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COMPARATIVE EFFICACY OF NILZAN PLUS®, WORMICIDE PLUS®, VERMITAN® AND IVOMEC® AGAINST GOAT NEMATODES

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Introduction

Gastrointestinal nematode infections due to Haemonchus, Oesophagostomum, Trichostrongylus and Bunostomum species have been demonstrated to be widespread in goats in Uganda and are associated with huge production losses¹. Chemoprophylaxis is the most available and most used method for controlling nematode infections in goats in Uganda. However, presence of anthelmintic resistant nematode populations or use of fake drugs could hamper the effectiveness of anthelmintics. Emergence of anthelmintic resistance has been reported in the neighbouring Kenya²,³,⁴,⁵. There is growing evidence in Uganda, though limited, suggestive of emergence of anthelmintic resistance among nematode populations in goats⁶. In view of this, studies were undertaken to assess the efficacy of common brands of anthelmintics sold in rural veterinary drug shops and farm stores in Uganda against nematode infections in goats.

Materials and Methods

Brands of anthelmintics tested

Nilzan Plus® (levamisole 1.5% + oxydazonanide 3.0%, Cooper Ltd., Nairobi, Kenya), Wormalide Plus® (levamisole 1.5% +
bithional 8.0%, Cosmos Ltd., Nairobi, Kenya), Vermitran® (albendazole 10%, Sanofi Ltd., Budapest, Hungary) and Ivomec® (Ivermectin, Merck Ltd., U.S.A.) were the common brands of anthelmintics sold. They were bought and applied according to the recommended dose rates on the labels.

Goats

The goats were of the Small East African breed of both sexes, aged 10 months to two years. They had been bought from several villages in Tororo District and kept for over six months at the Livestock Health Research Institute (LIRI). They were managed by daily herding on open pastures and housed at night.

Experimental protocol

Initially, faecal worm egg counts were performed on all 57 goats on the farm using the modified McMaster technique as described by Hansen and Perry⁷. Then 43 goats with egg counts of 200 and above were selected for the study. The selected goats were then randomly allocated to five groups, namely, untreated control⁷, Nilzan group⁸, Wormicid group⁸, Vermitran group¹¹ and Ivomec group⁹. On day 0, goats in respective groups were treated with anthelmintic at the manufacturer’s recommended dose rate. Nilzan Plus®, Wormicid Plus® and Vermitran® were given orally, while Ivomec was given as an injection subcutaneously. Nilzan Plus® was applied at 5 mg/kg body weight, Wormicid Plus® at 6.0 mg/kg body weight, Vermitran® at 5 mg/kg body weight and Ivomec® at 0.2 mg/kg body weight. Faecal egg counts were performed on goats in each group on day 0, 10, 14, 21, 28, 35, 49 and 56.

Larval cultures

Larval cultures were carried out on day 0, 10, 14 and 28 on faecal samples collected from goats treated with Nilzan Plus®, Wormicid Plus®, Vermitran® and Ivomec®. Faecal samples from all goats in each treatment group were pooled together and incubated at 27°C for seven to 10 days. Gastrointestinal nematodes larvae were then extracted and identified according to the characteristics described by Hansen and Perry⁷.

Data analysis

Anthelmintic efficacy was assessed using the faecal egg count reduction test as described by Coles and colleagues⁸. The percentage reduction was estimated based on the arithmetic mean eggs per gram of faeces (EPG). It was expressed as 100 \((1 - T_1/T_2)\) where \(T_1\) and \(T_2\) were the Treated and Control group egg counts on day

<table>
<thead>
<tr>
<th>Experimental Date</th>
<th>Untreated group</th>
<th>Nilzan Plus®</th>
<th>Wormicid Plus®</th>
<th>Vermitran®</th>
<th>Ivomec®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=11)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>Day 0</td>
<td>575 (200-1800)</td>
<td>1275 (600-2700)</td>
<td>1800 (200-6500)</td>
<td>663 (200-2000)</td>
<td>1955 (200-5400)</td>
</tr>
<tr>
<td>Day 10</td>
<td>514 (0-600)</td>
<td>225 (0-800)</td>
<td>64% 0</td>
<td>100% 0</td>
<td>0 100%</td>
</tr>
<tr>
<td>Day 14</td>
<td>757 (0-600)</td>
<td>50 (0-100)</td>
<td>64% 0</td>
<td>100% 0</td>
<td>0 100%</td>
</tr>
<tr>
<td>Day 21</td>
<td>357 (0-1600)</td>
<td>375 (0-1600)</td>
<td>0 100% 0</td>
<td>100% 0</td>
<td>0 100%</td>
</tr>
<tr>
<td>Day 28</td>
<td>314 (0-800)</td>
<td>64 (0-200)</td>
<td>79% 0</td>
<td>100% 0</td>
<td>0 100%</td>
</tr>
<tr>
<td>Day 35</td>
<td>785 (0-2400)</td>
<td>163 (0-600)</td>
<td>79% 0</td>
<td>100% 0</td>
<td>18 (0-100) 98% 0 100%</td>
</tr>
<tr>
<td>Day 49</td>
<td>228 (0-600)</td>
<td>163 (0-600)</td>
<td>28% 0</td>
<td>100% 0</td>
<td>0 100%</td>
</tr>
<tr>
<td>Day 56</td>
<td>342 (0-800)</td>
<td>275 (0-275)</td>
<td>19% 100 (0-400)</td>
<td>70% 0 100%</td>
<td>28 (0-200) 92% 0 100%</td>
</tr>
</tbody>
</table>

Nilzan Plus® had totally lost efficacy that the treated goats had higher epg than the controls.
10, 14, 21, 35, 49 and 56, respectively. Anthelmintics that demonstrated efficacy rates of less than 95% were considered ineffective.

Results

Anthelmintic efficacy results are shown in Table 1. Ivomec® maintained an efficacy of 100% for seven weeks then dropped to 92%. Vermitan® maintained an efficacy of 100% for four weeks then dropped to 98%. Wormicid Plus® maintained an efficacy of 100% for seven weeks then dropped to 70%. In contrast, Nilzan Plus® had an efficacy of 85% after ten days thereafter the efficacy drastically declined to 19% by the eighth week.

The predominant nematodes encountered in Nilzan Plus® treated goats pre-treatment were Trichostrongylus spp., Haemonchus spp., Bunostomum spp. and Cooperia spp. (Fig. 1). However, on day ten, the predominant nematodes were Trichostrongylus spp. and Oesophagostomum spp. and on day 14 they were Haemonchus spp., Trichostrongylus spp. and Oesophagostomum spp., respectively. By day 28, the nematode species composition had almost reverted to the pre-treatment structure and the predominant nematode species were Haemonchus spp., Bunostomum spp., Trichostrongylus spp., Oesophagostomum spp. and Cooperia spp.

The predominant nematode species in the Vermitan® treated goats pre-treatment were Trichostrongylus spp., Haemonchus spp., Oesophagostomum spp., Cooperia spp. and Nematodirus spp. (Fig. 2). However, all nematode species were cleared after treatment.

The Wormicid Plus® treated goats had predominantly Trichostrongylus spp., Haemonchus spp. and Cooperia spp. pre-treatment but all nematode species were cleared after treatment (Fig. 3).

![Figure 1: Composition of nematode genera in faecal cultures for goats treated with Nilzan Plus®.](image-url)
Figure 2: Composition of nematode genera in faecal cultures for goats treated with Vermintan®.


Figure 3: Composition of nematode genera in faecal cultures for goats treated with Wormicid Plus®.

Discussion

Whereas Ivomec®, Vermihan® and Wormicid Plus® had high efficacy against nematode infections in goats for about eight weeks, Nilzan® was grossly ineffective since it exhibited efficacies of less than 95% right from day ten up to the eighth week post-treatment. However, it is not always clear to what extent failure may be due to drug resistance, underdosing or the use of substandard or fake drugs. Many factors could contribute to the ineffectiveness of Nilzan Plus® such as adulteration or use of fake drugs. However, development of resistance against Nilzan Plus® is likely. Presence of worm eggs and Trichostrongylus and Oesophagostomum species larvae in faecal cultures of samples taken on day ten from the Nilzan Plus® treated goats, was evidence that adult worms had not been cleared and were still producing eggs.

Faecal cultures of samples taken on day 14, indicated presence of Haemonchus species worms in addition to Trichostrongylus and Oesophagostomum. This suggested that the treated goats probably harboured a pre-selected population of Haemonchus that was resistant to Nilzan Plus®. Moreover, resistance against Nilzan® has been reported in neighbouring Kenya10. Benzimidazole, levamisole and ivermectin resistances have been reported in nematodes of sheep and goats and anthelmintic resistance is likely to develop wherever anthelmintics are frequently used11. Probably a nematode population resistant to Nilzan® has emerged, since Nilzan® has been frequently used over a long period in Uganda, mainly because of its broad-spectrum effect against Fasciola parasites and nematodes, both in small ruminants and cattle.

