. Each animal was systematically examined using a conventional clinical examination for general health and musculoskeletal diseases. The checklist contained the following information:

a. Farm checklist
   - Herd size and composition
- Management conditions - housing, feeding, hygiene, herd health, veterinary services, AI, etc.

b. Animal checklist
- Animal identification
  - Age, sex, class or category and physiological status
  - General health status and condition of the limbs
  - Description of lameness: limb affected, site affected, severity, etc.

Animals with abnormal gait, or that were unable to rise or stand normally were considered as lame. The degree of lameness was scored as severe (if all limbs were affected or the animal was unable to rise and walk) moderate (if the animal showed abnormal gait) or mild (when there was little or no signs of abnormality in the gait).

The limb structure affected by any lesion was recorded as the site of infection. Excessive length of the toe was recorded as hoof over-growth.

Cultures were made from lesions of affected limbs in blood agar with incubation at 37°C overnight and smears from the colonies were stained with Gram's stain and examined under oil immersion. Identification of bacteria was done by the method described by merchant packer.

Data analysis
Data on prevalence rate of musculoskeletal disorders; limb, claw and site affected were computed for problem animals and were analysed against the production system, herd size, class of animals as independent variables. Chi-square was used to test differences.

Results and Discussion

Prevalence of lameness
The prevalence of musculoskeletal disorder, including hoof over-growth and interdigital skin hyperplasia that did not cause lameness, for all categories of animals was 16.3%. Of the total number of animals examined, 7.6% (73/964) exhibited clinical signs of lameness in at least one limb. The percentage of cows affected was twice as high (12.1%) compared to the preliminary results reported (ILCA 1994, unpublished).

The incidence of lameness varied between farms, with quoted figures for well-recorded herds varying between (4-55%) 55%. The prevalence rate surveys in some European countries have revealed that 25 to 40% of dairy cows are annually examined and treated for lameness. Even though the prevalence rate of lameness in this study was lower than values reported in those countries. The problem will definitely have an influence on productivity of dairy animals in these emerging production systems.

Farm conditions, hygiene and veterinary services
All farms under this study practice the tie-stall system. The floor type in all farms, except three, was either bare concrete or flat stone, and none of the farms provided bedding material to their animals. The barn hygiene was found to be acceptable in about three-quarter of the farms. Provision of footbath and the practice of hoof trimming were observed in only three of the farms. Generally, large farms had better access to veterinary services.

Factors influencing the occurrence of lameness
Nearly half of the study population was comprised of cows in different physiological status. The highest prevalence rate of lameness was observed in cows (12.1%) followed by breeding bulls (11.2%), youngstock 2.6%) and heifers (2.1%). The
differences between these groups of animals were significant (P<0.01, Table 1). With exception of laminitis, most cases of foot and leg lameness occur in mature and high producing cows\textsuperscript{4,7,8}. This could be associated with the relatively heavier body weight of this group of animals, the longer period of confinement, and change of management during pregnancy and lactation.

Significant differences (P<0.01) in the rate of infection between the fore and hind limbs, and between the medial and the lateral claw were recorded (Tables 3 and 4). The hind limb and lateral claw were more affected. In intensive dairy enterprises common in West Europe and North America 80% of digital lameness occur in the hind limb and 70% affect the horny tissue and the outer (lateral) claw\textsuperscript{4}. The fact that the weight bearing stress, particularly during pregnancy and lactation, is more intense on the hind limb and the lateral claw could explain the relatively higher prevalence of the problem in these two areas. In addition, the poor design of stalls and damaged and pitted floor surfaces observed in some of the farm areas, puts a lot of stress on the hind part of the animals.

The site of infection in the majority of lame cases (68.5%) was joint alone or the joint and other areas of the limb (Figure 1). Out of the 193 lesions, recorded 133 (68.9%) were observed in the horny tissue and 29(15%) in the joint. The remaining involved the interdigital area, bulb, skin, tendon, and muscle in decreasing order (Table 5). Bare concrete that interfere with the normal hoof growth and wet conditions that weakens the hoof are primary reasons for higher rate of infection of the horny tissue in most intensive farms\textsuperscript{6}. Disorders of angulations in the hock joint, particularly 'sickle-hock' and 'cow-hocked', were frequently observed in older cows and seem to be associated with heavy weight and long periods of confinement. Both have negative impact on the supporting structures of the limb and the hoof condition, consequently leading to joint deformity and hoof over-growth. As a result, these conditions could be potential predisposing factors to lameness.

Carpitis, carpal hygroma, tarsitis, tarsal cellulitis, peri-tarsitis, arthritis in the fetlock and foot rot were the predominant diseases of the joint. With the exception of carpal hygroma and some forms of tarsal cellulitis, severe cases of lameness were associated with diseases of the joint mentioned above. Even though micro-organisms isolated as causes of arthritis are the group of \textit{Mycoplasma} species, the sample taken from a case of peri-tarsitis proved positive to \textit{Streptococcus} species, which might have been from external invasion.

The prevalence of lameness did not vary significantly between farms with different herd sizes (Table 2). However, considering only tissue involvement, disorders of horny tissue were found to be highest (81.2\%, Table 5) in large farms. In addition, the proportion of tissue lesions associated with musculoskeletal disorders was higher (82.9\%) in large farms and among the most productive group of animals.

\textbf{Conclusion}

The artificial environment seen in many intensive husbandry practices intended for higher production is the source of most of the predisposing factors to lameness. For instance, reports of some studies\textsuperscript{6} confirm that poor designs of cubicles, bare concrete floor, wet conditions in the barn, and overcrowding favors higher incidence of lameness. This suggests that locomotory disorders become more important with increasing level of intensification. However, follow-up studies are required to determine the major infective organisms and the
Table 1. Prevalence of lameness in urban and peri-urban dairy farms in the Addis Ababa milk shed

<table>
<thead>
<tr>
<th>Classes of animals</th>
<th>Number examined</th>
<th>Lame cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>507</td>
<td>61</td>
<td>12.1</td>
</tr>
<tr>
<td>Bulls</td>
<td>17</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Young stocks</td>
<td>152</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>Heifers</td>
<td>288</td>
<td>6</td>
<td>2.1</td>
</tr>
<tr>
<td>Total</td>
<td>964</td>
<td>73</td>
<td>76</td>
</tr>
</tbody>
</table>

\[X^2 = 17.4; P<0.01\]

Table 2. Lameness prevalence in terms of intensification in urban and peri-urban dairy farms in the Addis Ababa milk shed.

<table>
<thead>
<tr>
<th>Farm size</th>
<th>Number of farms</th>
<th>Category</th>
<th>Number examined</th>
<th>Lame cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>7</td>
<td>Cows</td>
<td>429</td>
<td>52</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>others</td>
<td>396</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>825</td>
<td>62</td>
<td>7.5</td>
</tr>
<tr>
<td>Medium</td>
<td>9</td>
<td>Cows</td>
<td>47</td>
<td>6</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>33</td>
<td>2</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>80</td>
<td>8</td>
<td>10.0</td>
</tr>
<tr>
<td>Small</td>
<td>17</td>
<td>Cows</td>
<td>31</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>28</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>59</td>
<td>3</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Table 3. Lame cattle in terms of limb affection

<table>
<thead>
<tr>
<th>Category</th>
<th>Lame case</th>
<th>Limb affected</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Front</td>
<td>Hind</td>
<td>Both</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Cows</td>
<td>61</td>
<td>9</td>
<td>14.8</td>
<td>34</td>
<td>55.7</td>
</tr>
<tr>
<td>Others*</td>
<td>12</td>
<td>3</td>
<td>25.0</td>
<td>9</td>
<td>75.0</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>12</td>
<td>16.4</td>
<td>43</td>
<td>58.9</td>
</tr>
</tbody>
</table>

*Others are bulls, heifers and calves

Significant differences were observed in the rate of affection of front and hind limb (P<0.01).

Table 3. Lame cattle in terms of claw affection

<table>
<thead>
<tr>
<th>Category</th>
<th>Lame case</th>
<th>Claw affected</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medial</td>
<td>Lateral</td>
<td>Both</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Cows</td>
<td>33</td>
<td>1</td>
<td>3.0</td>
<td>8</td>
<td>24.2</td>
</tr>
<tr>
<td>Others*</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>1</td>
<td>2.9</td>
<td>9</td>
<td>25.7</td>
</tr>
</tbody>
</table>

*Others are bulls, heifers and calves

The rate of affection of medial and lateral claw was significantly different (P<0.01).
Table 6. Type of tissue lesions recorded in animals with musculoskeletal disorders found in different herd size.

<table>
<thead>
<tr>
<th>Tissue affected</th>
<th>Type of lesion</th>
<th>Large farm</th>
<th>Medium farm</th>
<th>Small farm</th>
<th>Total</th>
<th>Lesions in lame cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Horny tissue</td>
<td>Hoof over-growth, claw deformity, curled toe</td>
<td>108</td>
<td>82.1</td>
<td>13</td>
<td>9.7</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>dermatitis, Foot rot, skin hyperplasia</td>
<td>15</td>
<td>75.0</td>
<td>4</td>
<td>20.0</td>
<td>1</td>
</tr>
<tr>
<td>Joint</td>
<td>Arthritis, Hygroma, Peritarsitis</td>
<td>27</td>
<td>93.1</td>
<td>1</td>
<td>3.4</td>
<td>1</td>
</tr>
<tr>
<td>Bulb</td>
<td>Heel erosion</td>
<td>4</td>
<td>80.0</td>
<td>1</td>
<td>20.0</td>
<td>-</td>
</tr>
<tr>
<td>Skin</td>
<td>Wound</td>
<td>4</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tendon and Muscle</td>
<td>Peritendinitis, Abscess</td>
<td>2</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>160</td>
<td>82.9</td>
<td>19</td>
<td>9.8</td>
<td>14</td>
</tr>
</tbody>
</table>

Fig 1. Lameness prevalence in terms of site affected in dairy farms in Addis Ababa milk shed.
impact of lameness on productivity of dairy animals in urban and peri-urban production systems.

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THE PREVALENCE AND ANATOMICAL DISTRIBUTION OF BOVINE ONCHOCERCIASIS IN BASSA LOCAL GOVERNMENT AREA OF PLATEAU STATE, NIGERIA

ANOSIKE, J.C."; OKOLI, I.C.2; OKOLI, CHIDI G.3 and EKEH.I.K.1

1. School of Biological Sciences, Imo State University P.M.B. 2000 Owerri, Nigeria
2. Department of Animal Science and Technology, Federal University of Technology, Owerri-Nigeria.
3. Department of Environmental Technology, Federal University of Technology, Owerri-Nigeria

LA PREVALENCE ET LA REPARTITION ANATOMIQUE DE L’ONCHOCERCOSE BOVINE DANS LA REGION DE BASSA, ETAT DU PLATEAU, AU NIGERIA

Résumé

Une étude a été entreprise de septembre 1995 à février 1996 pour évaluer l’influence saisonnière sur les espèces et la répartition anatomique de l’onchocercose bovine dans la région de Bassa, Etat du Plateau, dans le Centre du Nigeria. Au total, 234 (46,8%) sur les 500 Bunaji Bos indicus étaient positifs aux diverses espèces d’onchocerca. Sur les 234 animaux infectés : 140 (59,8%) étaient des femelles alors que 94 (40,2%) étaient des mâles ; par ailleurs, 81,2%, 18,4% et 0,4% de ces animaux avaient des microfilariaires onchocerca gutturosa, O. duckei et O. gibsoni respectivement. Le taux de prévalence le plus élevé (63%) était enregistré en septembre, suivi de 48,6% en novembre et 45,9% en octobre, tandis que le taux le plus faible (32%) était relevé en février. La prévalence des microfilariaires par rapport aux différents mois couverts par l’étude a montré une variation considérable (P < 0,05). De plus, 46,4% d’échantillons ombicaux étaient positifs, alors que 37,1% et 11,8% d’échantillons de nuque et de cou étaient également positifs respectivement. Le taux d’infection était très élevé (P <0,05) chez les animaux âgés de 5 à 6 ans (59,84%), suivi par ceux âgés de 3 à 4 ans (36,75%), tandis que l’on a enregistré le plus faible taux d’infection (3,41%) chez les animaux âgés de 7 ans et plus. Sur un total de 1.216 microfilariae recueillies, 58,7%, 34,9% et 6,4% étaient collectées de la région ombilicale, du haut de la nuque et de la partie du cou respectivement. Il y avait une différence significative (P < 0,05) de la répartition des microfilariae dans les diverses parties anatomiques.

Mots-clés: Onchocercose bovine, répartition anatomique, prévalence, le Centre du Nigeria

Summary

A study was carried out from September 1995 to February 1996 to assess seasonal influence on the species and anatomical distribution of bovine onchocerciasis in Bassa Local Government Area of Plateau State, Central, Nigeria. A total of 234 (46.8%) of 500 Bunaji Bos indicus cattle were positive for various Onchocerca species. Of the 234 infected animals, 140 (59.8%) were females while 94 (40.2%) were males. Of these, 81.2%, 18.4% and 0.4% of the positive animals had microfilariae of Onchocerca gutturosa, O. duckei and O. gibsoni respectively. Highest prevalence rate (63%) was recorded in September, followed by November (48.6%) and October (45.9%) while February recorded the lowest rate of 32%. The prevalence of microfilariae in relation to the different months under study showed significant variation (P<0.05). In addition, 46.4% of umbilical snips were also positive while 37.1% and 11.8% of nuchal and neck snips respectively were also positive. Infection rate was significantly (P<0.05) higher in animals aged 5 to 6 years (59.84%), followed by those aged 3 to 4 years (36.75%) while those aged 7 years and above recorded the lowest rate (3.41%). Of the 1216 total microfilariae recorded, 58.7%, 34.9% and 6.4% were recorded from umbilical region, nuchal crest, and neck region respectively. Distribution of microfilariae in the differed anatomical sites differed significantly (P<0.05).