It appears that nematodes have developed resistance towards levamisole 1.5%, which is the main active ingredient in Nilzan Plus® against nematodes. Earlier studies on village goats in Uganda similarly found levamisole 1.5% (Wormicid®, Cosmos Ltd., Nairobi, Kenya) ineffective against goat nematodes8. The
excellent efficacy of Wormcid Plus® (levamisole 1.5% + bithionol 8%) against nematodes probably depended much on the effect of the 8% bithionol or the synergistic effect of the two active ingredients.

There have been reports of resistance to benzimidazoles such as thiabendazole and fenbendazole in nematode parasites in sheep in neighbouring Kenya. However, albendazole (Vermitran®, a benzimidazol, displayed very high efficacy against nematode parasites in goats in this study just as observed in earlier studies on albendazole (Vermitran®, Laboratorois Hipra, S.A., Giroma, Spain) in village goats in Uganda.

There have been reports on ivermectin resistance in goats in Denmark. However, ivermectin (Ivomec®) maintained high efficacy against nematode parasites of goats for nine weeks in this study. High efficiencies displayed by Ivomec®, Vermitran® and Wormcid Plus® encourage their use as alternative anthelmintics in areas where Nilzan Plus® is ineffective.

In conclusion, development of resistance against Nilzan Plus® is highly suspected. However, the fact that the Nilzan Plus® in question could probably be fake or adulterated should not be ignored. In view of these findings, there is need for the government to ensure strict quality control of veterinary drugs in general and anthelmintics in particular sold in many rural veterinary drug shops and farm stores in Uganda.

Acknowledgements

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References


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BOVINE AND CAPRINE FASCIOILIASIS IN ENUGU STATE, NIGERIA: RETROSPECTIVE ANALYSIS OF ABATTOIR RECORDS (1993-97) AND SIX MONTHS PREVALENCE STUDY
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2Department of Environmental Technology, Federal University of Technology P.M.B. 1526, Owerri, Imo State, Nigeria.
3Department of Animal Production and Health Technology, Michael Okpapa College of Agriculture, Umugwo P.M.B. 1472 Owerri, Imo State, Nigeria


Résumé
Une étude de la prévalence de la distomiasie chez les bovins et les caprins à l’aide d’une analyse rétrospective des rapports mensuels d’abattoir (1993-1997) et d’une inspection de la viande pendant six mois à l’abattoir de Nsukka, pour la période janvier-juin 1998, a été menée dans l’Etat d’Enugu. L’analyse des rapports portant sur 168.104 bovins et 319.427 caprins a montré que 14.351 (8,54%) et 3.183 (0,99%) étaient porteurs de Fasciola gigantica respectivement. La prévalence mensuelle globale variait entre 7,2% pour janvier et 9,4% pour août et novembre chez les bovins et 0,16% pour mars et 1,8% pour septembre chez les caprins. L’étude pendant six mois à l’abattoir de Nsukka a révélé une prévalence relativement plus élevée (5,8%) chez les caprins. On a également obtenu un résultat similaire (5%) chez les bovins. Ces chiffres étaient très différents (P<0,05) de ceux émanant des rapports officiels pour le même abattoir pendant la période 1993-1997. Il a été conclu que les rapports sur l’inspection de la viande dans l’Etat d’Enugu ne relatent pas bien les réalités sur le terrain et devraient, par conséquent, être améliorés.

Abstract
A prevalence study of fascioliasis in cattle and goats involving retrospective analysis of monthly slaughter house records (1993 to 1997) and six months meat inspection at Nsukka Urban slaughter house from January to June 1998 was carried out in Enugu State. Analysis of the records of 168,104 cattle and 319,427 goats showed that 14,351 (8.54%) and 3,183 (0.99%) harboured Fasciola gigantica respectively. Overall monthly prevalence ranged between 7.2% for January and 9.4% for August and November in cattle and 0.16% for March and 1.8% for September in goats. The six months study at Nsukka abattoir presented a relatively higher prevalence (5.8%) in goats. A similar result (5.0%) was also obtained in cattle. These figures differed significantly (P<0.05) from official records returned for the same abattoir from 1993 to 1997. It was concluded that meat inspection records in Enugu State may not represent the true picture on the ground and need to be improved upon.

Introduction
Fascioliasis is caused by fasciolidae trematodes of the genus Fasciola (large flukes) which migrate in the hepatic parenchyma and establish and develop in the bile ducts. It has been reported in many parts of the world1,2,3,4,5,6. The economic importance of the disease to livestock production is predicated on the prodigious losses it causes to food animal industry through liver condemnation and the morbidity produced by the liver fluke infestation3,5,7,8. Different published reports have established the prevalence of F. gigantica in some parts of Nigeria5,9,10,11. Most of these studies were based on data gathered passively from slaughter house records which gave a positive score citing the presence of adult worms in the bile ducts. Although hundreds of thousands of cattle and goats are processed for human consumption in Enugu State each year, no study has been carried out to determine the prevalence of fascioliasis in the two species of food animals.

The present study was designed to ascertain the actual prevalence of fascioliasis in cattle and
goats processed for human food in Enugu State.

**Materials and Methods**

A two-part study consisting of the examination and analysis of meat inspection records from 1993 to 1997 in seven local government areas of Enugu State and six months post-mortem inspection of cattle and goats slaughtered at Nsukka urban abattoir for the presence of *F. gigantica* was conducted.

In the first part, data on the numbers of cattle and goats handled in each local government area from 1993 to 1997 and the number infected with *F. gigantica* were analysed. Monthly and annual prevalence rates were then calculated for each local government area and for the entire state. Seasonal distribution of cases was also noted.

Information on age, sex and breed of the animals was not available.

In the second part of the study, the Nsukka urban abattoir which handles about 3,000 cattle and 6,000 goats annually was visited daily from 2nd to 30th June 1998 for post-mortem inspection of slaughtered animals. Before slaughter, the age, sex and breed of the animals were noted. The age of each animal was ascertained by observing the incisor teeth. All the cattle slaughtered were White Fulani (Bunaji) breed while the goats were the Red Sokoto (Maradi), originating from different parts of the State.

On a daily basis, post-mortem examination of whole and incised liver was carried out for

**Table 1**: Monthly prevalence of fascioliasis in cattle and goats slaughtered in Enugu State from 1993-97.

<table>
<thead>
<tr>
<th>Month</th>
<th>Cattle</th>
<th>No. Slaughtered</th>
<th>No. Infected (%)</th>
<th>Goats</th>
<th>No. Slaughtered</th>
<th>No. Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>14,247</td>
<td>1,044 (7.3)</td>
<td>13,973</td>
<td>308</td>
<td>1,044 (7.3)</td>
<td>13,973</td>
</tr>
<tr>
<td>Feb.</td>
<td>13,767</td>
<td>1,122 (8.1)</td>
<td>19,415</td>
<td>250</td>
<td>1,122 (8.1)</td>
<td>19,415</td>
</tr>
<tr>
<td>March</td>
<td>13,484</td>
<td>1,191 (8.8)</td>
<td>25,169</td>
<td>394</td>
<td>1,191 (8.8)</td>
<td>25,169</td>
</tr>
<tr>
<td>April</td>
<td>12,928</td>
<td>1,078 (8.3)</td>
<td>26,032</td>
<td>141</td>
<td>1,078 (8.3)</td>
<td>26,032</td>
</tr>
<tr>
<td>May</td>
<td>13,106</td>
<td>1,157 (8.8)</td>
<td>24,760</td>
<td>160</td>
<td>1,157 (8.8)</td>
<td>24,760</td>
</tr>
<tr>
<td>June</td>
<td>13,867</td>
<td>1,103 (8.0)</td>
<td>31,784</td>
<td>317</td>
<td>1,103 (8.0)</td>
<td>31,784</td>
</tr>
<tr>
<td>July</td>
<td>13,823</td>
<td>1,280 (9.3)</td>
<td>28,463</td>
<td>331</td>
<td>1,280 (9.3)</td>
<td>28,463</td>
</tr>
<tr>
<td>Aug.</td>
<td>13,478</td>
<td>1,268 (9.4)</td>
<td>29,860</td>
<td>246</td>
<td>1,268 (9.4)</td>
<td>29,860</td>
</tr>
<tr>
<td>Sept.</td>
<td>13,743</td>
<td>1,063 (7.7)</td>
<td>27,264</td>
<td>491</td>
<td>1,063 (7.7)</td>
<td>27,264</td>
</tr>
<tr>
<td>Oct.</td>
<td>14,370</td>
<td>1,230 (8.6)</td>
<td>27,603</td>
<td>213</td>
<td>1,230 (8.6)</td>
<td>27,603</td>
</tr>
<tr>
<td>Nov.</td>
<td>14,302</td>
<td>1,246 (9.4)</td>
<td>27,676</td>
<td>130</td>
<td>1,246 (9.4)</td>
<td>27,676</td>
</tr>
<tr>
<td>Dec.</td>
<td>16,984</td>
<td>1,466 (8.6)</td>
<td>27,613</td>
<td>203</td>
<td>1,466 (8.6)</td>
<td>27,613</td>
</tr>
<tr>
<td>Total</td>
<td>168,104</td>
<td>14,351 (8.54)</td>
<td>319,427</td>
<td>3,183</td>
<td>1,466 (8.6)</td>
<td>27,613</td>
</tr>
</tbody>
</table>

Overall prevalence of infection in the different local government areas (Table 2) show that the highest rates in cattle (16.31%) were recorded in Ezza followed by Abakiliki (11.62%) while Nsukka recorded the lowest rate (0.08%). There were thus significant differences (P<0.05) between the prevalence rates recorded at Nsukka and that of the other local government areas. Prevalence rates in goats slaughtered at the different local government areas were low with Abakiliki recording the highest rate (2.8%) while the lowest rate (0.24%) was recorded at Awgu.

Of the 1,267 cattle and 2,597 goats inspected at Nsukka Municipal abattoir over a period of six months, 63 (5.0%) and 151 (5.81%) were found to be positive for fascioliasis respectively (Table 3). These results are at variance with the 0.08% and 0.44% prevalence rates recorded for cattle and goats respectively in the same abattoir during the period 1993 to 1997.
### Table 2: Overall prevalence rates of fascioliasis in cattle and goats slaughtered in different Local Government areas of Enugu State from 1993-97.

<table>
<thead>
<tr>
<th>Local Govt Area</th>
<th>No. Examined</th>
<th>No. Infected (%)</th>
<th>No. Examined</th>
<th>No. Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udi</td>
<td>11,582</td>
<td>678 (5.5)</td>
<td>811</td>
<td>231 (28.5)</td>
</tr>
<tr>
<td>Enugu</td>
<td>128,723</td>
<td>11,877 (9.2)</td>
<td>229,747</td>
<td>1,568 (0.2)</td>
</tr>
<tr>
<td>Awgwu</td>
<td>8,133</td>
<td>324 (4.0)</td>
<td>8,807</td>
<td>21 (0.24)</td>
</tr>
<tr>
<td>Abakiliki</td>
<td>9,773</td>
<td>1,136 (11.6)</td>
<td>39,972</td>
<td>1,128 (2.8)</td>
</tr>
<tr>
<td>Oji River</td>
<td>2,751</td>
<td>191 (6.9)</td>
<td>4,709</td>
<td>75 (1.6)</td>
</tr>
<tr>
<td>Ezza</td>
<td>478</td>
<td>78 (16.3)</td>
<td>1,722</td>
<td>18 (1.1)</td>
</tr>
<tr>
<td>Nsukka</td>
<td>7,409</td>
<td>6 (0.08)</td>
<td>33,832</td>
<td>102 (0.44)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>168,852</strong></td>
<td><strong>14,290 (8.46)</strong></td>
<td><strong>319,427</strong></td>
<td><strong>3,183 (0.99)</strong></td>
</tr>
</tbody>
</table>

### Table 3: Prevalence rates of fascioliasis in cattle and goats slaughtered at Nsukka Slaughterhouse from January to June 1998.

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Examined</th>
<th>No. Infected (%)</th>
<th>No. Examined</th>
<th>No. Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>221</td>
<td>15 (6.79)</td>
<td>215</td>
<td>40 (18.60)</td>
</tr>
<tr>
<td>Feb</td>
<td>77</td>
<td>6 (7.9)</td>
<td>401</td>
<td>20 (4.98)</td>
</tr>
<tr>
<td>March</td>
<td>259</td>
<td>7 (2.70)</td>
<td>521</td>
<td>6 (1.15)</td>
</tr>
<tr>
<td>April</td>
<td>220</td>
<td>10 (4.50)</td>
<td>480</td>
<td>7 (1.46)</td>
</tr>
<tr>
<td>May</td>
<td>210</td>
<td>7 (3.33)</td>
<td>550</td>
<td>30 (5.45)</td>
</tr>
<tr>
<td>June</td>
<td>280</td>
<td>18 (6.43)</td>
<td>430</td>
<td>48 (10.00)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,267</strong></td>
<td><strong>63 (5.00)</strong></td>
<td><strong>2,597</strong></td>
<td><strong>151 (5.8)</strong></td>
</tr>
</tbody>
</table>

Monthly prevalence rates for both cattle and goats were significantly different (P<0.05). The highest rates (7.79% and 18.60%) were recorded in February and January for cattle and goats respectively, while the lowest rates (2.70% for cattle and 1.15% for goats) were recorded in March. An increase in infection rates with the onset of rains was observed.

Tables 4 and 5 indicate the sex and age related prevalence rates for cattle and goats. There was no significant difference (P>0.05) in infection rates in the male and female animals. While some level of age dependence in infection rates was observed among the animals, these were however not significant (P>0.05).

### Table 4: Sex-related prevalence rates of fascioliasis in cattle and goats slaughtered at Nsukka Slaughterhouse from January to June, 1998

<table>
<thead>
<tr>
<th></th>
<th>Cattle</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Examined</td>
<td>No. Infected (%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td><strong>Male</strong></td>
<td><strong>Female</strong></td>
</tr>
<tr>
<td>Male</td>
<td>1,048</td>
<td>52 (4.9)</td>
</tr>
<tr>
<td>Female</td>
<td>219</td>
<td>11 (5.00)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,267</strong></td>
<td><strong>63 (5.0)</strong></td>
</tr>
</tbody>
</table>
Table 5: Age-related prevalence rates of fascioliasis in cattle and goats slaughtered at Nsukka Slaughterhouse from January to June, 1998.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Cattle</th>
<th>No. examined</th>
<th>No. infected (%)</th>
<th>Goats</th>
<th>No. examined</th>
<th>No. infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 – 3</td>
<td>172</td>
<td>12 (7.0)</td>
<td></td>
<td>1 – 2</td>
<td>1,044</td>
<td>69 (6.5)</td>
</tr>
<tr>
<td>3 – 4</td>
<td>173</td>
<td>11 (6.3)</td>
<td></td>
<td>2 – 3</td>
<td>580</td>
<td>34 (60)</td>
</tr>
<tr>
<td>4 – 5</td>
<td>509</td>
<td>23 (4.5)</td>
<td></td>
<td>3 – 4</td>
<td>343</td>
<td>18 (5.3)</td>
</tr>
<tr>
<td>5 – 6</td>
<td>339</td>
<td>14 (4.1)</td>
<td></td>
<td>&gt;4</td>
<td>630</td>
<td>31 (4.9)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>75</td>
<td>3 (4.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,267</td>
<td>63 (5.0)</td>
<td></td>
<td>2,597</td>
<td>151 (5.8)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Analysis of the meat inspection records from Enugu State presented here showed that *F. gigantica* was relatively common in cattle and goats in the State. 8.54% and 0.99% prevalence rates were shown to occur in cattle and goats respectively. These figures are not in agreement with the 1.09% prevalence reported in cattle for northern Nigeria and the 14.7% reported also in cattle for the Jos Plateau Area. Similarly, the 0.99% prevalence in goats is also at variance with the 0.003% reported in goats for northern Nigeria.

A seasonal pattern of infection could not be established from the present results, rather, there seemed to be a consistent fluctuation in the prevalence rates through the months. This again is at variance with reports of higher prevalence rates towards the end of the rainy season highlighted by earlier workers.

Wide differences in prevalence rates for cattle and goats (8.5%) and (0.99%) for the period 1993 to 1997 could be due to the effects of differences in the original habitats of the animals. However, these results did not agree with the prevalence rates obtained from the second part of the study, 5.00% and 5.81% in cattle and goats respectively. It is probable that unsatisfactory meat inspection by veterinary personnel may have contributed to these differences. This is further highlighted by the sharp differences in other results obtained from Nsukka abattoir. For example, while overall prevalence in cattle for 1993 to 1997 was 0.08%, the rates for the first three months of 1998 in the same abattoir were 6.79%, 7.79% and 2.70% respectively.

Furthermore it is unlikely that the progressive increase in annual prevalence rates in cattle during the period 1993 to 1997 (not shown) was due to any important change in husbandry method. Very poor meat inspection facilities and unco-operative attitude of butchers has been reported in Nigerian abattoirs and may have contributed to the present discrepancies. It is equally probable that official figures returned for the other local government areas in the State also significantly underestimated the true situation of the disease. In addition, the slaughter figures recorded in the present study may not represent the true picture for the State since large numbers of animals are slaughtered privately in Nigeria, especially during festivities.