Keyword: Bovine Onchocercosis, anatomical distribution prevalence, Central Nigeria.
Introduction

Bovine onchocerciasis is a chronic infection of the skin and subcutis of cattle caused by microfilarial parasites of *Onchocerca* species and is transmitted by biting insects particularly simulid and culicoid flies. The disease had been reported in many parts of the world. The infection, though usually subclinical in nature is known to contribute to significant proportion of losses in hides and skin and meat rejections at meat inspection. There are specific predilection sites for skin dwelling microfilarial species within the bovine host. Previous studies have identified the cervical spine, ears, umbilical, tarsal and carpal regions as common predilection sites of the microfilariae. The highest concentrations of microfilariae are mainly found in the umbilical region. It is known that microfilariae of different species of *Onchocerca* congregate at different locations of the body. *O. gutturosa* congregates mainly at the umbilical region where *S. ornatum* prefers to feed. *O. gibsoni* congregates around the large nodules in the neck and flanks, while *O. duckei* are found along the dorsal midline areas.

In Nigeria, available reports show that *Onchocerca gibsoni, O. duckei, O. gutturosa* and *O. armiliata* have been isolated from different breeds of cattle in western and northern parts of the country. However, not much is known about the epidemiology of the disease in Central Nigeria, as many endemic areas in the country are not yet identified. Equally, not fully elucidated is seasonal influence on the infection rates and microfilariae concentrations in relations to the predilection sites of the various *Onchocerca* species. A survey on bovine onchocerciasis in various rural communities is very necessary to draw up a comprehensive map of the disease prevalence in Nigeria.

This paper reports the findings of a six months study designed to establish the prevalence and seasonal influence on the infection rates and anatomical distribution of onchocercal species of cattle in Bassa Local Government Area of Plateau State, Central Nigeria.

Material and Methods

The study Area

Bassa Local Government Area of Plateau State, Central Nigeria lies within 7°51' and 11°02' N and within 7°51' and 10°08'E. The mean annual rainfall ranges from 1520 to 2050mm while the temperature ranges between 20 and 21°C and may rise to 25°C in April. The vegetation type is mainly guinea savanna (few scattered trees, grassland and cactus hedges). The topography is that of chains of granite hills interrupted by stretches of valleys containing fast flowing rivers and streams, which provide good ecological conditions for the breeding of simulium species. There are two distinct seasons, the wet and dry seasons. The wet season extends from May to September while the dry season runs through the remaining part of the year. The inhabitants practice a mixture of subsistence arable farming and rearing of goats, sheep, pigs and cattle.

Collection of Samples and Analysis

A total of 500 Bunaji (*Bos indicus*) cattle brought for slaughter at the Local Government slaughter slab between September 1995 and February 1996 were examined for *Onchocerca* microfilariae. Before slaughter, data were collected on sex, age and breed of the cattle. On daily basis, bloodless skin biopsies (2mm) from shaved nuchal crest, umbilical and the neck region were collected from freshly...
slaughtered cattle. Each skin biopsy was placed in microtitre plate (flat bottom 96 wells) containing Tyrode’s solution with 20% new born calf serum and incubated for one hour at 37°C to encourage rapid emergence of microfilariae.

Microfilariae were then isolated and counted to determine their density. Identification of microfilariae was based on the morphological features as well as the anatomical site of location. It was not possible to concurrently recover and identify the adult worms because the carcasses were not available to the team. The results were analyzed using the analysis of variance (ANOVA) which was

Table 1: Prevalence of Bovine onchocerciasis in relation to species of Onchocerca in Bassa Local Government Area.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. Of Cattle</th>
<th>% of cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of infected</td>
<td>% infected</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>O. gutturosa</td>
<td>190</td>
<td>81.2</td>
</tr>
<tr>
<td>O. duckei</td>
<td>43</td>
<td>18.4</td>
</tr>
<tr>
<td>O. gibsoni</td>
<td>1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 2: Seasonal variation of Bovine onchocerciasis in Bassa Local Government Area.

<table>
<thead>
<tr>
<th></th>
<th>No Examin</th>
<th>No Infected</th>
<th>Percentage Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>Sept.</td>
<td>34</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>Oct.</td>
<td>11</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>Nov.</td>
<td>21</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>Dec.</td>
<td>30</td>
<td>107</td>
<td>137</td>
</tr>
<tr>
<td>Jan.</td>
<td>32</td>
<td>51</td>
<td>83</td>
</tr>
<tr>
<td>Feb.</td>
<td>32</td>
<td>68</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3: Prevalence and infection of Bovine onchocerciasis in Bassa Local Government Area.

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of cattle Examined</th>
<th>No. (%)</th>
<th>No. of snips Collected</th>
<th>No. (%) snips Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept.</td>
<td>100</td>
<td>633 (63)</td>
<td>266</td>
<td>99 (37.0)</td>
</tr>
<tr>
<td>Oct.</td>
<td>30</td>
<td>14 (46.6)</td>
<td>90</td>
<td>30 (33.0)</td>
</tr>
<tr>
<td>Nov.</td>
<td>50</td>
<td>24 (48.0)</td>
<td>126</td>
<td>52 (41.0)</td>
</tr>
<tr>
<td>Dec.</td>
<td>137</td>
<td>63 (45.9)</td>
<td>317</td>
<td>94 (29.6)</td>
</tr>
<tr>
<td>Jan.</td>
<td>83</td>
<td>38 (45.78)</td>
<td>187</td>
<td>70 (37.4)</td>
</tr>
<tr>
<td>Feb.</td>
<td>100</td>
<td>32 (32.00)</td>
<td>245</td>
<td>62 (25.3)</td>
</tr>
</tbody>
</table>

Table 4: Aged related prevalence of Bovine onchocerciasis in Bassa Local government Area.

<table>
<thead>
<tr>
<th>Estimated Age Range in years</th>
<th>Examined</th>
<th>No. Infected</th>
<th>% of infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-6</td>
<td>248</td>
<td>140</td>
<td>59.84</td>
</tr>
<tr>
<td>3-4</td>
<td>231</td>
<td>86</td>
<td>36.75</td>
</tr>
<tr>
<td>&gt;7</td>
<td>21</td>
<td>8</td>
<td>3.41</td>
</tr>
</tbody>
</table>

Table 5: Microfilarial distribution and infection rate in relation to anatomical sites.

<table>
<thead>
<tr>
<th>Anatomical Site</th>
<th>No. of Snips</th>
<th>No. (%) Snips</th>
<th>No. (%) Positive Snip</th>
<th>No. (%) Microfilariae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilicus</td>
<td>442</td>
<td>205 (46.4)</td>
<td>714 (58.7)</td>
<td></td>
</tr>
<tr>
<td>Nuchal Crest</td>
<td>429</td>
<td>159 (37.1)</td>
<td>424 (34.9)</td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>365</td>
<td>43 (11.8)</td>
<td>78 (6.4)</td>
<td></td>
</tr>
</tbody>
</table>
double-checked with the student T-test to establish statistical significance.17

Results

The results of the 500 Bunaji (Bos indicus) cattle examined for bovine Onchocerciasis at different anatomical sites showed that 234 (46.8%) were infected. Of the infected cattle, 81.2% had O. gutturosa, 18.4% had O. duckei and 0.4% had O. gibsoni (Table 1). A mixed infection rate of 7.2% was also recorded. The highest prevalence rate (63%) was recorded in September followed by November (48%) and October (46.6%) while December and January had 45.9%, and 45.7% respectively. February recorded the lowest rate of 32% (Table 2).

Of the 234 infected cattle, 140 (59.8%) were females while 94 (40.2%) were males. The disease prevalence in the females was significantly (P<0.05) different from that of the males. The highest number of positive snips (99 and 94) were recorded in September and December respectively while the lowest was observed in October (Table 3). The prevalence of microfilariae across the different months under study showed a significant variation (P<0.05).

Data in Table 4 show that infection was highest in cattle aged 5-6 years (59.84%) followed by those aged 3-4 years (36.75%) while those above 7 years recorded the lowest rate (3.41%). About 46.4% of the total 442 snips taken from the umbilicus were positive for microfilariae, while 37.1% of 426 snips and 11.8% of 365 snips taken from the nuchal crest and neck region were positive for microfilariae respectively (Table 5).

A total of 1216 microfilariae were recovered from the 234 positive cattle of which 58.7%, 34.9% and 6.4% were recovered from the umbilical region, nuchal crest and neck respectively (Table 5). The distribution of microfilarial parasites across the anatomical sites differed significantly (P<0.05). A mean microfilarial density of 2.78 per skin snip was also calculated.

Discussion

The present investigation revealed that bovine onchocerciasis due to O. gutturosa, O. duckei and O. gibsoni infections exist in Bassa Local Government Area of Plateau State in Central Nigeria. This supports the reported precence of the filarial nematodes in Nigeria.13,14,16,18 These results showed a clear seasonal influence on prevalence and infection rate of microfilariae in the area. The low values recorded during the dry months and the high values recorded during wet month of September was in agreement with earlier reports on the effect of rainfall, temperature and wind in the transmission of O. gutturosa.19,20 These studies highlighted the periodic variations in the bionomics of the vectors due to seasonal and climatic conditions and other host factors such as host location in relation to breeding sites of vectors as possible explanations for this types of results. In addition, under optimum conditions of temperature, rainfall and wind S. ornatum feeds actively and hence microfilarial transmission is enhanced.10 The high umbilical counts recorded in the present study also confirm previous observations that African Simulium species bite low on the body within a meter from the ground, and that the natural vector of O. Gutturosa feeds specifically on the umbilical region.21

Sex related differences in prevalence were significant in the present study. This differs from the result of Nwaiko,20 which reported no significant differences between the male and female cattle. The present result may be explained by the fact that Fulani nomads dispose female animals only after their reproductive life has expired. It is probable that most of the
female animals fell within the middle-aged cohort when the infection is usually highest. This could as well explain why more middle-aged animals were more infected than others.

A low mean microfilarial density was estimated and the low estimate may have resulted from escapes into the preservation fluid and death in the course of field collection. The mixed infection rate of 7.2% recorded from different anatomical sites could be explained by the fact that the number of valid species in cattle is a debatable subject since the specific characteristics are variable. The present results showed that bovine onchocerciasis was relatively low in the study area. This could be attributed to continuous alterations of ecological sites of *Simulium* by cyclic climatic condition, activities of farmers in bush burning and various activities, which polluted the streams and rendered them unsuitable for survival of *Simulium* eggs.

References


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INFLUENCE OF IVERMECTIN AND CLORSULON STRATEGIC TREATMENTS ON LIVEWEIGHT GAIN AND HELMINTH INFECTIONS OF GRAZING CALVES IN KENYA

R.M. WARUIRU* and J.W. NGOTHO

Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi (Kabete Campus), P.O. Box 29053, Nairobi, Kenya

EFFETS DES TRAITEMENTS STRATEGIQUES A L'IVERMECTINE ET AU CLORSULON SUR LE GAIN PONDERAL ET LES INFECTIONS PAR LES HELMINTHES DES VEAUX EN PATURAGE AU KENYA

Résumé
Les taux de croissance des veaux en pâturage ont été évalués après des traitements stratégiques sous-cutanés à l'ivermectine (IVM) en association avec le clorsulon (CLS). Trente veaux au sevrage infectés par Fasciola gigantica et souffrant de nématodose infrclinique gastro-intestinale (GI) ont été affectés au hasard à l'un des trois groupes sous traitement ci-après : le groupe I composé de 10 veaux non-traités (groupe-témoin), le groupe II comprenant 10 veaux traités avec 0,2 mg IVM/kg de poids vif, le groupe III constitué de 10 veaux traités avec 0,2 mg IVM/kg de poids vif et 2 mg CLS/kg de poids vif. Les traitements stratégiques étaient effectués en juin, octobre 1999 et en février 2000. On a relevé les poids individuels et les échantillons de fèces au début de l'étude et ensuite à trois semaines d'intervalle. Les nématodes gastro-intestinaux et les douves du foie étaient transmis aux veaux pendant toute la durée de l'étude avec une prévalence globale de 34% et 63% respectivement. Les œufs apparaissent de Strongyle étaient très répandus pendant la saison des pluies, tandis que la prévalence des œufs de douve du foie était très forte durant la saison sèche. En dépit de l'infection constante par les helminthes, à la fin de l'étude : les veaux du groupe III avaient gagné en moyenne (± Ecarts type) 157,6 ± 5,8 kg (P<0,05) par rapport aux veaux du groupe II avec un gain pondéral de 125,6 ± 6,3 kg et à ceux du groupe I avec un gain moyen de 95,7 ± 7,1 kg. D'après ces résultats, un programme qui utilise trois traitements stratégiques des bovins à IVM/CLS a permis d'obtenir un meilleur contrôle des nématodes et des douves du foie, ce qui a entraîné un gain pondéral plus élevé comparé aux veaux non-traités.

Summary
The growth rates of grazing calves were evaluated after subcutaneous strategic treatments using ivermectin (IVM) or IVM in combination with clorsulon (CLS). Thirty weaner calves harbouring infections of Fasciola gigantica and subclinical gastro-intestinal (GI) nematodosis were randomly assigned to one of three treatment groups: group I comprising 10 unmedicated controls, group II, 10 calves treated with 0.2 mg IVM kg⁻¹ body weight; group III, 10 calves treated with 0.2 mg IVM kg⁻¹ body weight and 2 mg CLS kg⁻¹ body weight. Strategic treatments were undertaken in June and October 1999 and February 2000. Individual weights and faecal samples were taken at study initiation and at 3-week intervals thereafter. Both GI nematodes and liver flukes were transmitted to the calves during the entire study period with an overall prevalence of 34% and 63%, respectively. Strongle-type eggs were most prevalent during the rainy seasons while prevalence of liver fluke eggs was highest during the dry months. Even in the face of continual helminth challenge, at study termination, group III calves had gained an average (± S.D.) 157.6 ± 5.8 kg (p < 0.05) compared to the group II gain of 125.6 ± 6.3 kg and the group I average gain of 95.7 ± 7.1 kg. These results indicate that a programme using three IVM/CLS strategic treatments of cattle provided significantly better nematode and liver fluke control resulting in a better weight gain than untreated calves.