Information on age, breed and sex of animals was lacking from the official records obtained for the period. However, incidence studies at Nsukka showed some sex and age related prevalence rates which though not significantly different were in agreement with earlier observations of similar patterns in animals examined in other parts of the world.

References

Bovine and Caprine Fascioliasis in Enugu State, Nigeria: Retrospective Analysis of Abattoir Records (1993-97) and Six Months Prevalence Study


Received for publication on 24th March, 2000.


Abstract

Introduction

Bovine brucellosis is a disease of cattle caused by infection mainly with *Brucella abortus* and characterized primarily by abortion late in pregnancy and the retention of the placenta which can lead to infertility. Brucellosis is a widespread and economically important zoonotic disease in tropical and sub-tropical regions and creates problems for the intensive and extensive animal production systems. The disease is prevalent in many countries in Africa and the prevalence is quite high. Lack of consorted plans and national policies in the control and prevention of the disease, close human-animal contacts and food consumption customs are mainly responsible for the widespread and maintenance of the disease under tropical conditions. Many countries have made considerable progress in limiting the occurrence of brucellosis. However, in East Africa the disease is still one of the serious cattle health problems and the sera-prevalence rate varies from 1.8% to 35%. Previous studies on bovine brucellosis in central and northern Ethiopia indicated that the disease is common. The present study describes the results of a study on bovine brucellosis in farms and ranches in South-eastern Ethiopia.
BOVINE BRUCELLOSIS IN RANCHES AND FARMS IN SOUTH-EASTERN ETHIOPIA

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LA BRUCELLOSE BOVINE DANS LES RANCHS ET LES FERMES DANS LE SUD-EST DE L’ETHIOPIE

Résumé

Au total, 4,243 bovins âgés de plus de six mois appartenant à trois systèmes d’élevage dans le sud-est de l’Éthiopie, ont été examinés pour déterminer la présence des anticorps anti-Brucella. Le Test d’agglutination sur lame au Rose Bengal (RBPT) a été utilisé pour un examen sélectif et le Test de fixation du complément (CFT) pour confirmation. Le taux de séro-prévalence de la brucellose bovine dans les unités de production variait entre 3,5% et 18,9% avec une prévalence globale de 4,9%. 19 des 43 troupeaux (44,2%) avaient un animal ou plus qui réagissait et un taux de prévalence plus élevé a été observé chez les grands troupeaux par rapport aux plus petits. On a trouvé un nombre plus important d’animaux qui réagissaient chez les bovins âgés de plus de 4 ans; ils ont été détectés dans quatre des huit (50%) unités de production ayant fait l’objet d’examen sérologique. Deux de ces unités de production sont des ranchs qui servent de centres de reproduction de croisements F₁ (Zébu x Frison) qui sont distribués aux fermiers individuels et aux associations d’éleveurs de bétail laitier en vue d’accroître la production laitière. Le dépistage d’animaux infectés dans les centres de reproduction montrent clairement qu’ils ont propagé la brucellose non seulement dans les ranchs mais aussi dans le pays.

Abstract

A total of 4,243 cattle above six months of age belonging to three management systems in South-eastern Ethiopia were examined for the presence of Brucella antibodies. The Rose Bengal Plate Test (RBPT) was used as a screening test while Complement Fixation Test (CFT) as a confirmatory test. The sero-prevalence rate of bovine brucellosis in the production units varied from 3.5 to 18.9% with an overall prevalence of 4.9 percent. Nineteen of 43 herds (44.2%) had one or more reactor animals and a higher prevalence rate was observed in bigger herd sizes than in smaller herds. A significant increase in the number of reactors was found in animals above four years of age. Reactors were detected in four of the eight (50%) production units examined serologically, of which two were ranches serving as breeding centres to produce F₁ crosses (Zebu x Friesian) to be distributed to individual farmers and dairy associations to increase milk production. The detection of infected animals in the breeding centres strongly suggested that they may have been spreading brucellosis not only in the ranchs but also in the country as well.

Introduction

Bovine brucellosis is a disease of cattle caused by infection mainly with Brucella abortus and characterised primarily by abortion late in pregnancy and the retention of the placenta which can lead to infertility¹. Brucellosis is a widespread and economically important zoonotic disease in tropical and sub-tropical regions and creates problems for the intensive and extensive animal production systems ²,³,⁴,⁵. The disease is prevalent in many countries of Africa and the prevalence is quite high²,⁶,⁷. Lack of concerted plans and national policies in the control and prevention of the disease, close human-animal contacts and food consumption customs are mainly responsible for the widespread and maintenance of the disease under tropical conditions³. Many countries have made considerable progress in limiting the occurrence of brucellosis. However, in East Africa the disease is still one of the serious cattle health problems and the sero-prevalence rate varies from 1.8 to 35%⁶,⁷. Previous studies on bovine brucellosis in central and northern Ethiopia indicated that the disease is common⁸,⁹,¹⁰,¹¹. The present study describes the results of a study on bovine brucellosis in farms and ranches in South-eastern Ethiopia.

*Corresponding Author
Materials and Methods

Study Area and Animals

A cross-sectional study of bovine brucellosis was carried out from November 1997 to April 1998 on cattle kept in eight production units in four zones of South-eastern Ethiopia. Blood samples were collected from 4,243 cattle above six months of age to detect Brucella antibodies. The animals were of two breeds: local Zebu (Arsi or Boran) and crosses (Boran x Friesian or Arsi x Friesian). Animal identification was made using ear tags. All animals had not been vaccinated against brucellosis. During blood sample collection data on age, sex and herd sizes were recorded.

Blood Sample Collection

About 10 ml of blood was collected from the jugular vein of each animal using plain vacutainer tubes and needles. The blood samples were identified by each individual animal and kept at room temperature overnight followed by siphoning. Sera obtained were deep-frozen at -20°C until tested. The volume of antigen and test sera required each day were removed from the refrigerator and kept at room temperature for 30 to 60 minutes before the test. In this study, the Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) were used. All sera were tested with RBPT for rapid screening of brucellosis and those with a positive reaction to the RBPT were subjected to CFT for confirmation.

Laboratory Tests

The reagents and control sera required for the RBPT and CFT were obtained from the Central Veterinary Laboratory, Weybridge, Surrey, England. The RBPT was conducted following standard methods. Any observed agglutination was considered positive.

The reagents for the CFT were evaluated by titration. A 3% sheep red blood cell suspension was prepared before being used in the test proper. The preparation of reagents, CFT test protocol and interpretation of test results were according to the techniques recommended by the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) Service Laboratory, Berlin. Sera with 75% (3+) fixation of complement at a dilution of 1:5, and at least with 50% (2+) fixation of the complement at a dilution of 1:10 and above were taken as positive.

Results

The prevalence rate of brucellosis in individual production units varied from 3.5% to 18.7%. The overall prevalence in the study area was 4.9% (Table 1). Four of eight (50%) of the study production units were found to consist of three or more reactor animals. Of the total number of serum samples examined, the RBPT and CFT detected 215 (5.1%) and 207 (4.9%) respectively.

Abernosa ranch had the highest number of

<table>
<thead>
<tr>
<th>System of rearing</th>
<th>Production unit</th>
<th>No. of animals tested</th>
<th>%positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>Agarfa</td>
<td>148</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Assela</td>
<td>204</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Yekatit-25</td>
<td>121</td>
<td>-</td>
</tr>
<tr>
<td>Ranch</td>
<td>Abernosa</td>
<td>705</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Dida Tuyura</td>
<td>910</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Gobe</td>
<td>2,006</td>
<td>-</td>
</tr>
<tr>
<td>Extensive grazing</td>
<td>Bokoji</td>
<td>64</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dire</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4,243</td>
<td>207</td>
</tr>
</tbody>
</table>
Table 2: Relationship of herd size and prevalence of brucellosis in South-eastern Ethiopia from November, 1997 to April, 1998

<table>
<thead>
<tr>
<th>Herd size</th>
<th>No. of herds</th>
<th>positive (%)</th>
<th>No. of animals in herd</th>
<th>tested</th>
<th>positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>6</td>
<td>1 (16.7)</td>
<td>231</td>
<td>7 (3.0)</td>
<td></td>
</tr>
<tr>
<td>51-100</td>
<td>14</td>
<td>5 (35.7)</td>
<td>784</td>
<td>31 (4.0)</td>
<td></td>
</tr>
<tr>
<td>101-150</td>
<td>13</td>
<td>6 (46.2)</td>
<td>1,419</td>
<td>81 (5.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;150</td>
<td>10</td>
<td>7 (70.0)</td>
<td>1,660</td>
<td>85 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>19 (44.2)</td>
<td>4,094</td>
<td>204 (5.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Animals in extensive grazing system not included.

Table 3: Prevalence of brucellosis according to age, sex and breed in South-eastern Ethiopia from November, 1997 to April, 1998

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>tested</th>
<th>No. of animals positive</th>
<th>%positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>321</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>3773</td>
<td>202</td>
<td>5.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-2</td>
<td>812</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>2-4</td>
<td>975</td>
<td>17</td>
<td>1.7</td>
</tr>
<tr>
<td>4-6</td>
<td>1168</td>
<td>67</td>
<td>5.7</td>
</tr>
<tr>
<td>&gt;6</td>
<td>1041</td>
<td>120</td>
<td>11.5</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross</td>
<td>1337</td>
<td>48</td>
<td>3.6</td>
</tr>
<tr>
<td>Local Zebu</td>
<td>2757</td>
<td>156</td>
<td>5.7</td>
</tr>
</tbody>
</table>

reactors (18.7%) followed by Agarfa dairy farm (10.8%). A statistically significant difference (P<0.05) in prevalence rate was observed among the infected production units. No reactor animal was detected from Assela, Yekatit 25, Gobe and Bokoji production units.

Nineteen of 43 herds (44.2%) examined had one or more reactor animals (Table 2). The prevalence of brucellosis with regard to sex, age, and breed of animals is summarised in Table 3. Although the proportion of female animals was 92.2% of the total animals examined, the number of female reactors was higher (5.4%) than males (0.6%). A higher prevalence rate was observed in animals above six years of age (11.5%) as compared to animals between four and six (5.7%) and two to four years of age (1.7%). The difference in reactor numbers between the age groups was significant (P<0.05). The prevalence rate was higher in local animals (5.7%) than in cross-bred animals (3.6%), Table 3. The difference in prevalence rate between the two breeds was not statistically significant (P>0.05).

It was also observed that the prevalence of brucellosis was higher in ranches (5.2%) and lower in the extensive grazing management (2%). The dairy farms had an intermediate prevalence (3.4%). However, no significant difference was observed among the three management systems (P>0.05).

Discussion

The sero-prevalence rates for brucellosis observed in this study showed that bovine
brucellosis is endemic in ranches, dairy farms and extensive grazing animal production systems in South-eastern Ethiopia. The results of the present study are in agreement with previous reports, which indicated that bovine brucellosis is prevalent in Ethiopia. Different prevalence rates were recorded as the surveys were undertaken in different production systems and also in different regions of Ethiopia. Meyer reported that of 1,010 dairy animals examined serologically at the Institute of Agricultural Research Stations, 39% were positive for brucellosis. Tekle et al. found an overall prevalence rate of 4.2% in indigenous cattle in central Ethiopia. Yilkal reported a prevalence rate of 8.1% in urban and peri-urban dairy production systems in and around Addis Ababa. In North-eastern Ethiopia in Chaffa state, a prevalence rate of 22.8% was reported in cross-bred cows.

Abernosa and Dida Tuyura ranches are centres in which local Zebu cows and heifers are bred using Friesian semen to produce F₁ crosses. The F₁ crosses are distributed to individual farmers or dairy associations to increase milk production. The fact that these ranches have infected animals (18.7% and 6.2%, at Abernosa and Dida Tuyura, respectively) and are breeding centres strongly suggest that they may have been spreading the disease not only in the ranches but also in the country as well. In the ranches, animals may abort or deliver in the pasture outside maternity pens without being detected. As a result there could be a high risk of contamination of the grazing land and common watering points with aborted foetuses and foetal membranes. Furthermore, the loose fence enclosing the ranches allows stray dogs to have access and take away the aborted foetus and afterbirth from place to place and contaminate the pasture which may also increase the chances of infection.

The higher prevalence rate observed in larger herd sizes is in agreement with other previous reports. The relationship between the proportion of Brucella infected animals and herd size could be attributed to the intense cattle contact within the herd. Hellman et al. observed a higher number of Brucella reactors in animals four to eight years of age in southern Sudan and Yilkal reported 4% infection rate in animals above four years and 1.3% in animals between six months and two years of age. It is widely accepted that sexually
immature cattle are resistant to exposure to brucellosis and susceptibility increases with sexual development and pregnancy\textsuperscript{16,17}. The greater the number of infected cows that abort or calve, the greater is the exposure risk to other cattle in the herd\textsuperscript{1}. Most of the production units were not fenced and the neighbouring nomadic herds share some of the watering points used for animals in the ranches. Crawford \textit{et al.} \textsuperscript{17} indicated that proximity to infected herds, water ways and scavengers could influence interherd transmission of brucellosis. Multitudes of gazelles and antelopes graze in these ranches and its surroundings. The possibility of involvement of wild life in maintenance and spread of brucellosis has been reported\textsuperscript{16,19}.

The results of the present study indicate that bovine brucellosis is endemic and is one of the important disease problems in South-eastern Ethiopia. The fact that infected ranches were being used as breeding centres definitely favours spread and maintenance of brucellosis in the ranches as well as in the country. The distribution of F, pregnant heifers from infected ranches to farmers or dairy associations can exacerbate the prevailing infection status and will, therefore, continue to spread brucellosis in the ranches and in the country unless an optimum control strategy such as vaccination is instituted sooner.

\textbf{Acknowledgements}

The authors thank Mrs. J.A. Stack at Central Veterinary Laboratory, Weybridge, Surrey, England for the kind provision of antigens required for the tests. The generous collaboration of staff members of Asella Regional Veterinary Laboratory is highly appreciated.

\textbf{References}


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THE PREVALENCE AND DISTRIBUTION OF SALMONELLA IN SLAUGHTER CATTLE, SLAUGHTERHOUSE PERSONNEL AND MINCED BEEF IN ADDIS ABABA, ETHIOPIA

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PREVALENCE ET REPARTITION DE SALMONELLA CHEZ LE BETAIL DE BOUCHERIE, LE PERSONNEL D'ABATTOIR ET LE BOEUF HACHE A ADDIS-ABABA (ETHIOPIE)

Résumé
La prévalence et la répartition de Salmonella chez le bétail de boucherie, le personnel d'abattoir et les échantillons de boeuf haché à Addis-Abéba ont été déterminées. On a analysé les prélèvements fécaux, les ganglions lymphatiques mésentériques, les muscles abdominaux et diaphragmatiques du bétail de boucherie, les échantillons de boeuf haché des supermarchés et les prélèvements de selles du personnel d'abattoir. Salmonella étaient isolées de 10,6% (5/47) et 19,6% (9/47) des prélèvements fécaux de bovins et des ganglions lymphatiques respectivement. Sur 235 échantillons simples de muscles abdominaux et diaphragmatiques, 23 (9,9%) étaient respectivement positifs à Salmonella. 193/300 (6%) des prélèvements de selles du personnel d'abattoir contenaient Salmonella. 263/330 (7,9%) des échantillons de boeuf haché des supermarchés étaient positifs à Salmonella. 98 souches de Salmonella composées de 6 différents sérovars étaient identifiées comme étant S. dublin (54,1%), S. anatum (27,6%), S. saintpaul (9,2%), S. meleagris (5,1%), S. muenchen (1%) et S. rough form (3,1%). Selon les résultats de cette étude, Salmonella est répandue chez le bétail de boucherie, le personnel d’abattoir et le boeuf haché, et il existe un lien entre les souches bovines et humaines dans la zone couverte par l'étude.

Abstract
The prevalence and distribution of Salmonella in slaughter cattle, slaughterhouse personnel and minced beef samples in Addis Ababa were determined between February, 1999 to September, 1999. Faecal samples, mesenteric lymph nodes, abdominal as well as diaphragmatic muscles from slaughtered cattle, minced beef samples from supermarkets and stool samples from slaughterhouse personnel were analysed. Salmonella were isolated from 10.6% (5/47) and 19.6% (9/47) of cattle pooled samples of faeces and mesenteric lymph nodes respectively. Of 235 single samples of abdominal and diaphragmatic muscles, 23 (9.9%) and 28 (11.9%) respectively were Salmonella positive. Eighteen (6.0%) of 300 stool samples from abattoir personnel contained Salmonella. Twenty-six (7.9%) of 330 minced beef samples from supermarkets were Salmonella positive. Ninety-eight Salmonella isolates consisting of six different serovars were identified as S. dublin (54.1%), S. anatum (27.6%), S. saintpaul (9.2%), S. meleagris (5.1%), S. muenchen (1.0%), and S. rough form (3.1%). The results from this study show that Salmonella are widespread in slaughter cattle, slaughterhouse personnel and minced beef and there exists a link between cattle and human Salmonella isolates in the study area.

Introduction
Salmonellosis has remained an important problem in industrialised and developing countries of the world. The increased global population coupled with massive production of animal and human food, the widespread occurrence of Salmonella in the natural environment and various sectors of the food industry could aggravate the problem1,2.

*Correspondence to Dr. Bayleyegn Molla.