* Corresponding author: fax 254-2-630985, e-mail: velpath@uon.ac.ke
Introduction

Fasciolosis is an important disease of domestic ruminants causing serious economic losses. It is generally accepted that acute infection with Fasciola gigantica can cause severe clinical disease and a high mortality rate in grazing calves, but the economic impact due to chronic infection may also be of considerable importance. This subclinical form of the disease generally remains undetected and hence may significantly reduce production without the producer’s knowledge. Assesment of the economic cost of chronic infections remains difficult because of the multiple factors that can occur in the management system.

Clorsulon, an injectable sulphonamide is highly effective against bile duct stages of liver flukes (F. hepatica and F. gigantica) in cattle. However, CLS must be used concurrently with nematocides as it is ineffective in controlling gastro-intestinal (GI) nematodes in cattle. Ivermectin (IVM) is highly effective against parasitic nematodes. The use of IVM concurrently with clorsulon does not impair the efficacy of the latter anthelmintic.

The present study was conducted to evaluate the effects of strategic dosing of IVM and CLS on the weight gain of weaner calves with naturally acquired GI nematode and F. gigantica infections.

Materials and Methods

Study area

The present study was conducted on a dairy farm between June 1999 and June 2000 in Kamae area of Lari Division, Kiambu District, about 70 km to the northwest of Nairobi. The area is endemic for GI nematodes and liver fluke infections.

Experimental animals

Thirty female Friesian crossbred calves were used in the study. The calves were 8 to 10 months of age and had previously been exposed to nematode and liver fluke contaminated pastures.

Experimental designs

The calves were individually identified by eartags and before treatment, they were weighed and rectal faeces taken from each animal. They were then assigned to one of the three treatment groups (10 calves per group) based on weight. Group I calves were the unmedicated controls; group II were treated with IVM subcutaneously at 0.2 mg kg⁻¹ liveweight and group III calves treated with IVM and CLS (Ivomec® Super, MSD Agvet, at the approved dose rate of 1 ml 50 kg⁻¹ liveweight). This provided dose levels of 0.2 mg IVM kg⁻¹ liveweight and 2 mg CLS kg⁻¹ liveweight.

Strategic treatments were undertaken in late June (29th June 1999) corresponding to the end of the long rains, in October before the onset of the short rains and February 2000, corresponding to the late dry season. The animals were grazed together on natural pastures comprising mainly Kikuyu grass (Pennisetum clandestinum) and had free access to water and mineral salt. Weight gains were assessed by weighing each animal at 3 weeks intervals. Rainfall readings were recorded throughout the study period.

Parasitological methods

Rectal faecal samples were collected at the start of the study and at a 3 week interval thereafter. A modified McMaster technique was used to determine the number of nematode worm eggs gram⁻¹ (epg) of faeces. Faecal cultures were made on pooled positive samples from each group and incubated for 14 days at 27°C and third stage larvae (L₃) identified to genus level. A faecal sedimentation procedure was used to detect the presence of F. gigantica eggs. Individual faecal samples were recorded as either positive or negative with actual counts of epg for F. gigantica not determined.
Fig 1: Monthly rainfall and seasonal distribution of strongyle and liver fluke infections of calves in Kamae area from June 1999 to June 2000.
Statistical analysis

Data on liveweight gains was analysed by one-way analysis of variance and differences between groups tested for significance using Students t-test. Statistical differences are reported at P<0.05.

Results

During the study, 3 calves died due to health problems unrelated to helminthosis; one in group I and two in group II died due to gastro-enteritis and pneumonia. No eggs of *Strongloides* sp., *Nematodirus* sp., and *Trichuris* sp. were found in the faecal examinations, therefore, counts on epg reflect only strongyle-type eggs. These counts were generally low throughout the study in all three groups of calves and overall, the abundance was less than 165 eggs with about 34% of the calves in the control group excreting nematode eggs at any given sampling period. Rainfall and epg trends were strikingly similar as the prevalence of strongyle eggs were higher during the two rainy peaks (Fig. 1). The pooled faecal cultures for positive epg identified *Haemonchus* sp. as the predominant nematode with an occurrence rate of 57.3% in the herd. The occurrence rate of *Trichostrongylus* sp. was 22.8%, *Cooperia* sp. 10.6% and *Oesophagostomum* sp. 9.3%.

Eighty-one percent of the calves were shedding *F. gigantica* eggs at study initiation. In December, faecal sedimentations showed that 6 of 9 (67%), 4 of 8 (50%) and 3 of 10 (30%) calves were positive in groups I, II and III, respectively. On study termination, these same groups had 4 of 9 (44%), 5 of 8 (63%) and 2 of 10 (20%) liver fluke eggs. The overall prevalence of liver fluke eggs was about 63% and was higher during the dry periods (Fig. 1). The average bodyweight of the calves are shown in Fig. 2. At the end of the study, group III calves had a mean liveweight gain advantage of 61.9 kg over group I (P<0.05) and 32.0 kg (P<0.05) over group II calves (Table 1).

Discussion

This study demonstrated that grazing cattle in liver fluke and nematode endemic area resulted in significantly lower weight gains of untreated calves as previously reported in sheep. When only GI nematodes were removed in the IVM treated groups, the calves gained less weight than those treated with a combination of IVM and CLS, probably due to the subclinical liver fluke infections as was reported by others.

The effect of climate on the prevalence of GI nematodes was evident, as levels of strongyle infection was higher during the long and short rains. This was in agreement with earlier observations of Waruiru and colleagues in cattle in Kiambu District. Liver flukes were also transmitted year-round with peaks during the dry months and was in accordance with the findings made by other workers in central Kenya. The occurrence of *F. gigantica* infections throughout the year is suggestive of the fact that essential requirements for the completion of the life cycle of the fluke, i.e., high moisture, moderate temperature and availability of the snail intermediate host, are continually present in the study area. Given the strongyle and liver fluke transmission patterns, combined IVM/CLS treatments were apparently required as they reduced the effect of parasitism in the calves by reducing nematode and liver fluke worm burdens, respectively. Other broad-spectrum anthelmintics, which have efficacy against liver flukes could also be used to prevent production losses caused by fasciolosis that may be superimposed upon existing GI nematode infections. However, results from this study also demonstrated that even a single strategically timed anthelmintic treatment
Fig. 2 Mean liveweights of calves strategically treated with ivermectin or ivermectin/clordulon compared with untreated controls.
Table 1: Mean weight gain of control (group I) and ivermectin or ivermectin/clorsulon (groups II and III) treated calves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Mean initial weight (kg)</th>
<th>Mean final weight (kg)</th>
<th>Mean gain (kg)</th>
<th>Mean daily gain (g day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>118.7±7.4</td>
<td>214.4±8.5</td>
<td>*95.7±7.1</td>
<td>312.8±11.6</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>118.5±6.7</td>
<td>244.1±8.7</td>
<td>b125.6±6.3</td>
<td>410.5±10.8</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>118.4±8.2</td>
<td>276.2±7.9</td>
<td>b,c157.6±5.8</td>
<td>515.1±15.2</td>
</tr>
</tbody>
</table>

Values without a common superscript letter within a column are significantly different (P<0.05).

in the face of continual parasite challenge could be advantageous. Other methods of control, for example grazing management, use of molluscicides biological control of fluke intermediate host and animal parasitic nematodes merit further consideration.

Acknowledgements

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STANDARDIZATION OF A TUBERCULIN TEST FOR THE DIAGNOSIS OF TUBERCULOSIS IN CAMELS IN EGYPT

S.A. SELIM¹, F.A. ABDUL RAZIK², E.A. NASR³, M. BALATA², M.O. EL-SHAZLY⁴ and M.E. HANAN¹

¹Department of Microbiology, Faculty of Veterinary Medicine, Cairo University.
²Desert Research Institute, Cairo Egypt.
³Veterinary Serum and Vaccine Research Institute, Abbassa, Cairo.
⁴Department of Pathology, Faculty of Veterinary Medicine, Cairo University

NORMALISATION DU TEST A LA TUBERCULINE POUR LE DIAGNOSTIC DE LA TUBERCULOSE CHEZ LES CHAMEAUX EN EGYPTE

Résumé

Quatre chameaux étaient infectés expérimentalement par l'inoculation intratrachéale de Mycobacterium bovis virulent. Deux d'entre eux étaient simultanément injectés avec du dexaméthazone par voie intramusculaire pour s'assurer qu'ils étaient effectivement infectés. Plusieurs tests à la tuberculine étaient effectués sur tous les chameaux pendant les 15 mois qu'a duré l'expérience en utilisant des tuberculines PPD (Dérivé protéique purifié) bovine et humaine préparées localement. Les chameaux étaient abattus et autopsiés, puis soumis à l'examen bactériologique.

Une nouvelle clé-type d'interprétation du test à la tuberculine chez les chameaux était établie. Selon cette clé-type, une différence de l'épaisseur du pli de la peau de 7 mm ou plus est considérée comme étant une réaction positive dans le cas de la tuberculine PPD humaine, tandis qu'une différence de 6,5 mm est considérée comme une réaction positive dans le cas de la tuberculine PPD bovine.

La validité de la nouvelle clé-type d'interprétation était confirmée par l'examen de 107 chameaux choisis au hasard à l'abattoir et qui faisaient l'objet de tests cutanés à la tuberculine, de nécropsie et d'examens bactériologiques, qui ont révélé une bonne corrélation entre eux.

Cette étude confirme et recommande l'utilisation de la tuberculine PPD bovine combinée avec la nouvelle clé-type d'interprétation comme un test de choix pour le diagnostic précis de la tuberculose bovine chez les chameaux en Egypte, par rapport à la clé-type actuelle utilisant la tuberculine PPD humaine pour le test unique intradermique à la tuberculine.

Summary

Four camels were experimentally infected by intratracheal inoculation of virulent Mycobacterium bovis. Two of them were simultaneously injected with dexamethazone intramuscularly to ensure that they were infected. Several tuberculin tests were performed on all the camels through 15 months of the experiment by using the locally prepared human and bovine Purified Protein Derivative (PPD) tuberculins. The camels were slaughtered and subjected to post-mortem and bacteriological examination.

A new standard key for interpretation of tuberculin test in camels was established. The key considers a difference in skin fold thickness of 7 mm or more as positive reaction in the case of human PPD tuberculin whereas a difference of 6.5 mm is considered positive in the case of bovine PPD tuberculin.

The validity of the new standard key was confirmed by the examination of 107 blindly selected field camels in the abattoir which were subjected to cervical tuberculin skin tests, post-mortem and bacteriological investigations, which revealed a good correlation between them.

This study confirms and recommends the use of bovine PPD tuberculin coupled with the new standard key for interpretation as a better test for the accurate diagnosis of bovine tuberculosis in camels in Egypt than the present key using human PPD tuberculin in the single intradermal tuberculin test.

¹ Corresponding author. Fax 202-5725240. E-mail Vbiotech@ega.net
Introduction

The standard diagnostic test for bovine tuberculosis in living animals is the intradermal tuberculin test (ITT), one of the oldest immunological tests still in widespread use\(^1\).

This test measures a delayed type hypersensitivity (DTH) response to injected mycobacterial antigens (tuberculins). But the efficiency of the test depends upon many standardized conditions in order to reduce the fallacy of the test which may develop during field investigations. According to direction 80/2/9/ECC, Annex B., it is concluded that no single tuberculin test and interpretation key or combination of tuberculin tests and interpretation keys is suitable for all environments and epidemiological situations. The need in each country or geographical area for properly designed field trials to determine the specificity and sensitivity of tuberculin tests is emphasized\(^2\).

The standard interpretation key of skin fold thickness in ITT is the decisive factor in detecting the diseased animals which are subjected to slaughtering using the eradication programs in different countries. Each country must have its own standard key for interpretation of tuberculin tests in each species of animals. For example, according to the USDA standards (in the USA), an increase in camel skin fold thickness greater than 5 mm can be considered as suspicious, while with Russian standards, an increase in skin fold thickness of 8 mm or more is considered as a positive reaction, whereas a reaction less than 8 to 5 mm is considered as suspicious and a reaction less than 5 mm as a negative reaction\(^3,4\).

In Egypt, previous investigators\(^5,6,7\) used the same standard key which is recommended by the Official Veterinary Authorities (Governmental Organization for the Veterinary Services 'GOVS') for the interpretation of single intradermal tuberculin test in cattle. This standard key was recommended by Weybridge Laboratories for interpretation in cattle and considers a difference in skin fold thickness of 4 mm or more as a positive reaction, difference from 3- to less than 4 mm as suspicious and less than 3 mm as a negative reaction. Previous studies in Egypt\(^5,7\) revealed the invalidity of this standard key for interpretation of tuberculin skin test in diagnosis of bovine tuberculosis in camels due to a high percentage of false positive reactors. They recommended the necessity of developing a strict interpretation key that is based on an accurate field experimental design.

In this study, standardization of tuberculin test for the diagnosis of tuberculosis in camels experimentally infected with *M. bovis* and blindly selected field camels was carried out.

Materials and Methods

Two groups of camels were used in the present investigation: Four, 3 years old tuberculin negative camels were supplied by the Desert Research Institute in Cairo and hospitalized in an isolated area in the hospital of the Faculty of Veterinary Medicine, Cairo University. The camels were inoculated intratracheally with 2 ml suspension of virulent *M. bovis* strain containing 0.3 mg (wet weight) as described previously\(^8\). Mycobacterial strain was kindly provided by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. To ensure that they contracted disease, two of the camels were intramuscularly inoculated with 2 ml dexamethazone as the disodium salt in concentration of 4 mg/ml as described previously\(^9\).

One hundred and seven field camels were blindly selected from the quarantine of an abattoir. They were tested by cervical
single intradermal tuberculin test by inoculation of human PPD tuberculin on the right side of the middle third of the neck, and bovine PPD tuberculin on the left side at the same time.

Human and bovine PPD tuberculins were provided by the Bacterial Diagnostic Products Research Dept., Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo. It was available in a concentration of 2 mg/ml (100,000 TU/ml) for human PPD tuberculin while for bovine PPD tuberculin it was in a concentration of 1 mg/ml (50,000 TU/ml). The dose of intradermal inoculation for both tuberculins was 0.1 ml and skin fold thickness was measured before and after inoculation of tuberculin by a skin caliper.

The four camels experimentally infected with *M. bovis* were subjected to four repeated tuberculin skin tests. The tests were performed after 2, 6, 12 and 15 months from the start of the experimental infection. At the same time, the 107 field camels were subjected once to skin tuberculin test with both human and bovine PPD tuberculins before necropsy. Post-mortem and bacteriological examinations were done on three of the four experimentally infected animals (the fourth camel died from generalized tuberculosis after 8 months), in addition to 107 field camels which were examined in the abattoir. Serial incision of all lymph nodes of the head, carcass and viscera and from lesions and lymph nodes were collected to be bacteriologically examined in the laboratory for isolation of causative organisms as described previously\(^{10}\).

### Results

Firstly, the results of the tuberculin testing of four experimentally infected camels are shown in Table 1. The maximum difference in skin fold thickness in the case of the human PPD tuberculin was 11.8 mm and the least difference was

<table>
<thead>
<tr>
<th>Tuberculin Test*</th>
<th>1st (2 M)</th>
<th>2nd (6 M)</th>
<th>3rd (12 M)</th>
<th>4th (15 M)</th>
<th>PM finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of camel</td>
<td>H</td>
<td>B</td>
<td>H</td>
<td>B</td>
<td>H</td>
</tr>
<tr>
<td>87 (Dexamethazone)</td>
<td>11.8</td>
<td>10.9</td>
<td>9.8</td>
<td>7.5</td>
<td>9.3</td>
</tr>
<tr>
<td>59 (Dexamethazone)</td>
<td>8.8</td>
<td>6.9</td>
<td>8.1</td>
<td>6.7</td>
<td>Dead*</td>
</tr>
<tr>
<td>88</td>
<td>7.0</td>
<td>6.6</td>
<td>8.4</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>89</td>
<td>9.3</td>
<td>7.6</td>
<td>7.8</td>
<td>7.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>


* Difference in skin fold thickness is expressed as (mm)

* This camel died after 8 months of experimental infection.

\( M = \) Month Post Infection
7.0 mm. In the case of bovine PPD tuberculin, the maximum skin reaction was 10.9 mm and the minimum was 6.5 mm. Post-mortem examination of the experimentally infected camels revealed that the two camels which received dexamethazone showed miliary tuberculosis (all lymph nodes, internal organs, Figure 1 and membrane showing tuberculous lesion-Figure 2). Camel No. (89) showed localized tuberculous lesions in the bronchial lymph nodes and no visible lesions could be observed in the last camel No. (88).

Secondly, the results of the tuberculin testing randomly selected 107 of the field camels as shown in Table 2. Thirty one camels were tuberculin positive while 19 camels out of them had no visible lesions and were negative bacteriologically. While 12 camels had tuberculous like lesions. *M. bovis* was isolated from three cases only with a percentage of 9.7% (3/31) (true positives) and the other nine cases considered as false positives with a percentage of 29% (9/31). *Corynebacterium pseudotuberculosis*, atypical *Mycobacteria* from one case and

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**Fig. 1:** Lung of camel showing multiple tuberculous lesions in both lobes.

**Fig. 2:** Abdominal pleura showing numerous raised plaques and grape like nodules.
Table 2: The result of post mortem findings, bacteriological examination in 12 tuberculin reactor camels showing tuberculosis like lesion

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Age of camel (Years)</th>
<th>Difference in skin fold thickness</th>
<th>PM findings</th>
<th>Bacteriological examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human PPD tuberculin</td>
<td>Bovine PPD Tuberculin</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>5.6</td>
<td>3.4</td>
<td>pygranulomatous lesion in prescapular L.N.</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>6.2</td>
<td>4.9</td>
<td>Cheesy material with pygranulomatous lesion prefemoral L.N.</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>9.8</td>
<td>7.0</td>
<td>Congested with cheesy material in both prefemoral L.N.</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>6.0</td>
<td>4.9</td>
<td>Pyogranulomatous lesion in prescapular L.N., pyogranulomatous with cheesy material in the intercostal L.N.</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>5.9</td>
<td>4.5</td>
<td>Pyogranulomatous prescapular L.N.</td>
</tr>
<tr>
<td>6</td>
<td>5.0</td>
<td>9.1</td>
<td>6.0</td>
<td>Calcification in superficial cervical L.N.</td>
</tr>
<tr>
<td>7</td>
<td>5.0</td>
<td>8.8</td>
<td>7.2</td>
<td>Prescapular L.N. congestion and contain cheesy material</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>8.4</td>
<td>5.3</td>
<td>Cheesy material in prescapular and intercostal</td>
</tr>
<tr>
<td>9</td>
<td>6.0</td>
<td>13.0</td>
<td>7.9</td>
<td>Caseation lesion (small nodules) in both prefemoral L.N. and congestion of lung tissues</td>
</tr>
<tr>
<td>10</td>
<td>6.0</td>
<td>7.6</td>
<td>5.8</td>
<td>Pyogranulomatous lesion (small nodules) in prescapular L.N.</td>
</tr>
<tr>
<td>11</td>
<td>7.0</td>
<td>8.9</td>
<td>6.0</td>
<td>Left lung contain one small calcified nodules</td>
</tr>
<tr>
<td>12</td>
<td>8.0</td>
<td>14.3</td>
<td>9.3</td>
<td>Miliary tuberculosis (lung is very large)</td>
</tr>
</tbody>
</table>

No isolates were obtained from the remaining two cases. So, the total false positive camels (28/31) had a percentage of 90.3%.

From our results, we recommended the new standard key for interpretation of single intradermal tuberculin test in camels in Egypt after experimental infection with *M. bovis*. P.M. findings and bacteriological examination as follows: 7 mm difference in skin fold thickness or more with human PPD tuberculin can be considered a positive reactor and 6.5 mm or more with bovine PPD tuberculin also is a positive reactor.

Application of this new recommended standard on 107 camels indicated that only 8 camels could be considered as positive with human PPD tuberculin while only (4/107) camels could be considered positive.
with bovine PPD tuberculin. With the bacteriological examination only 3 animals No. (3, 9, 12) gave positive culture of *M. bovis*. The results are shown in Table 2.

**Discussion**

To standardize single intradermal tuberculin tests in camels under the local conditions in Egypt, four negative tuberculin test camels were experimentally infected by intratracheal inoculation with virulent *M. bovis*.

The maximum reaction readings with human PPD tuberculin showed that the difference in skin fold thickness reached 11.8 mm and the least reaction was 7 mm. At the same time, the maximum skin fold reaction with bovine PPD tuberculin was 10.9 mm while the least reading was 6.5 mm. We consider the least reading of the difference in skin fold thickness in the case of using human PPD tuberculin as the new recommended standard key and it was 7 mm or more, and 6.5 mm or higher in case of using bovine PPD tuberculin for the interpretation of single intradermal tuberculin test in Egypt.

In the case of applying the new recommended standard key, only 8 camels could be considered as positive reactors with the human PPD tuberculin with a percent of 7.4% and 4 cases were positive reactors for the bovine PPD tuberculin with a percent of 3.7%.

For confirmation of tuberculin results obtained, all the camels were slaughtered, examined for pathological changes and any organ or tissue showing suspected tuberculous lesions was cultured for bacteria. Typical Mycobacteria could be isolated from 3 cases only with a percent of 2.8% (true positives) and *C. pseudotuberculosis* or MOTT or mixed infection could be isolated from 7 lesions, and no bacteria were isolated from two cases which showed tuberculous lesions.

The same situation has been experienced in the USA and Canada where in the case of Bactrian camels with positive skin reactions, no Mycobacteria were isolated or seen either in biopsied or necropsied tissues. The authors attributed failure of isolation of mycobacteria from lesions to some atypical mycobacteria, which are very difficult to isolate. They found poor correlation between the precence or absence of lesions or acid fast bacilli (AFB) and the intradermal tuberculin and Bactrian camels in an established herd based on pathological findings and correlative post-mortem cultures. But the tuberculous lesion was not found in 12 post-mortem examinations to positive intradermal tuberculins in many camels after being tested by tuberculin test in the same herd. In comparing the new standard key considered 28.9% cases as positive reactors with the human PPD tuberculin, the new recommended key considered only 8 positive reactors with a percent of 7.4% only which is nearer to the true (2.8%), while with the bovine PPD tuberculin, only 4 animals, a percent of 3.7% could be detected as tuberculin positive reactors in comparison to 16.8% with the previous standard related to true positives. Also, it was observed that the human PPD tuberculin detected more positive reactors (7.4%) than the bovine PPD tuberculin (3.7%). So bovine PPD tuberculin is nearer to the true positives (2.8%). This means that human PPD tuberculin is more sensitive but the bovine PPD tuberculin is more specific. It has also been demonstrated that the bovino PPD was approximately one and a half times more potent than the human PPD per unit of weight.

Similar results for interpretation key of tuberculin testing of camels in Egypt have been reported. The strictly recommended 8.5 mm or more as positive reaction with human PPD tuberculin and 7 mm
difference or more skin fold thickness as positive reaction with bovine PPD tuberculin. The difference may be attributed to obtaining data from randomly selected camels from field cases which are not strictly controlled as experimentally infected animals in our study.

In conclusion, results of the present investigation necessitate the use of bovine PPD tuberculin coupled with the new standard key for interpretation of single intradermal tuberculin test as a better test for diagnosis of bovine tuberculosis in camels under the Egyptian conditions due to high sensitivity and specificity.

We recommended the application of serological tests for evaluation of the recommended new standard interpretation key of single intradermal tuberculin test on camels for the diagnosis of bovine tuberculosis in Egypt.

Acknowledgement

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References


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positive reactors with a percent of 7.4%.

In conclusion, the human skin TST is a relatively simple, non-invasive, and reliable test for the diagnosis of tuberculosis. In contrast, the PPD test is more invasive and requires an intradermal injection. It was observed that the human skin TST is more sensitive than the bovine PPD test, with 25% of positive reactions, whereas the bovine PPD test is considered less sensitive, with only 2% of positive reactions.

These results highlight the importance of using the appropriate test for the specific diagnostic needs. The TST is particularly useful for detecting latent tuberculosis infection, while the PPD test is more suitable for diagnosing active tuberculosis in populations with a low prevalence of infection.
EFFETS DE LA VARIETE ET DE LA SAISON SUR LES CARACTERISTIQUES DU SPERME ET LES EVALUATIONS DE CERTAINS PARAMETRES GENETIQUES CHEZ LA PINTADE A COURONNE (NUMIDA MELEAGRIS GALEATA)

Résumé

Cette étude a examiné l'effet de la variété et de la saison sur les caractéristiques du sperme et le poids vif, ainsi que la corrélation et les répétabilités des paramètres importants chez les pintades à couronne (N.m. galeate). Deux variétés de pintade : une variété exotique nacrée (VEN) et une variété locale noire (VLN) étaient utilisées pendant trois mois chacune durant la saison sèche et la saison des pluies. Les caractéristiques évaluées étaient le volume d'éjaculat, la concentration du volume du sperme, la quantité de sperme, le taux de motilité, les spermatozoïdes morts et anormaux, et le poids vif. On a obtenu avec la variété VEN de meilleurs résultats (P < 0,001) en ce qui concerne toutes les caractéristiques de la qualité du sperme et le poids vif. La saison des pluies a aussi favorisé certaines caractéristiques (P < 0,01) beaucoup plus que la saison sèche. La variété x la saison avaient des effets significatifs (P < 0,001) sur la concentration de sperme, la quantité de sperme et le pourcentage de spermatozoïdes morts avec la VLN montrant une plus grande sensibilité. La plupart des caractéristiques du sperme étaient en corrélation les unes avec les autres, et avec le poids vif. Les coefficients de répétabilité étaient élevés (> 0,3) pour toutes les caractéristiques, à l'exception du taux de motilité qui était moyen (0,297 ± 0,197). On a conclu que la VEN, avec moins de sensibilité aux influences saisonnières, serait une meilleure source de sperme de qualité que la VLN, tandis que la saison pluvieuse serait la meilleure période pour la collecte de sperme. En outre, la répétabilité élevée et les coefficients de corrélation parmi les caractéristiques observées peuvent servir de base pour l'amélioration génétique.

Mots-clés : Pintade, variété, saison, caractéristiques du sperme, évaluation des paramètres génétiques

Summary

This study investigated the effect of variety and season on semen characteristics and body weight and as well correlation and repeatabilities of relevant parameters in helmeted guinea fowl (N.m. galeata). Two varieties of birds: exotic pearl variety (EPV) and local black variety (LBV) were used over a period of three months each in the dry and wet seasons. Ejaculate's volume, sperm volume concentration, sperm count, percent motility, dead and abnormal spermatozoa and body weight were traits evaluated. Results showed that variety significantly (P<0.01) affected all semen quality traits and body weight in favour of EPV. Wet season also significantly (P<0.001) favoured some traits more than dry season. Variety x season had significant (P<0.001) effects on sperm concentration, sperm count and percent dead spermatozoa with LBV showing greater susceptibility. Most semen traits correlated with each other and body weight. Repeatability coefficients were high (>0.3) for all traits, except percent motility which was medium (0.297 ± 0.197). It was concluded that EPV; with less susceptibility to seasonal influences would be better source of quality semen than LBV, while wet season would be the best time for semen collection. Besides, the high repeatability and correlation coefficients among the observed traits suggest a basis for genetic improvement.

Keywords : Guinea fowl, variety, season semen characteristics and genetic estimates.

* Corresponding author E-mail: ibutswat@atbu.edu.ng, Fax: 234-77-541842
Introduction

Guinea fowls are medium sized gallinaceous birds from any of the genera Agelastes, Numida, Guttera and Acryllium. It is commonly accepted that the domestic guinea fowls was derived from the helmeted guinea fowl (Numida meleagris). Nigeria is estimated to have about 4.7 million guinea fowls which are ranked second to chickens and there are no cultural or religious edicts preventing their consumption nationally. Their production has, therefore, been advocated to partly remedy the animal protein deficiency in the nation. However, two problems limiting the popularity of guinea fowls production are seasonality of breeding and low fertility which are concomitantly responsible for their low reproductive capacity.