Although the incidence of salmonellosis seems to vary across countries, it is one of the most widespread food-borne zoonoses. It is currently difficult to evaluate the status of salmonellosis in developing countries due to lack of a co-ordinated epidemiological surveillance and reporting systems3,4,5.

Salmonella infection in adult animals tends to be limited to a healthy carrier state. Stress associated with transport of animals from rearing farms to abattoir augments shedding of
Abattoirs have also been demonstrated as potential sources of contamination of meat destined for human consumption. Poorly disinfected knives and other slaughtering equipment and poor hygiene among abattoir personnel could also contribute to carcass contamination. Human salmonellosis is widespread in young children, in elderly citizens frequently afflicted with underlying chronic diseases and in immunosuppressed individuals.2,3

The distribution and level of contamination of Salmonella in the food chain in Ethiopia is not known except that the agent has been isolated from poultry, cattle, minced beef and humans.5,9,10 The present study was undertaken to determine the prevalence and distribution of Salmonella in the food chain from cattle to consumer using a cross-sectional study design.

Materials and Methods

Study area and collection of samples

A cross-sectional study was carried out in Addis Ababa, at the main abattoir and available supermarkets in the city between February 1999 to September 1999. At the abattoir an average of 600 heads of cattle and 500 sheep and goats are slaughtered daily of which 20 to 25 cattle were randomly selected and sampled weekly. Faecal, mesenteric lymph node, abdominal and diaphragmatic muscle samples were taken from 235 apparently healthy slaughtered cattle earlier examined ante-mortem by authorised veterinarians over a period of 24 weeks. Faecal and mesenteric lymph node samples were pooled separately (five single samples per pool) whereas abdominal and diaphragmatic muscles were analysed as single samples. Three hundred stool samples (out of 700 abattoir workers) from apparently healthy slaughterhouse personnel were randomly collected in collaboration with the medical personnel in the abattoir clinic. A total of 330 minced beef samples were also randomly collected from 22 supermarkets in Addis Ababa. Samples were taken aseptically and transported immediately in a cool box to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Addis Ababa University for analysis.

Isolation and identification of Salmonella

For the cultural method, the techniques described by the International Organisation for Standardisation: ISO 65791 and Quinn et al.7 were used. Briefly, the following procedures were employed:

Level 1: Pooled faecal samples (containing five single samples each) were collected from slaughtered cattle at the Addis Ababa abattoir from 235 animals. About 2-3 g samples of faeces were added to 10 ml of Rappaport-Vassiliadis (RV) enrichment broth (Merck) and incubated at 42°C for 24 h. The following day, 0.1 ml was transferred to another 10 ml of RV and incubated at the same temperature and duration. This was followed by streaking onto two different plates of Brilliant green phenol-red lactose-sucrose (BPLS) and MacConkey agar (Merck) and incubated at 37°C for 18 to 24 h. If growth was slight or if no typical colonies of Salmonella were present, the plates were re-incubated for a further 18 to 24 h and re-examined for the presence of typical colonies of Salmonella.

Level 2: Pooled samples of mesenteric lymph nodes (containing five single samples each) were collected from the same number of animals (n=235). Twenty-five grams of the sample, after trimming off the fascia and fat, was added to 225 ml of buffered peptone water (BPW, Merck) and incubated at 37°C for 16 to 20h. About 0.1 ml of the culture was transferred to 10 ml of RV and incubated at 42°C for 18 to 24 h. Another 1 ml of this culture in BPW was transferred to 10 ml of selenite cystine (SC) broth (SIFIN, Berlin) and incubated at 37°C for 18 to 24 h. On the third day, streaking was done onto selective agar media (BPLS and MacConkey) and incubated at the same temperature and duration as in level 1.

Level 3: Single beef cut samples from diaphragm (n=235) and abdominal muscles (n=235) were collected separately. Twenty-five grams of sample from each of the 235 animals was pre-enriched in 225 ml of BPW and treated as in level 2.

Level 4: Stool samples from 300 abattoir workers: About 2-3 g were taken and treated as in level 1.
Level 5: 330 minced beef samples from supermarkets: Twenty five grams of each sample was taken and pre-enriched in BPW and treated as in level 2.

Suspected Salmonella colonies were tested biochemically. The “Enterotube” II (Roche-Diagnostica®, Basel, Switzerland), a ready-to-use biochemical testing system for the identification of Salmonella was used as described by the manufacturer. Screened Salmonella isolates were further tested using polyvalent I and II Salmonella anti-sera (SIFIN®, Berlin). Serotyping of Salmonella isolates was kindly done by the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Berlin, Germany.

To compare the pool prevalence for faeces and mesenteric lymph nodes with the Salmonella prevalence of individual muscle samples, it was calculated according to Cowling et al.\textsuperscript{12} as: Prevalence = 1 - (1 - pool prevalence)\textsuperscript{\textit{n}} where \textit{n} is pool size; in our case \textit{n}=5.

Results

Table 1 shows the distribution and sources of Salmonella isolates from slaughter cattle, slaughterhouse personnel and minced beef samples. Out of the 47 pooled faecal and mesenteric lymph node samples, Salmonella was isolated in 5 (10.6%) and 9 (19.6%) samples respectively. Of the total 235 single samples of the abdominal muscle, 23 (9.8%) and of the diaphragmatic muscle, 28 (11.9%) contained Salmonella. Eighteen (6.0%) of the 300 human stool samples examined from slaughterhouse personnel had Salmonella isolates.

Twenty six (7.9%) of the minced beef samples were found to contain Salmonella (Table 2). The range was 0-3 isolates per 15 samples from each supermarket. The number of samples from supermarkets detected as Salmonella positive with regard to meat source was 14 (7.8%), 9 (7.5%) and 3 (10.0%) from Addis Ababa abattoir, Kara and Kaliti butcheries respectively(Table 2).

From pooled samples of faeces and mesenteric lymph nodes, S. dublin, S. muenchen and S. anatum were isolated. Similar to cattle faeces, S. dublin dominated in samples of abdominal and diaphragmatic muscles. Salmonella isolates from slaughterhouse personnel consisted of S. anatum, S. meleagrisid and S. dublin (Table 3).

Of the Salmonella serovars isolated from minced beef samples, S. anatum was dominant followed by S. saintpaul and S. dublin. With regard to source of origin of minced beef samples, three Salmonella serotypes (S. anatum, S. dublin and

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Total Examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled faeces</td>
<td>47</td>
<td>5</td>
<td>10.6</td>
</tr>
<tr>
<td>Pooled mesenteric lymph nodes</td>
<td>47</td>
<td>9</td>
<td>19.2</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>235</td>
<td>23</td>
<td>9.8</td>
</tr>
<tr>
<td>Diaphragmatic muscle</td>
<td>235</td>
<td>28</td>
<td>11.9</td>
</tr>
<tr>
<td>Slaughterhouse Personnel:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human stool</td>
<td>300</td>
<td>18</td>
<td>6.0</td>
</tr>
<tr>
<td>Supermarket:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced beef</td>
<td>330</td>
<td>26</td>
<td>7.9</td>
</tr>
</tbody>
</table>
Table 2: *Salmonella* isolates in minced beef samples from supermarkets in Addis Ababa, Ethiopia - February, 1999 - September 1999

<table>
<thead>
<tr>
<th>Sources of Meat</th>
<th>Number of supermarkets</th>
<th>Number of samples examined</th>
<th><em>Salmonella</em> positive (%)</th>
<th>Serovars by source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addis Ababa</td>
<td>12</td>
<td>180</td>
<td>14 (7.8)</td>
<td><em>S. anatum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. dublin</em></td>
</tr>
<tr>
<td>Kara</td>
<td>8</td>
<td>120</td>
<td>9 (7.5)</td>
<td><em>S. anatum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. dublin</em></td>
</tr>
<tr>
<td>Kaliti</td>
<td>2</td>
<td>30</td>
<td>3 (10.0)</td>
<td><em>S. anatum</em></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>330</td>
<td>36 (7.9%)</td>
<td><em>S. anatum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. dublin</em></td>
</tr>
</tbody>
</table>

Table 3: *Salmonella* serovars isolated from slaughter cattle, slaughterhouse personnel and minced beef samples

<table>
<thead>
<tr>
<th><em>Salmonella</em> Serovar (stool samples)</th>
<th>Sources from which <em>Salmonella</em> isolated</th>
<th>Faeces</th>
<th>Mesenteric lymph nodes</th>
<th>Abdominal muscle</th>
<th>Diaphragmatic muscle</th>
<th>Abattoir personnel</th>
<th>Minced beef</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. anatum</em></td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>12</td>
<td>27 (27.6)</td>
<td></td>
</tr>
<tr>
<td><em>S. dublin</em></td>
<td>4</td>
<td>2</td>
<td>18</td>
<td>21</td>
<td>4</td>
<td>4</td>
<td>53 (64.1)</td>
<td></td>
</tr>
<tr>
<td><em>S. muenchen</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td><em>S. melagris</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 (5.1)</td>
<td></td>
</tr>
<tr>
<td><em>S. saintpaul</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>9 (9.2)</td>
<td></td>
</tr>
<tr>
<td><em>S. rough form</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>5</td>
<td>21</td>
<td>23</td>
<td>18</td>
<td>26</td>
<td>98 (98.0)</td>
<td></td>
</tr>
</tbody>
</table>

*S. saintpaul* originated from Kara butchery whereas from Kaliti all three strains belonged to *S. saintpaul*. *Salmonella anatum* and *S. dublin* were also isolated from minced beef samples which originated from Addis Ababa abattoir (Table 2). In all samples collected from Addis Ababa abattoir (faeces, mesenteric lymph nodes, abdominal and diaphragmatic muscles and minced beef), no *S. saintpaul* was isolated.

**Discussion**

In the present study *Salmonella* isolates from the diaphragm (11.9%) and abdominal muscles (9.8%) showed that beef cuts were the most important sources of *Salmonella*. This is in comparison to estimated prevalence of 2.2% and 4.2% for individual faecal and mesenteric lymph node samples respectively. The estimated prevalence of these single samples was determined from pooled samples according to Cowling et al.\textsuperscript{12}. Collective sample testing can be used to determine herd status (infected or non-infected) or, if multiple pools per herd are used, the individual animal prevalence can be estimated with the help of the results obtained from pooled samples\textsuperscript{12}. Low prevalence in live animals, indicated by low isolation rates of *Salmonella* in faeces and lymph nodes suggests that the contamination of beef cuts by *Salmonella* does not originate from live animals. There must have been cross-contamination during the skinning process as a result of poor hygienic conditions during subsequent dressing operations. One probable source could be from infected abattoir personnel as 6.0% of them had *Salmonella* isolated from their stool. This may be interpreted as an indication of chain contamination between cattle carcass and people. On the other hand, in human faeces *S. melagris* was detected, a serovar which was not found in cattle and minced beef samples.

The relatively high prevalence rate of
Salmonella infection observed in slaughterhouse personnel could be partly attributed to the habit of consuming raw meat while slaughtering. Mache and Mengistu isolated 45 Salmonella serogroups including S. typhi (15.6%), Salmonella group A (8.9%), B (24.4%), C (31.1%), D (13.3%) and E (6.7%) from 700 stool samples collected from adult outpatients with diarrhea in Addis Ababa. A study conducted in Kampaala District (Uganda) showed Salmonella organisms in stool samples of 8.1% of the patients with acute diarrhoea which belonged to S. typhimurium and S. enteritidis.

Various reports indicated that the incidence of Salmonella infection in apparently healthy cattle in abattoirs varies with the sample type, sampling techniques and culturing method employed. Collection of samples from slaughterhouse, beef cuts, abattoir personnel and minced beef serves to indicate whether there exists a link between different serovars of potential public health hazard. The predominant Salmonella serotypes found were S. dublin followed by S. anatum. These results agree with works carried out by various authors in apparently healthy slaughtered animals in Africa and other parts of the world.

The major Salmonella isolates from slaughterhouse personnel were S. anatum, S. dublin and S. meleagridis. Although not fully serotyped, Mache and Mengistu also isolated Salmonella serogroups B, C, D and E from stool samples in Addis Ababa. Salmonella anatum and S. dublin were also isolated from slaughtered cattle which indicate the possible existence of a link between the serovars in the meat chain and those isolated from slaughterhouse personnel. Chambers also reported these serovars from abattoir workers and children in Zimbabwe. Salmonella meleagridis was detected only from stool samples of slaughterhouse workers. Its origin could have been other contaminated food products as it was not identified in any sample of the slaughter cattle. It should also be pointed out that there was no case of contamination of the 330 beef cut samples by S. meleagridis.

Salmonella anatum, S. dublin and S. saintpaul were the most frequent serotypes in minced beef samples from the supermarkets. The dominance of S. anatum and S. dublin in minced beef is in agreement with the work of Molla et al. except that S. typhimurium was not encountered in any of the samples analysed in this study. The source of the two serotypes found in minced beef from supermarkets could have been from the Addis Ababa abattoir as these strains were isolated from samples collected at that slaughterhouse. The origin of S. saintpaul appears to be from Kaliti and Kara butcheries supplying meat to supermarkets to be processed into minced beef.

This study indicated that Salmonellae are widespread in slaughter cattle, slaughterhouse personnel and minced beef in the study area. Furthermore, it suggested the possible existence of a link between the serovars in the meat chain and those isolated from slaughterhouse personnel.

Acknowledgements

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DIETARY LEVELS OF ENERGY AND PROTEIN FOR OPTIMAL GROWTH OF CROSSBRED ANGLO-NUBIAN GOATS IN SAMOA

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TAUX ENERGETIQUE ET PROTEINIQUE DES ALIMENTS POUR LA CROISSANCE OPTIMALE DES CHEVRES CROISEES ANGO-NUBIENNES A SAMOA

Résumé

Dix-huit chèvres croisées anglo-nubiennes, âgées de 20-24 mois avec un poids vif de 20,9 ± 0,12 kg avant l’expérience, ont été utilisées pour déterminer les taux énergétiques et protéiniques requis pour la croissance optimale des chèvres dans le milieu tropical de Samoa. Les chèvres étaient réparties au hasard en trois groupes, en fonction de leur poids, et soumises à trois régimes expérimentaux avec trois taux protéiniques et énergétiques : 14,6%, 13,4% et 12,4% de protéine brute (PB) et 12,3 ; 13,4 et 14,4 MJ EB/kg pendant 8 semaines. Les régimes étaient classés comme suit (a) régime à forte teneur en protéine et à faible taux énergétique (HPLE) (b) régime à teneur moyenne en protéine et à taux moyen en énergie (MPME) et (c) régime à faible teneur en protéine et à taux élevé en énergie (LPHE). Les lettres P et E signifient respectivement protéine et énergie. Les valeurs de la consommation volontaire de concentré et de fourrage de 734 ; 680 et 650 g/chèvre/jour et 512 ; 498 et 446 g/chèvre/jour ont été relevées pour les chèvres soumises aux régimes HPLE, MPME et LPHE respectivement. La consommation volontaire de concentré était plus élevée (P < 0,05) chez les chèvres servies de HPLE, suivie de MPME et LPHE. La consommation de concentré a baissé de façon linéaire avec l’augmentation du taux énergétique. La consommation de fourrage a suivi la même tendance que celle de concentré (P<0,05). La consommation alimentaire volontaire totale (concentré + fourrage) diminuait avec l’accroissement des taux énergétiques et non pas avec celui de la teneur en protéine. Les gains pondéraux moyens/jour étaient de 95 ; 130 et 89 g/chèvre/jour pour les régimes HPLE, MPME et LPHE respectivement. Il y avait des différences significatives (P<0,05) quant aux gains de poids quotidiens. Les chèvres nourries de régime MPME avaient un gain pondéral plus élevé (P<0,05) que celles servies de régimes HPLE et LPHE. Les valeurs de la digestibilité de la matière organique apparaissent de 63,8 ± 0,04% ; 66,2 ± 0,02% et 62,2 ± 0,08% ont été obtenues pour les chèvres servies de régimes HPLE, MPME et LPHE respectivement. Les coefficients de digestibilité des substances nutritives apparaissant pour la protéine brute et l’énergie brute étaient beaucoup plus élevés pour les chèvres nourries de régime HPLE et MPME respectivement. Cependant, le régime HPLE était plus faible (P>0,05) que les régimes LPHE et MPME quant aux coefficients de digestibilité apparente de l’énergie brute. La digestibilité de la fibre brute était nettement plus faible chez les chèvres servies de régime HPLE. Selon ces résultats, la teneur optimale en protéine et les besoins en énergie dans un régime de concentré pour avoir une performance optimale des chèvres croisées anglo-nubiennes est d’environ 13,4% PB et 13,4 MJ EB/kg de poids vif puisque c’est ainsi que l’on a obtenu le meilleur taux de croissance.