For guinea fowls to give their maximum contribution to Nigeria's protein supply, their seasonality of breeding must be overcome in addition to improving their fertility. Artificial insemination has much to contribute as vital a reproductive technological tool for rapid and efficient breeding improvement. However, on guinea fowls reared in the country, only a few studies on semen characteristics have been reported, and all these reports came from humid zone. There is no report on the semen characteristics of the bird from arid or semi-arid zone of the country where the birds are not only numerous, but are also an integral part of households, especially in rural areas. The objectives of this study, therefore, were:

1. To investigate the effects of variety and season on semen characteristic of grey breasted helmeted guinea fowls (Numida meleagris).

2. To estimate correlation and repeatability of semen characteristic. The results are hoped to contribute to the development of sustainable artificial insemination programmes for the species and provide a basis for genetic improvement.

Materials and Method

Study area

Bauchi State occupies the center of the northeastern region in the Sudan Savannah (arid and semi arid) ecological zone of Nigeria. It's center is on at latitude 10°19' and longitude 9°49' at an altitude of 590 above sea level. There are two distinct seasons in a year: the rainy season (between May-October) and dry season (between November and April). The mean annual rainfall is 1091 mm, detailed climatic description of Bauchi is well documented.

Experimental birds and their management

Three each of the exotic (improved) pearl variety (EPV) and local (unimproved) black variety (LBV) of the male grey breast helmeted guinea fowl (N.M. galeata), aged about 9 months, were used. The EPV were sourced from the National Veterinary Research Institute, Vom, while the LBV were from farmers in the Bauchi metropolis. The birds were housed singly in well ventilated, netted poultry pens and were fed commercial layer mash (crude protein 17%) and clean drinking water ad libitum throughout the experimental period.

Semen Collection and Examination

A total of 6 ejaculates were collected per season i.e twice per bird each third week of February, March and April (dry season) and June, July and August for the rainy season. The time of collection usually was between 0600 and 0900 hours GMT.
Prior to the start of the experiment there was a 3-weeks daily pre-experimental training of semen collection using the lumbar massage method of the cocks\textsuperscript{12}.

The ejaculate volume was read using a 1mililliter syringe used for semen collection, sperm motility was assessed subjectively immediately after every ejaculation by visual estimation under cover-slip using a light microscope\textsuperscript{13,14}.

The abnormal and dead spermatozoa were determined using supravital staining technique with a stain mixture of nigrosin-eosin as described by Evans and Maxwell\textsuperscript{15}.

The live-dead ratio was differentiated and counted in smear of the semen and stain mixture\textsuperscript{16} while the percent abnormal spermatozoa was microscopically evaluated by random observation of 200 spermatozoa under a high magnification (x 1000). Sperm concentration was determined haemocytometrically\textsuperscript{17}. Sperm counts were obtained as product of sperm concentration and ejaculate volume\textsuperscript{16}. The cocks body weight were determined using a weighing scale.

Data Analysis

Data obtained were analyzed using Analysis of Variance (balanced design) following angular transformation of percent motile, dead and abnormal spermatozoa. All traits were analysed using a fixed model containing variety, season, variety and season interactions.

Relationships between the semen traits were determined using Pearson’s correlation’s using MINITAB\textsuperscript{®} computer software\textsuperscript{19}.

Repeatability coefficients were calculated using the following expression\textsuperscript{20}.

\[
R = \frac{\sigma B^2}{\sigma B^2 + \sigma W^2}
\]

where

\[
R = \text{Repeatability coefficient}
\]

\[
\sigma B^2 = \text{Variance components due to differences between individuals}
\]

\[
\sigma W^2 = \text{Variance components due to differences within individuals}
\]

The standard error for repeatability was estimated using the expression proposed by Backer\textsuperscript{21}.

\[
\text{SER (R)} = \frac{2(1 - R[1 + (K - 1)R]^2}{K(K - 1)(N - 1)}
\]

where

\[
K = \text{Number}
\]

\[
N = \text{Number of individuals}
\]

\[
R = \text{repeatability}
\]

Results and Discussion

The effects of variety, season, variety seasonal interactions on semen quality characteristics and body weights are presented in Table 1, while the correlation between them in Table 2. Table 3 shows the repeatability coefficients of semen characteristics.

Effects of variety on semen traits and body weights

From Table 1 the results showed a significant (P<0.001) effect of variety on all semen characteristics and body weight in favour of EPV. The superiority of the EPV over LBV with respect to ejaculate volume is consistent with the findings of Egbutunike
Table 1. Variety and seasonal influences on semen characteristic and bodyweight of the Guinea fowl.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>EJV (ml)</th>
<th>SCN (X109/ml)</th>
<th>SCT (X109)</th>
<th>Motility</th>
<th>Dead spz</th>
<th>Abnormal spz</th>
<th>Weight (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>LBV</td>
<td>0.065 ± 0.002</td>
<td>1.36 ± 0.20</td>
<td>0.092 ± 0.14</td>
<td>38.5 ± 2.8</td>
<td>26.3 ± 1.2</td>
<td>33.4 ± 0.76</td>
<td>1.26 ± 0.005</td>
</tr>
<tr>
<td>EPV</td>
<td>0.081 ± 0.001</td>
<td>4.61 ± 0.08</td>
<td>0.376 ± 0.11</td>
<td>51.6 ± 1.7</td>
<td>14.2 ± 0.5</td>
<td>19.5 ± 0.01</td>
<td>1.70 ± 0.005</td>
</tr>
<tr>
<td>Season</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Dry</td>
<td>0.070 ± 0.002</td>
<td>2.21 ± 0.34</td>
<td>.172 ± 0.027</td>
<td>32.7 ± 1.2</td>
<td>25.0 ± 1.5</td>
<td>31.5 ± 2.00</td>
<td>1.47 ± 0.039</td>
</tr>
<tr>
<td>Wet</td>
<td>0.076 ± 0.002</td>
<td>3.75 ± 0.22</td>
<td>.296 ± 0.023</td>
<td>57.3 ± 1.9</td>
<td>15.5 ± 0.8</td>
<td>21.4 ± 1.10</td>
<td>1.49 ± 0.039</td>
</tr>
<tr>
<td>Variety x season</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LBV x Dry</td>
<td>0.061 ± 0.002</td>
<td>0.23 ± 0.07</td>
<td>0.014 ± 0.009</td>
<td>25.2 ± 2.6</td>
<td>33.2 ± 0.5</td>
<td>39.7 ± 1.48</td>
<td>1.24 ± 0.006</td>
</tr>
<tr>
<td>LBV x Wet</td>
<td>0.068 ± 0.002</td>
<td>2.49 ± 0.0</td>
<td>0.170 ± 0.009</td>
<td>51.8 ± 2.6</td>
<td>19.5 ± 0.5</td>
<td>27.1 ± 1.48</td>
<td>1.27 ± 0.006</td>
</tr>
<tr>
<td>EPV x Dry</td>
<td>0.079 ± 0.002</td>
<td>4.20 ± 0.97</td>
<td>0.329 ± 0.009</td>
<td>40.2 ± 2.6</td>
<td>16.8 ± 0.5</td>
<td>23.3 ± 1.48</td>
<td>1.69 ± 0.006</td>
</tr>
<tr>
<td>EPV x Wet</td>
<td>0.084 ± 0.002</td>
<td>5.02 ± 0.07</td>
<td>0.422 ± 0.009</td>
<td>62.8 ± 2.6</td>
<td>11.5 ± 0.5</td>
<td>15.7 ± 1.48</td>
<td>1.71 ± 0.006</td>
</tr>
</tbody>
</table>

** = P<0.01  
*** = P<0.001  
NS = Not significant  
EJV = Ejaculate Volume  
SCN = Sperm Concentration  
SCT = Sperm Count  
SPZ = Spermatozoa  
LBV = Local Black Variety  
EPV = Exotic Pearl Variety

and Nkanga\(^22\) and Gbadamosi and Egbinike\(^23\) in local and exotic chickens. These observed differences might be attributed to differences in body weight which favoured the EPV, which possibly had larger reproductive organs, more so that there was a high (P<0.001) correlation between body weight and ejaculate volume.
Table 2. Correlation coefficients between semen characteristics and bodyweight of Guinea fowl

<table>
<thead>
<tr>
<th></th>
<th>Ejaculate Sperm concentration</th>
<th>Sperm count</th>
<th>Motility spermatozoa</th>
<th>Dead spermatozoa</th>
<th>Abnormal sperma tozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration</td>
<td>0.715</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm Count</td>
<td>0.813</td>
<td>0.979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility</td>
<td>0.447</td>
<td>0.611</td>
<td>0.566</td>
<td>-0.906</td>
<td>-0.911</td>
</tr>
<tr>
<td>Dead Spermatozoa</td>
<td>-0.677</td>
<td>-0.931</td>
<td>-0.906</td>
<td>-0.911</td>
<td></td>
</tr>
<tr>
<td>Abnormal Spermatozoa</td>
<td>-0.608</td>
<td>-0.766</td>
<td>-0.768</td>
<td>-0.758</td>
<td>0.764</td>
</tr>
<tr>
<td>Bodyweight</td>
<td>0.707</td>
<td>0.896</td>
<td>0.908</td>
<td>0.404</td>
<td>-0.771</td>
</tr>
</tbody>
</table>

All were significant (P<0.001).

(Tables 2).

The mean ejaculate volumes of 0.065 ± 0.002 and 0.081 ± 0.001 mls for LBV and EPV respectively, in this study were both higher than the mean volume of 0.03 ± 0.001 mls reported by Nwakalor et al.,6 for golden sovereign guinea fowl, the values were however lower than 0.12 ± 0.002 mls reported by Ayorinde7. These differences might be due to genetic and environmental differences.

Sperm concentration and counts varied significantly (P<0.001) with variety. The EPV maintained superiority over the LBV. This finding also corroborates others findings22,23,24,25 who associated higher spermatogenetic efficiency with exotic chickens. The mean concentration of 1.36 X 10^9 ± 0.20/ml for LBV and 4.61 X 10^9 ± 0.08/ml for EPV, were higher than those reported by Ledec et al.,26 of 0.25 X 10^9 ± 0.05/ml, but lower than the findings of Nwakalor8 and Ayorinde8. The differences in values obtained were expected as sperm concentration is known to vary with breed, nutrition, season and method of semen collection inter alia8,9.

Semen motility is essential for fertility. In this study EPV had a higher (P<0.001) percentage motile spermatozoa than the LBV, even though the percentages for both varieties were sub-optimal18,25, artificial insemination would favour the use of semen from the EPV than LBV.

In this study EPV had lower (P<0.001) percent dead sperms than LBV. Similar trend was maintained for percentage abnormal sperms in both varieties. From the results, only the semen of EPV was within the accepted range for good fertility of less than 20% abnormal or dead sperms18,25,26.

Effects of season on semen traits and body weight

From Table 1, the effect of season on ejaculate volume, sperm concentration, sperm count, % sperm motility, % dead and abnormal spermatozoa were significant (P<0.001). Wet (rainy) season favoured all semen traits (semen volume, concentration, count, motility, low% dead and abnormal spermatozoa). These findings support other past reports on
guinea fowls in the sub-humid zones of Nigeria, but contradict the findings of Saed and Al-soudi in Iraq, and Egbuga and Nkanga on chickens in sub-humid zones of Nigeria who observed a reduced volume and concentration in the wet season. The hottest period (dry season) had the most adverse effect on semen morphology and characteristics of the guinea fowl in this study. This could have been due to high ambient temperature and low relative humidity which might have concomitantly affected the feed intake and the spermatogenetic process. Onuora established a positive correlation between ejaculate volume, sperm concentration and motility with all seasonal factors, except high ambient temperature, which negatively affect semen, but positive in respect of dead or abnormal spermatozoa proportions. Dry seasons in Bauchi are characteristically hot and very dry. From the results obtained in this study, significant differences in body weight between the two seasons with wet season favouring heavier weight, could have been due to reduced feed intake in the dry hot weather in the dry season in Bauchi.

**Effects of variety x season**

The interaction between variety and season had no significant effect on ejaculate volume, percent mortality and abnormal spermatozoa as well as body weight. On the other hand, sperm concentration, sperm count and percent dead spermatozoa were significantly (P<0.001) affected by the interaction, with LBV exhibiting greater susceptibility to the effects of season than EPV. This was probably because LBV was semiwild, whereas the EPV was fully domesticated, thus partially corroborating the reports of Lorenz that the effect of season on semen quality is more striking in wild birds than in fully domesticated ones. In addition unlike EPV, LBV had no history of artificial selection for any trait such as body weight or out of season breeding.

The semen of the LBV was highly oligospermatic on the average in the dry season relative to that of the EPV, with mean sperm concentration of 0.23 X 10^9/ml and 4.2 X 10^9/ml, respectively. The sperm count of the LBV in the dry season was also too low to effect fertility as it averaged only 14 ± 9 X 10^9 spermatozoa which was far below the minimum 62 million spermatozoa needed for high level of fertility in poultry. The greater adverse effect of seasons on semen live-dead ratio in LBV than EPV contradicts the findings of Egbuga and Nkanga in chicken cocks. This variation could be attributed to species differences. There are similar reports on seasonal variations in morphological abnormalities in different bulls species of semen, in Northern Nigeria.

**Correlation between semen traits and body weight**

Table 3 shows the correlation among semen characteristics and between semen characteristics and bodyweight. The correlation coefficients were all highly significant (P<0.001), except for percent mortality which bore medium correlation with ejaculate volume and body weight. Percent dead and abnormal spermatozoa were negatively correlated with all other traits, but positively correlated with one another. These results corroborate the earlier findings of Onuora. The high and positive genetic correlation of most semen traits with bodyweight favours selection for better reproductive ability on the basis of body weight.

**Repeatability estimates of semen traits**

The repeatability coefficients for all the semen traits varied from low to high. Repeatability for sperm count, concentration, percent dead spermatozoa,
Table 3. Repeatability coefficients of semen characteristics of the Guinea fowl

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>Number of Birds</th>
<th>$\sigma_{B}^2$</th>
<th>$\sigma_{W}^2$</th>
<th>Repeatability (SER (R))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate Volume</td>
<td>6</td>
<td>0.000780</td>
<td>0.0000866</td>
<td>0.474 ± 0.242</td>
</tr>
<tr>
<td>Sperm Concentration</td>
<td>6</td>
<td>3.149</td>
<td>0.834</td>
<td>0.791 ± 0.244</td>
</tr>
<tr>
<td>Sperm Count</td>
<td>6</td>
<td>0.239</td>
<td>0.0057</td>
<td>0.807 ± 0.0239</td>
</tr>
<tr>
<td>Motility</td>
<td>6</td>
<td>98.817</td>
<td>234</td>
<td>0.656 ± 0.0197</td>
</tr>
<tr>
<td>Dead Spermatozoa</td>
<td>6</td>
<td>49.983</td>
<td>34.2</td>
<td>0.656 ± 0.055</td>
</tr>
<tr>
<td>Abnormal Spermatozoa</td>
<td>6</td>
<td>70.500</td>
<td>55.0</td>
<td>0.562 ± 0.262</td>
</tr>
</tbody>
</table>

SER (R) = Standard Error of Repeatability

and abnormal sperms and ejaculate volume were generally high. While that of percentage sperm mortality was low. More work on these lines is needed to ascertain selection based on these criteria.

Conclusion and Applications

It can be concluded from these results that:

1. The EPV had heavier body weight and were less affected by seasonal influence. They can be used, therefore, as source of quality semen in the dry season.

2. The wet season would be best time for semen collection for both varieties.

3. The high correlation and repeatability coefficient among semen traits and bodyweight could provide the basis for genetic improvement through selection, but more work has to be done on this aspect of the study.

Acknowledgements

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References


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OEDEMA DISEASE OF SWINE: A TOXAEMIA OR AN INFECTIOUS DISEASE?

T.A. NGATIA1, C.M. MULEI2, P.K. GATHUMBI1 and J.K. WABACHA2

1 Department of veterinary pathology and microbiology,
2 Department of clinical studies, Faculty of Veterinary Medicine, University of Nairobi,

P.O. Box 29053, Nairobi, Kenya

L’OEDEME PORCIN : UNE TOXEMIE OU UNE MALADIE INFECTIEUSE ?

Résumé

Vingt-et-un porcs Large White âgés de 2 à 3 mois provenant de dix fermes différentes ont été autopsiés. L’historique clinique disponible révélait la mort soudaine des porcs en bonne santé après le sevrage (8 – 14 jours). Les principaux résultats macroscopiques étaient l’œdème sous-cutané de la tête, la sous-mucosité de la paroi de l’estomac, le mésentère du colon spiral, le cerveau et les méninges caractérisés par la dégénérescence cellulaire, la congestion du poumon, l’œdème, la consolidation et l’affaissement. A l’examen microscopique, les principales lésions constatées chez tous les animaux étaient l’œdème aux points spécifiques, la pneumonie interstitielle de divers degrés de gravité et l’encéphalite ventriculaire non-suppurative. Sur la base de l’historique clinique et des résultats histopathologiques et macroscopiques, les animaux étaient diagnostiqués comme ayant succombé à l’œdème porcin. Les lésions histopathologiques remarquées dans le poumon et les tissus du cerveau étaient caractéristiques d’infections virales. Par conséquent, il a été conclu que l’œdème porcin décrit dans cet article pourrait être lié aussi bien à un agent infectieux qu’à l’entérotoxémie.

Summary

Twenty-one pigs of the large white breed, aged between 2 and 3 months from ten different farms were examined at post mortem. The consistent clinical history was sudden death of health pigs after weaning (8-14 days). The main macroscopic findings were subcutaneous oedema of the head, submucosa of the stomach wall, mesentery of the spiral colon and the brain and the meninges characterised by cloudy appearance, lung congestion, oedema, consolidations and collapse. Microscopically, the main lesions observed in all animals were oedema in the predilection sites, interstitial pneumonia of varying degrees of severity and non-suppurative ventricular encephalitis. On the basis of the clinical history and the gross and histopathological findings, the animals were diagnosed to have died of oedema disease of swine. The histopathological lesions observed in the lung and the brain tissues were characteristic of viral infections. Consequently it was concluded that oedema disease of swine described in this communication could have been associated with both an infectious agent and enterotoxaemia.

Introduction

Oedema disease of swine is an acute commonly but not invariably fatal disease of pigs aged between 8-14 weeks1,2,3. Although it has been considered to be an enterotoxaemia associated with some serotypes of Escherichia coli (mainly 0138, 0139 and 0141), its etiopathogenesis is still not completely understood1,3. Clinically it is characterised by sudden death or

* Corresponding author
development of nervous signs\textsuperscript{1,2,3,4,5,6,7}.

The major neurological signs that are observed in the affected pigs include incoordination, tremors, paralysis and convulsions, in addition to these nervous signs some pigs may have dyspnoea. In pigs, in which clinical signs are observed, death commonly occurs within a number of hours or a day or two\textsuperscript{1,2,3,4,5,6,7}. At necropsy, many pigs have no gross lesions; however, a few may show subcutaneous oedema of the head, and abdomen among other places. In such cases there may be excessive amount of fluid, as well as oedema of the stomach wall and mesentery of the spiral colon. The main microscopic lesions consist of oedema in the predilection sites, necrotizing arteritis (angiopathy) and encephalomalacia\textsuperscript{1,2,3,4,5}.

The purpose of this paper is to report the occurrence of interstitial pneumonia and non-supportive ventricular encephalitis in pigs with oedema disease of swine and to discuss their significance in relation to the aetiology, pathology and clinical signs of the disease.

Materials and Methods

The pigs described here originated from ten farms near the Faculty of Veterinary Medicine, University of Nairobi, Kenya. They were of the large white breed and included both males and females. Generally they had been fed on commercially prepared feeds from different manufacturers. The animals were brought to the post mortem facility (Department of Veterinary Pathology and Microbiology) at different times, either dead or in extremis whereby they were sacrificed. During autopsy, each animal was systematically examined and any morphological changes noted. Tissues for histopathology were collected from the lungs, brain, kidneys, liver and the stomach wall. The collected tissues were fixed in 10% formalin solution, processed routinely, sectioned at 6\(\mu\) thickness and stained with haematoxylin and eosin. The diagnosis of oedema disease in these cases was based on the clinical and epidemiological history and/or clinical signs and gross lesions.

Results

Clinical Features

On the bases of clinical history, 45 piglets aged 2-3 months, from a population of 529 were suspected to have died of oedema disease. The case history indicated that the morbidity and mortality rates of the disease ranged from 1-100\% and 86-100\%, respectively. Seven out of the 45 piglets were reported to have died suddenly without any clinical signs being observed. In the piglets that were seen alive, death occurred within two days. The clinical signs observed in these piglets included a combination of the following: dullness, anorexia, weakness, shivering, fever, dyspnoea, squealing, ataxia, staggering, difficult in walking, incoordination, lameness, inability to stand, recumbency, paralysis, convulsions and peddling movements. Bacterial examination of the colonic and rectal contents of two pigs with typical symptoms that had died earlier had yielded pure cultures of haemolytic \textit{Escherichia coli}. However, it was not possible to type the organism.

Pathological Findings

Macroscopic Findings

A total of 21 piglets all in good body condition were examined at post-mortem. In some of the piglets there were no gross lesions encountered. When the gross lesions were observed they varied from case to case. They were either one or combinations of the following; subcutaneous oedema of the head region\textsuperscript{3};
ventral abdomen\(^1\); legs\(^1\); lateral sides of the body\(^1\); oedema of stomach wall\(^3\); oedema of the mesentery of the spiral colon\(^13\); effusions in the body cavities\(^5\); brain oedema and congestion\(^3\); lung congestion\(^1\); lung oedema\(^10\); lung consolidations\(^5\) and lung collapse\(^19\). The consolidation of the lungs occurred either in patches or affected large areas of the lungs. The lung collapse also occurred in patches spread over the entire lung lobes or both lungs were completely collapsed.

**Histopathological Findings**

In all the affected pigs there was alveolar collapse and alveolar wall thickening of varying degrees of severity. The alveolar wall thickening was attributed to mononuclear cell infiltration. In some cases, some areas showed marked interstitial reaction (i.e. interstitial pneumonia). Air passages especially those without cartilages were either completely or partially collapsed. In all cases there was no exudate in the alveoli or the lumen of the air passages (Figs 1 and 2). In some cases there was perivascular and peribronchial lymphoid proliferations. In the brain, there were areas of mononuclear cell infiltration. The inflammatory reaction started beneath the ependyma and spread outwards destroying the brain tissue (ependymitis or ventricular encephalitis) (Figs 3, 4 and 5). In other cases, the blood vessels were engorged and the perivascular spaces dilated. The areas around the neurones were also dilated. In all the cases, the choroid plexuses, the meninges and the blood vessel walls were not involved.

Other changes observed were mild to moderate degenerative changes in the liver and the kidneys, as well as oedema in the predilection sites. In addition to oedema, the stomach and the colonic walls showed marked dilatation of the lymphatic vessels, some of which also contained fibrin clots in the lumens.

**Discussion**

The pigs described in this communication were all young (2-3 months) and in good body condition. Some of them had died suddenly without clinical signs being observed. When seen alive, the clinical signs observed were mainly neurological, although some also had dyspnoea and/or fever. The morbidity and mortality rates were variable. Such features have previously been described in association with oedema disease of swine\(^1\,^2\,^3\,^4\,^5\,^6\,^7\).

At necropsy, some pigs had no gross lesions; others showed only lung collapse and/or pneumonia as the only abnormalities. Besides these, other piglets showed one or more of the following changes: subcutaneous oedema in various sites, oedema of the stomach wall and mesentery of the spiral colon and effusions in the pleural, peritoneal and pericardial cavities. Except for lung collapse and pneumonia, the lesions were typical of oedema disease of swine in accordance with the descriptions given by various authors\(^1\,^2\,^3\,^4\,^5\,^6\,^7\,^8\,^9\,^10\,^11\,^13\). The lung collapse (which in some cases accompanied by consolidations and/or oedema) resembled the lung collapse observed in animals dying of an overdose of general anaesthetics such as pentobarbitone sodium. In such cases, death occurs as a result of paralysis of the respiratory centre in the brain\(^14\).

When examined microscopically, the lungs showed alveolar collapse and interstitial pneumonia of varying degrees of severity (Figs. 1 and 2).

On the basis of clinical, epidemiological and pathological features, the present authors were dealing with oedema disease of swine. Although the etiopathogenesis of oedema disease is still not completely understood it is commonly considered to be an enterotoxaemia associated with some serotypes of haemolytic *Escherichia*
Fig. 1: Lung from pig No. 96 suffering from oedema disease showing moderate alveolar collapse and alveolar wall thickening (right hand side). Some alveoli are emphysematous (left had sie-empty spaces) HE X 100.

Fig. 2: Lung from pig No. 73 suffering from oedema disease showing, consolidations due to interstitial pneumonia (dark areas). Some alveoli have become emphysematous (irregular clear spaces). Note lack of exudate in the alveoli and air passages (A). HE X 100.
Fig. 3: Brain from pig No. 96 suffering from oedema disease showing cellular infiltration (i) beneath the ependyma (e). HE X 100

Fig. 4: Brain from pig No. 96 suffering from oedema disease showing, inflammatory reaction (i) beneath the ependyma (e) and spreading outwards to affect the brain tissue. HE X 100.

Fig. 5: Brain from pig No. 96 suffering from oedema disease showing, mononuclear cell infiltration beneath the ependyma. HE X 400.
coli. However, although the toxaemia theory is still widely accepted, a review of the literature together with the findings of this report, indicate that other agent(s) may also be involved in the pathogenesis of the oedema disease of pigs. In this regard, some investigators have suggested a possible involvement of an infectious agent in the pathogenesis of oedema disease.

The main microscopic changes that are observed in the oedema disease of swine are generally degenerative in nature are basically angiopathy and encephalomalacia. However, in our study the histological changes observed in the brain and the lungs tissues of the affected and characteristic of viral infections. The encephalitis, however, appeared to differ from other types of porcine encephalitides in terms of distribution of the lesions in the brain. Except for lack of demyelination and the non-involvement of the spinal cord, the distribution of the inflammatory lesions was similar to that of Visna in sheep, i.e. beneath the ependyma. Kurtz and others also described similar lesions, but unfortunately they did not consider them of much significance. Nieberle and Cohrs also mention occurrence of small glial infiltrates, but they too, did not attach any significance to them. Unlike Nieberle and Cohrs and Kurtz and associates we consider the encephalitis observed significant. Therefore, it is our opinion that the encephalitis was responsible for the neurological signs and the dyspnea observed clinically in the affected pigs.

The observations made by Underdahl and others tend to suggest that, the causal agent of oedema disease is to be found in higher concentrations in the blood and lung tissue than in the intestinal contents. This seems to support our observations that all pigs we examined had some degree of interstitial pneumonia. The results of this study seem to suggest that, the causal agent of oedema disease of swine is more likely to be an infectious agent (possibly a virus) in addition to the E. coli enterotoxaemia. Therefore, more work is needed to elucidate further the aetiology, pathogenesis, pathology as well as the epidemiology of the oedema disease of swine in Kenya. This is fundamental as the incidence of suspected cases of oedema disease of swine is increasing in Kenya and hence becoming a limiting factor in pig production.

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Oedema Disease of Swine: A Toxaemia or an Infectious Disease?


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The researchers wish to thank other members of the Department of Clinical Nutrition and Development who presented the talks on post harvest organization. The technical as well as the secretariat assistance given to the members of the Department of Veterinary Pathology and Microbiology is highly appreciated.

The authors thank the staff of the Department of Clinical Nutrition and Development who presented the talks on post harvest organization. The technical as well as the secretariat assistance given to the members of the Department of Veterinary Pathology and Microbiology is highly appreciated.
SHORT COMMUNICATION

AN ASSESSMENT OF THE PREFERENCE OF MUCUNA PRURIENS VAR COCHINCHINENSIS, MUCUNA PRURIENS VAR UTILIS AND GLIRICIDIA SEPIMUM BY SHEEP AT NYANKPALA IN THE MOIST GUINEA SAVANNA AGROECOLOGY.

F. AVORNYO*, N. KARBO and A. ADDO-KWAFO

Animal Research Institute, P.O. Box 52, Nyankpala, Tamale, Ghana

A preference study was undertaken with Mucuna pruriens var cochinensis, Mucuna pruriens var utilis and Gliricidia sepimum using eight sheep from The Animal Research Institute, Nyankpala. The average age and weight of the sheep were 7 months and 15 kg, respectively. The sheep were each assigned to a pen and a kilo each, fresh weight, of the test feeds were hung separately in every pen. Water was available to the animals throughout the trial. Consumption of the feeds by individual sheep was monitored from 9:00 a.m., and the amounts consumed by each animal were measured after 15 min, 30 min, 1 hour and 2 hour, each day. The procedure was repeated on the next two days. A randomized complete block design was used to test differences between the amounts of the different feeds consumed (treatment) after accounting for the variation due to day (block). Coefficient of preference (COP) was calculated for each feed as:

\[
\text{COP} = \frac{\text{amount of feed consumed}}{\text{average amount of all feeds consumed}}
\]

On the first day, no feed was consumed within 15 min. Data collected after 1 hour revealed that a significantly higher quantity of the Gliricidia had been consumed compared with the Mucuna, with the difference between Gliricidia and Mucuna pruriens var cochinensis achieving statistical significance (P<0.05). Data collected at 2 hours showed that preference was now for the two varieties of Mucuna. At the end of the observations on day one, Gliricidia sepium and Mucuna pruriens var cochinensis emerged the preferred feeds (Table 1). On the second day, no feed was consumed in the first 30 min. Throughout the observation period, Gliricidia was not eaten. At 2 hour significantly higher amounts of the Mucuna had been eaten (P<0.05) when compared with Gliricidia. On the third day, more Mucuna was consumed than Gliricidia with the difference in the consumption of Mucuna pruriens var utilis and Gliricidia sepimum attaining statistical significance after 30 min of observation (P<0.05). This order was maintained after 2 hours.

Significantly more feed (P<0.05) was consumed on day three compared with day 1 or with day 2 (Table 1). A significant interaction between the type of feed most consumed and the day of observation was noted (P<0.05). The overall COP values were 1.36, 1.18 and 0.45 for Mucuna pruriens var cochinensis, Mucuna pruriens var utilis and Gliricidia sepimum, in this order.

The animals displayed preference for Gliricidia on the first day probably because they were familiar with Gliricidia. Gliricidia, however, was not palatable hence the sheep explored other available feeds1. On the second day, preference was no longer for

* Corresponding author
Gliricidia but for Mucuna, with Mucuna pruriens var utilis slightly preferred to Mucuna pruriens var cochinchinesis. This may be indicating that animals will shift from a known to a new feed gradually.

Other trials have also indicated low intakes of Gliricidia sepium\(^2\) probably because the plant has a repulsive odour due to the presence of coumarin\(^4\) and other photochemicals\(^5\) in the leaves and stems. The study, however, has shown that consumption of Gliricidia by sheep at the Animal Research Institute, Nyankpala may have increased over the years since the data on Gliricidia consumption from this study (87.5 after 2 hours of observation) is higher when compared with similar data (0 g after 3 hours of observations\(^9\)). The consumption of Mucuna by sheep was at levels (Table 1) comparable to that reported for Cajanus cajan (225 g after 3 hours), which was considered the most preferred by sheep, among four species\(^2\).

**Acknowledgements**

The authors wish to acknowledge IITA for the collaboration with CCROPNET. We are also indebted to Mr. Adam Mohammed who helped with the collection of data.

<table>
<thead>
<tr>
<th>Gliricidia</th>
<th>Mucuna</th>
<th>Mucuna</th>
<th>Average for day</th>
</tr>
</thead>
<tbody>
<tr>
<td>sepium</td>
<td>pruriens var</td>
<td>pruriens var</td>
<td>cochinchinesis</td>
</tr>
<tr>
<td>Day 1</td>
<td>162.5</td>
<td>150</td>
<td>112.5</td>
</tr>
<tr>
<td>Day 2</td>
<td>0(^b)</td>
<td>175(^a)</td>
<td>262.5</td>
</tr>
<tr>
<td>Day 3</td>
<td>100(^b)</td>
<td>450(^a)</td>
<td>300(^a)</td>
</tr>
<tr>
<td>Average for feed</td>
<td>87.5(^a)</td>
<td>258.3(^a)</td>
<td>225(^a)</td>
</tr>
<tr>
<td>COP at end of experiment</td>
<td>0.45</td>
<td>1.36</td>
<td>1.18</td>
</tr>
</tbody>
</table>

\(^a,b\) Values with different superscript letters in the same row but excluding the last column are statistically different, \((P \leq 0.05)\) values with \((P \leq 0.05)\) different superscript letters in the last column are statistically different; COP = coefficient of preference.
Received for publication on 20th August, 2001.

References


Dematiaceous fungi include a large group of organisms grouped together primarily on the basis of their dark pigmentation. In dark brown, olivaceous or black. In most cases, the pigment is melanin, specifically dihydroxyphenylalanine meloid (CHN) and appears to be common in many medically important dematiaceous fungi. The melanin is present in the outer walls of the hyphal and sexual states of these fungi.

A number of opportunistic fungal infections proved to be dematiaceous fungi that were previously known only to be agents of plant diseases or soil-woody matter saprophytes and were not considered to be potentially pathogenic to man or lower animals. Dematiaceous fungi are widely distributed in nature and mycotic infections caused by these fungi have been reported from all parts of the world. They are more frequently found in tropical and subtropical regions. One dematiaceous fungus, Phaeophlockia gregata, was isolated from soybean cyst nematode Heterodera glycines. Infections caused by these fungi include chronic mycosis, phaeohyphomycosis, mycoses, mycotoxicosis, eczematoid and keratomycoses. Infection is said to be by traumatic implantation of the fungi from the environment.

Materials and Methods

Four urban areas belonging to four different local government areas of Anambra State, South eastern Nigeria...
The study was conducted in the Department of Animal Research, University of(X), where the animals were housed under standard conditions. The study was designed to assess the effect of different diets on the performance of dairy cattle. The results showed that the diet containing corn had a higher average daily gain (ADG) than the diet containing soybean meal (SBM). The average ADG for the corn diet was 3.5 kg/day, while the ADG for the SBM diet was 2.8 kg/day. The data were analyzed using a two-way ANOVA, and the results indicated a significant difference between the two diets (P<0.05).

Table 1: The mean daily gains of dairy cattle on different diets and the coefficient of performance.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mean ADG (kg/day)</th>
<th>Coefficient of Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>3.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>2.8</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Acknowledgements

The authors wish to acknowledge the KWA Research Council for providing the funds for this study. We also thank Mr. Adam Mohammed, who helped with the collection of data.
SHORT COMMUNICATION

OCCURRENCE OF DEMATIACEOUS FUNGI ISOLATED FROM CAT DROPPINGS IN ANAMBRA STATE, NIGERIA.

UMEH, C.N.* ODIPBO, F.J.C. and OKEKE, E.M.

Department of Applied Microbiology and Brewing Nnamdi Azikiwe University P.M. 5025, Awka, Nigeria.

Dematiaceous fungi include a large group of organisms grouped together primarily on the basis of their dark pigmentation-light to dark brown, olivaceous or black. In most cases, the pigment is melanin, specifically dihydroxynaphthalene melanin (DHN) and appears to be common to many medically important dematiaceous fungi[]. The melanin is present in the cell walls of the hyphae and/or conidia of these fungi.

A number of aetiologic agents of opportunistic fungal infections proved to be dematiaceous fungi that were previously known only to be agents of plant diseases or soil woody matter saprophytes and were not considered to be potentially pathogenic to man or lower animals[]. Dematiaceous fungi are widely distributed in nature and mycotic infections caused by them have been reported from all parts of the world even though they are more frequently found in tropical and subtropical regions. One dematiaceous fungus, *Phialophora gretata*, was isolated from soybean cyst nematode *Heteroderaglycines*. Infections caused by these fungi include chromomycosis, phacochyphomycosis, mycetoma, onychomycosis oculomycosis and keratomycosis. Infection is said to be by traumatic implantation of the fungi from the environment.

Pathogenic dematiaceous fungi infections have been reported in lower animals[]. Okeke and Gugnani[] reported isolation of *Exophiala jeanselmei* from bat guano and the isolates were found to be pathogenic to laboratory mice. The organism *Wangiella dermatitidis* has also been isolated from the liver of bats showing no macroscopic lesion[]. The dematiaceous fungi *Exophiala spinifera*, the organism responsible for phacochyphomycosis has been isolated from a cutaneous lesion on the paw of a male domestic short hair cat and from nasal exudate and abscess contents of female short hair cat[]. *Xylohyphabantiana* has also been reported from a cat where they cause cerebral phacochyphomycosis[].

Cats are kept as pets in many homes and any disease conditions limit the appreciation of these cats as pets and for other values for which they are kept. There have been reported cases of mycotic infections in animals such as the cats and therefore it is necessary to understand the relationship between cats and dematiaceous fungi. The present study was initiated to investigate dematiaceous fungi as they occur in cats.

**Materials and Methods**

Four urban areas belonging to four different local government areas of Anambra State, south eastern Nigeria

---

* Corresponding author: E-mail: cnumehe@infoweb.abs.net
Figure 1: Dematiaceous fungi isolated from different areas of Anambra State

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples</th>
<th>Total No. of positive samples</th>
<th>Cladosporium sp</th>
<th>Alternaria sp</th>
<th>Exophiala werneckii</th>
<th>Exophiala spinifera</th>
<th>Fonseca pedrosol</th>
<th>Curvularia sp</th>
<th>Phialophora parasitica</th>
<th>Phaeococymyces exophilale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awka</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nafinia</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nawgu</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ebenebe</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>35</strong></td>
<td><strong>11</strong></td>
<td><strong>4</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

Cat droppings (1-3g) were obtained from a total of 35 cats. Samples were collected from the cats by the researcher immediately they were voided to avoid possible contamination. Samples were carefully labelled transported to the laboratory and processed within the hour. Each sample was placed in a sterile test-tube and 9ml of sterile water added to it. The samples were processed according to the method of Umeh and Anekwe 1998.

The identification of fungi was based on the morphological characteristics and certain physiological attributes. Colony morphology was studied in subcultures on Sabouraud dextrose agar and microscopic characters i.e. hyphal morphology and sporulation pattern were studied in lactophenol mounts prepared from slide cultures. Physiological and biochemical tests via growth at 37°C and inability to hydrolyze 12% gelatin were carried out according to Espinel-Ingroff et al.
Results and Discussion

Out of the 35 samples of cat droppings examined, dematiaceous fungi were isolated from different locations. These included Cladosporium sp 4 isolates and one isolate each for Alternaria sp, Exophiala werneckii, Exophiala spinifera, Fonsecaea pedrosoi, Curvularia sp, Phialophora parasitica and Phaeococcomyces exophilaei. The dematiaceous fungi isolated from the various locations were not evenly distributed both in species and frequency of occurrence (Table 1).

Cladosporium (2 species) were recovered from samples collected from Nawgu and one each from Awka and Nawfia. Phialophora parasitica and Phaeococcomyces exophilaei were isolated from Nawfia. Fonsecaea pedrosoi and Curvularia sp were isolated out of the 5 samples collected from Nawgu and Exophiala spinifera from Awka.

From the results of this study, dematiaceous fungi appear to be commoner in cat droppings than in goat droppings as has previously been investigated by Umeh and Anekwe. Some of the dematiaceous fungi may have come from the materials which the cats fed on like palm kernel chaff and vegetable matter which are good sources of dematiaceous fungi or the occurrence of the fungi might be due to contact with soil or plant debris which are also sources of dematiaceous fungi. It will be interesting to call attention of the occurrence of these fungi in cats since there are reported cases of pathogenic dematiaceous fungi in cats. These fungi as well as others have been implicated in difficult medical conditions when they enter animal and human tissues. Little information can be obtained in Nigeria concerning human infections from these fungi. However, Ajello et al., had confirmed these fungi as agents of mycotic infections in man and lower animals. Cats have long been regarded as 'man's best friend' and therefore it can be said that the infection of cats will ultimately affect man. Since these fungi had been isolated from cats and they have been shown to produce human mycotic infections, then it can be said that there is a potential risk of man getting infected through his infected pet.

References


Received for publication on 21st March, 2001.
SHORT COMMUNICATION

LEFT ABOMASAL DISPLACEMENT AND INDIGESTION FOLLOWING WIRE NAIL INGESTION IN A ZERO-GRAZED FRIESIAN COW

E.S. BIZIMENYERA1  MK SAIMO2  P. NSAMBA1  J. KABAGAMBE and A. SENGGOOBA2.

1Department of Veterinary Medicine,  2Department of Anatomy, Faculty of Veterinary Medicine, Makerere University, Box 7062, Kampala.

Livestock management changes in Uganda have increased the incidence of 'diseases of intensification' such as traumatic reticuloperitonitis, displacement of the abomasum, mastitis, grain overload, overgrown hooves, polyethylene ingestion and tuberculosis. In peri-urban areas of Kampala city, dairy cattle are kept on a zero-grazing system. This is an intensive management system especially popular with women farmers where a few cows (1-10) are housed and fed cut herbage supplemented with domestic raw crop residues, and commercial dairy meal or spent brewers' grain. Banana (Matooke) skins and peelings of cassava and sweet potatoes (all common staple foodstuffs), are collected from households, restaurants, schools, markets or waste garbage heaps and fed to cows. During collection of these feed by-products and crop residues, foreign objects such as metals and fish bones are also carried along to the feeding troughs1,2,3, where they are eventually ingested by the cows. Most of the ingested objects especially heavy or metallic pieces are retained in the reticulum and often penetrate that organ resulting to reticulitis, peritonitis or indigestion4,5.

An adult, multiparous Friesian cow raised on a zero-grazing dairy cattle unit near Kampala was presented with anorexia and a sudden drop in milk yield (from eight litres to zero) within 12 hours. The owner had fed her cows banana skins (peelings), nappier grass and brewers grains the previous evening. The four other adult Friesian cows in the unit were clinically normal. On clinical examination, the cow was dull, depressed, staring at the feed and water and had a normal temperature but rapid pulse. The cow was grinding teeth and pinching of the withers elicited a grunt. There was complete ruminal stasis and palpation through the paralumbar fossa revealed a slightly distended firm and doughy rumen. Faeces were scanty, slightly pasty with grain particles and malodourous. On percussion and auscultation of the left flank, ping sounds were heard indicative of abomasal displacement. When a metal detector (Vet-Tecâ) was applied on the xiphoid area, a continuous ringing sound was emitted, suggesting the presence of a foreign metallic object in the reticulum. The breath had aromatic odour typical of carbohydrate engorgement. A tentative diagnosis of left abomasal displacement, traumatic peritonitis complicated with grain engorgement was made.

A left paralumbar laparotomy was
performed on the standing animal under regional anaesthesia through a paravertebral nerve block (T13, L1 and L2) using lignocaine HCl. On abdominal exploration a left abomasal displacement was confirmed with the organ squeezed between the left abdominal wall and the lower left side of the rumen. The rumen was lifted up and the abomasum manipulated by pushing it downward and exteriorising it through the laparotomy wound so as to expose its parietal surface. An abomasopexy using a one metre long non absorbable No 2 suture (Supramid®, Burns Veterinary Supply) was used to fix the abomasum to the ventral abdominal wall. One suture was fixed in the abomasal serosa. One end of the suture material was threaded to an S-shaped needle and carefully manipulated down through the ventral abdominal wall while pushing down the abomasum to its normal position. The other end of the suture was similarly threaded and manipulated down to go through the ventral abdominal wall close to the first one while holding on and gently pulling the two ends of the sutures, the abomasum was placed in its normal position on the ventral abdominal wall to the right. The sutures were tied and fixed in a surgeon's note at the the skin on the ventral abdominal wall.

A rumenotomy was performed and the rumen was sutured around the laparotomy skin incision to minimise leakage of the contents into the peritoneum. The rumen was found to be impacted with doughy aromatic smelling contents with brewer's grains. Half of the contents were evacuated and 500g sodium bi-carbonate in 2 litres of water were poured into the rumen. Following a manual exploration of the reticulum, a 10cm (4 inches)-wire nail was pulled out of the reticulum wall where it had stuck after perforating the organ. The ruminal wall was closed routinely using catgut no.2 (Kruuse®️️) in a continuous pattern and the abdominal wall closed in three layers using catgut no.2 (Kruuse®️️) and no.3 (Supramid®, Burns Veterinary Supply) for the skin in a horizontal mattress pattern. The postoperative treatment consisted of 15mg/kg penicillin-streptomycin injectable (PenStrep®, Norbrook Laboratories) and oxytetracycline-gentian violet wound spray (Alamycin aerosalá, Norbrook Laboratories), all repeated for 4 days. Indigestion powder (Brema digest®️️) was given and 5 litres of ruminal fluids from a donor cow administered by stomach tube the next day. Skin stitches were removed 10 days postoperative by which time the milk production had stabilized to 8 liters per milking.

This was a difficult case in terms of both differential diagnosis and treatment. Although many cases of abomasal displacement occur around parturition (either a few days before or few weeks after), this cow was nursing a three-month-old calf. The displacement must have been acute, as the displaced organ did not contain much fluid and gas, and so was easily manipulated back into its normal position. Abomasal drainage was not necessary in the reported case. In many cases of left abomasal displacement seen by the authors, the affected animals maintained selective feeding preferring grass to grain or concentrates but the reported case had complete anorexia. It has been observed that when dairy cows get access to dairy meal or any concentrate, they may engorge themselves to death. Grain tends to increase peristaltic bowel movements, but later atony develops due to lactic acid accumulation. Ruminal atony leads to the impaction and delayed emptying of the organ. This case of left abomasal displacement was possibly triggered by the grain engorgement and the ingested wire nail. This case could be a rare incident of
left abomasal displacement associated with grain engorgement and wire nail ingestion and was definitely the first such documented report in Uganda. The wire nail that was removed from the reticulum was still rusty meaning it had been freshly ingested. The authors’ experiences show that nails that have been exposed to ruminal fluids for longer periods appear shiny and smooth. It would appear that the nail was possibly ingested with the banana skins (peelings). The brewers grains led to increased peristalsis that could have caused the nail to perforate the reticulum. The pain and discomfort following the reticular perforation and the grain engorgement could have precipitated the left abomasal displacement.

The authors’ recommend that farmers in Uganda recognize the dangers posed by intensification in dairy husbandry practices. Crop residues like banana skins (peelings) should be carefully sorted out to remove any foreign objects before being fed to cattle. Alternatively rumen magnets could be administered to the cows as a preventive measure since they have been found to be useful. However, rumen magnets are largely unknown to Uganda’s farming community at the moment.

Acknowledgements

The authors thank the clinicians and laboratory staff of the Ambulatory Clinic of Faculty of Veterinary Medicine, Makerere University.

References:


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A ventral midline incision was made in the abdominal wall. The rumen was opened and the ruminal contents were removed. The contents were then spread out and examined for any foreign material. The contents were then returned to the rumen and the incision was closed in layers. The animal was allowed to recover and was observed for any signs of discomfort or pain. The incision site was cleaned and dressed with an antiseptic solution. The animal was then sent back to the farm to continue its normal activities.
SHORT COMMUNICATION

CLINICAL FEATURES OF NATURALLY ACQUIRED DOG RABIES IN SOUTH WEST NIGERIA

O.A. OLAWALE, C.A AGUNLOYE

Faculty of Veterinary Medicine
University of Ibadan, Ibadan 200213 Nigeria.

There is need for public enlightenment on rabies as a zoonosis in Nigeria and proper legislation on dog control and vaccination.

Rabies is an acute neurotropic disease of man and other warm blooded animals particularly carnivores, felids and some bats. Dogs are the major reservoirs of rabies in Nigeria\(^1\). The disease is invariably fatal in clinically affected animals. In humans, early post-exposure vaccination is associated with a high survival rate\(^4\).

In some parts of the world where there is intensive vaccination of dogs and control of stray dogs, the disease is minimal in human populations.

In Nigeria, previous reports have shown that dog rabies is highly prevalent\(^1\) and campaigns to control the disease have not received adequate support from individuals and government (Adeyemi, G.A. personal communication). Also there are indications that the disease is on the increase\(^2\).

This paper presents various clinical and epidemiological findings in 21 cases of dog rabies presented at veterinary clinics in 3 major towns in south west Nigeria between 1993 and 1998.

A questionnaire survey of dog rabies was carried out in three towns in south west Nigeria, namely Lagos, Abeokuta and Ibadan over a five-year period (1993-1998). The Information was collected from 21 veterinary clinics located in these three towns.

The questionnaire was structured into two sections. Section A dealt with general information such as routine preventive measures against rabies and actions taken on suspecting rabies cases. Section B dealt with specific information on dog rabies cases, including breed, sex and age of rabid cases, major clinical, laboratory and epidemiological findings, vaccination record and actions taken by clinician -in-charge. Cases lost to follow-up (10 cases) were excluded from the study.

The data collected were analysed using proportions. Suspected cases of rabies were confined by veterinary clinics. Diagnosis was based on clinical signs of rabies in affected dogs, death of suspected case within 10 days after showing clinical signs and the presence of Negri bodies in brain smears at post mortem or animal inoculation.

Based on the above criteria, 21 dogs were confirmed rabid during the survey period (1993-1998) in the three towns. Fig 1 presents the sex distribution of the cases of rabies. In all, more male dogs 14/21 (66.7%) were diagnosed as rabid. Using clinical signs at presentation, thirteen (61.9%) were classified as the dumb / paralytic form of rabies. Twelve (57.1%) of the dogs were reported to roam freely and 16/21 (76.2%) were household pets.

* Corresponding author
Majority of rabid dogs 13/21 (61.9%) were more than one year in age (Table 1). Only one of the cases was reported to have been vaccinated against rabies in previous twelve-month period. All but one of the dogs was of the local mixed breed of mongrel dog. A total of 6,796 dogs were reported to have been vaccinated against rabies in the three towns.

There is clinical evidence of rabies in the population of dogs surveyed with 21 cases confirmed as rabid. This finding is similar to those previously reported\(^2\),\(^3\) which indicate that dog rabies predominates in Nigeria. In countries with intensive animal rabies and stray dog control, dog transmitted rabies has been reduced to the bearest minimum\(^4\),\(^6\). The reasons why more cases of rabies\(^10\) were reported in Ibadan is unclear but it may have been because it is the only town with a Veterinary School and diagnostic facilities for rabies among the three towns surveyed and so there is improved diagnosis.

Although natural resistance to rabies is uncommon in dogs\(^7\), a large proportion of the rabid dogs in this study were over one year in age. This consistent with the epidemiology of the infection, since maternally derived immunity would have been on the wane by this time. Similarly, some workers\(^8\) have reported more cases of rabies in dogs six months and above, stressing the need to vaccinated dogs early so as to prevent rabies. The 21 cases of rabies reported from this study may be an underestimation since some cases were lost to follow-up.

In this study, a high proportion of the dogs were reported to roam freely without being confined thereby becoming a source of infection to other animals. Generally, dogs in low income areas in Nigeria roam freely and absence of enabling laws in Nigeria may be a contributing factor to the endemicity of rabies in Nigeria.

Predominantly more male dogs were

**Figure 1:** Sex distribution of rabies cases in survey towns.
Table 1: Characteristics of Dog Rabies cases in three towns in South West Nigeria.

<table>
<thead>
<tr>
<th>Town/Location</th>
<th>Age (Yrs)</th>
<th>Use</th>
<th>Methods of Keeping</th>
<th>Type of Rabies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1</td>
<td>&gt;1</td>
<td>Pet</td>
<td>Guard</td>
</tr>
<tr>
<td>LAGOS</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>ABEOKUTA</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>IBADAN</td>
<td>7</td>
<td>3</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL (%) 8(38.1) 13(61.91) 16(76.19) 5(23.81) 9(42.86) 12(57.14) 8(38.10) 13(61.91)

* Based on clinical signs

** Semin - stray dogs move around without restraint.

reported rabid in this study. This might be related to their roaming, mating behaviour or frequent fights with other dogs\(^7,8\). Rabies is traditionally transmitted by contact with infective saliva\(^7,9\) although recently there have been reports of human rabies without a bite exposure\(^4\). The use of many of the dogs as household pets may pose a human health risk to rabies.

The higher number of paralytic form of rabies may reflect the terminal stage of the illness\(^7\). Also dogs in the furious form of rabies tend to roam\(^7\) and may get lost if they move far away from home. Most Nigerians recognise the furious form of rabies as reflected in local languages\(^3\).

There is urgent need for an enabled legislative enforcement of dog control laws and campaigns on dog vaccination as dogs are the natural host of rabies in Nigeria and most developing countries\(^9\).

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We are grateful to the participating Veterinarians and dog owner for their cooperation.

References

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BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

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Objet
Le Bulletin de la Santé et de la Production animales en Afrique contient des articles de recherches originales traitant d'activités en matière de santé et de production animales visant à assurer le développement de l'industrie animale et une meilleure utilisation des ressources du bétail en Afrique. Le Bulletin est un périodique trimestriel.

Présentation des articles
Deux exemplaires des articles doivent être adressés à Monsieur le Rédacteur en Chef, Bulletin de la Santé et de la Production Animales en Afrique, Organisation de l'Unité Africaine/Bureau interafrique des Ressources animales, P.O. Box 30786, Nairobi, Kenya.


Un article ne peut être soumis pour publication que s'il n'a pas encore été proposé ailleurs; il fera l'objet de quelques modifications par le Comité de Rédaction.

Genres d'articles publiés dans le Bulletin
- des communications originales.
- des brèves communications.
- analyse des articles proposés par le Rédacteur.
- des éditoriaux.
- le courrier des lecteurs.
- analyse d'ouvrages.
- informations et annonces.

Format des articles
Les manuscrits doivent respecter les conditions suivantes: Le titre doit être concis et ne pas dépasser plus de 15 mots, il est suivi du (des) nom(s) de l'auteur (ou des auteurs) et des établissements où le travail a été effectué, ainsi que de l'adresse pour les correspondances si elle n'est pas la même.

Le résumé ne doit pas dépasser 200 mots. Son texte bref et concis comprendra les principaux résultats et la (les) conclusion(s) de l'étude.

L'introduction expose le but de la recherche.

Le matériel et les méthodes utilisés.

Les résultats présentés brièvement.

Un débat sur l'importance de l'article.

Remerciements éventuels.

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Le nom de l'auteur (ou des auteurs) suivi des initiales du ou des prénoms, l'année de parution (entre parenthèses), l'abréviation du titre du périodique suivant la "World List of Scientific Periodicals" (souligné), le numéro de la première page. Le titre de l'article ne doit pas être inclus.

2. Revue
Le nom de l'auteur (ou des auteurs) suivi des initiales du ou des prénoms, l'année de parution (entre parenthèses), le titre exact (souligné), la ville où elle a été publiée, les éditeurs, le numéro de la première page.

3. Rapport annuel
Le nom du pays, l'année faisant l'objet du rapport, puis le nom du service ou de l'organisation, le numéro de la première page.

Si le même auteur est cité plus d'une fois, ses publications seront indiquées dans l'ordre chronologique dans la liste bibliographique et s'il y a plus d'une publication, les lettres "a, b, c," seront ajoutées aussi bien dans la liste bibliographique que dans le texte.

Illustrations
Les tableaux et les titres doivent être en nombre aussi réduit que possible. Un tableau d'une trop grande dimension est difficile à lire même s'il peut être reproduit. Les tableaux et les figures doivent être numérotés dans l'ordre, respectivement Tableau 1, etc., ou Fig. 1 etc. et joints à la fin du texte. Les références aux tableaux et aux figures dans le texte doivent être numérotées et non pas indiquées "tableau ci-dessous" ou "figure ci-dessous". Les illustrations en couleurs ne sont reproduites qu'aux frais de l'auteur (ou des auteurs).

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