Abstract

Eighteen crossbred Anglo-Nubian does, 20 to 24 months and having a pre-trial average live weight of 20.9±0.12kg were used to investigate dietary levels of energy and protein optimal for growth in the tropical environment of Samoa. The goats were assigned on the basis of weight, to three treatment groups in a completely randomized design experiment and three experimental diets were offered at three protein and energy levels: 14.6, 13.4 and 12.4% crude protein (CP) and 12.3, 13.4 and 14.4 MJ GE/kg for eight weeks. The diets were designated as high protein low energy (HPLE), medium protein medium energy (MPME) and low protein high energy (LPHE). The letters P and E stand for protein and energy, respectively. Voluntary concentrate and forage intake values of 734, 680 and 650 g/doe/day and 512, 498 and 446 g/doe/day were obtained for the goats on diets HPLE, MPME and LPHE, respectively. Voluntary concentrate intake was higher (P<0.05) in goats offered HPLE followed by MPME and LPHE. Concentrate intake decreased linearly with increasing value of energy. Forage intake followed the same trend as concentrate intake (P<0.05). Total voluntary feed intake (concentrate + forage) decreased with increase in the levels of energy and not with protein levels. Average daily live weight gains were 95, 130 and 89 g/doe/day for HPLE, MPME and LPHE diets, respectively. There were significant differences (P<0.05) in daily live weight gains. The goats that had the MPME diet were higher (P<0.05) in live weight gain than those on HPLE and LPHE diets. Apparent organic matter digestibility values of 63.8±0.04, 66.2±0.02 and 62.2±0.08% were obtained for the goats offered HPLE, MPME and LPHE diets, respectively. Apparent nutrient digestibility coefficients for crude protein and gross energy were significantly higher for goats on HPLE and MPME diets, respectively. However, HPLE diet was lower (P<0.05) than LPHE and MPME diets in apparent digestibility coefficients of gross energy. Crude fibre digestibility was significantly lower in the goats on HPLE diet. From these results the estimated optimal protein and energy requirements in a concentrate diet for optimum performance of crossbred Anglo-Nubian goats was 13.4% CP and 13.4 MJ GE/kg BW since growth rate was best at that level.
Introduction

All over the world, there are different breeds of goats and each is adapted to particular environmental conditions and has different nutritional requirements. The Anglo-Nubian goats and their crosses found in the Pacific Island Countries (PICs) today, were introduced some years ago. Information on dietary levels of protein and energy of tropical breeds of goats is scant. However, few attempts have been made to determine their requirements for growth and maintenance, reproduction and lactation.

In the PICs, the traditional system of feeding goats is based on the use of kitchen waste, browsing and to a less extent on the use of agricultural by-products. This practice is perhaps the major cause of low productivity from these animals, as the feeds consumed may be inadequate both in quality and quantity. There are no data on the feed intake and nutrient utilization for this breed of goats under the traditional or organized systems of production. Therefore, it has not been possible to formulate complete rations adequate with respect to all nutrients, particularly energy and protein that are capable of adequate growth and efficient meat production.

No attempts have been made to establish the dietary levels of energy and protein requirements of the crossbred Anglo-Nubian goats in the Pacific Island countries. An estimate of the optimal dietary protein and energy levels at which efficiency of utilization is optimal is essential for the understanding of the nutritional requirements for the growth of crossbred Anglo-Nubian goats in the tropical environment of Samoa, a Pacific Island country. The objective of this trial, therefore, was to contribute to the knowledge of understanding the dietary levels of energy and protein for optimal growth of crossbred Anglo-Nubian goats in Samoa.

Materials and Methods

Animals, feed preparation and management

Eighteen growing female crossbred Anglo-Nubian goats between 20 to 24 months of age and having a pre-trial average live weight of 20.9±0.12 kg were divided, on the basis of weight, to three treatment groups in a completely randomized design experiment. In each treatment there were six replicates. Two animals were housed in a pen. For each treatment, three pens that had previously been disinfected were allocated. Pens had concrete floors covered with wood shavings as litter. Feed and water troughs for group feeding were provided in each pen. Also attached to each pen was a secured plastic container for forage.

The eighteen goats were fed experimental diets at the following three protein and energy levels: 14.6, 13.4 and 12.4% crude protein (CP) and 12.3, 13.4 and 14.4 MJ GE/kg for eight weeks. The diets were designated as high protein low energy (HPLE), medium protein medium energy (MPME) and low protein high energy (LPHE). The letters P and E stand for protein and energy, respectively.

The feed ingredients used in the preparation of the experimental diets (concentrates) were cassava flour, dried brewers' grains, urea (46% N), mineral and vitamin premix and salt. Cassava tubers of a sweet variety (Manihot dulcis) were harvested from the Crop Science Discipline Farm located in the School of Agriculture. The tubers were peeled and the pulp cut into chips. These were sun-dried and ground in a hammer chipper passing through a 6mm screen. The brewers' grains were obtained wet from the Western Samoa Breweries Limited, Apia. These were spread on a concrete floor and sun-dried to a constant moisture content. These ingredients were used with others to compound the experimental diets. The percentage composition of experimental diets offered to the goats is provided in Table 1. The concentrate portion for each goat was weighed out for a week. The concentrate portion of each diet was offered ad libitum to the goats (10% in excess of the previous day's intake) during which they also had free access to fresh clean water. The forage portion (2.0-kg) was fed in two portions at 8:30 and 17:30 h. Records of individual feed intake and weekly body weight changes were kept. Feeds not consumed within 24 hours were collected, weighed, the amount recorded and feed residue discarded. Before the feed residue was...
discarded a sample was taken for dry matter and chemical analysis. The animals were allowed a five-days adaptation period to get used to the concentrate supplement followed by 56 days of feeding. The difference between the initial and final average live weights was used to compute live weight gain.

**Table 1: Composition of Experimental Diets (% Air Dry)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>HPLE</th>
<th>MPME</th>
<th>LPHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava flour</td>
<td>39.50</td>
<td>48.50</td>
<td>60.50</td>
</tr>
<tr>
<td>Dried brewers’ grains</td>
<td>54.00</td>
<td>46.00</td>
<td>35.50</td>
</tr>
<tr>
<td>Urea (46%N)</td>
<td>4.50</td>
<td>3.50</td>
<td>2.00</td>
</tr>
<tr>
<td>Mineral/vitamin</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Digestibility study**

At the end of the experiment, all the animals in each treatment group were used for metabolic studies. Since they were still on the same diet and environment, the digestibility study started two days after the end of the growth phase. Each goat was given 2.5 kg of concentrate diets and 2.0 kg of forage (guinea grass – *Panicum maximum*) in two equal portions at 8:30 and 17:30 h. The total faecal collection method was used for faeces. Faeces voided were collected for seven days. Total daily faecal outputs for animals in each pen were weighed and a 25% sample was removed for dry matter determination. Samples of forage, experimental diets and faeces were later dried in a forced draught oven at 70°C for 48 hours. The dried daily samples of forage, experimental diets and faeces were then bulked separately and milled with a simple laboratory mill and stored in air-tight bottles until required for analysis.

**Analytical Procedures**

The AOAC method was used for nutrient contents of feed ingredients, forage, experimental diets and faeces. All analyses were done in triplicate. Dry matter was determined by drying at 102°C for 24 hours, ash by firing at 600°C for 24 hours, protein by the micro-Kjeldahl procedure (N x 6.25). Gross energy values were determined by a bomb calorimeter (Adiabatic bomb, Parr Instrument Co., Moline, IL) using thermochemical benzoic acid as standard. Crude fibre of the feed ingredients, forage, experimental diets and faeces were determined according to the procedure of Naumann and Bessler. Apparent nutrient digestibility coefficients were calculated by difference.

**Statistical Analysis**

Voluntary feed intake, growth rate and apparent nutrient digestibility coefficients were subjected to analysis of variance for completely randomized designs. Where significant differences were observed treatment means were compared by Duncan’s New Multiple Range Test.

**Results**

Table 2 presents nutrient values of feed ingredients, forage (guinea grass – *Panicum maximum*) and experimental diets. The treatment means for all performance characteristics are presented in Table 3. Voluntary concentrate and forage intake values of 734, 680 and 650 g/day/doe and 512, 498 and 446 g/day/doe were obtained for the goats on HPLE, MPME and LPHE, respectively. Voluntary concentrate intake was higher (P<0.05) in goats offered HPLE followed by MPME and LPHE. It was observed that concentrate intake decreased linearly with increasing value of energy. Forage intake also followed the same trend as concentrate intake (P<0.05).

Average daily live weight gains were 95, 130 and 89 g/doe/day for HPLE, MPME and LPHE, respectively. There were significant differences (P<0.05) in daily weight gains. The goats on MPME diet were better (P<0.05) in live weight gain than the goats on HPLE and LPHE diets. There were slight differences in live weight gains between goats on HPLE and LPHE diets, however, the difference was of no statistical significance (P<0.05). Feed efficiency was significantly better (P<0.05) in the MPME fed goats.

Table 4 presents data on apparent nutrient digestibility coefficients of goats offered the experimental diets. Apparent digestibility
coefficients for crude protein was significantly higher (P<0.05) for goats on HPLE and MPME diets, respectively and gross energy was better digested in LPHE and MPME compared to the HPLE diet. Crude fibre digestibility was significantly lower (P<0.05) in the goats on HPLE diet. Apparent organic matter digestibility (OMD) values of 63.8 ± 0.04, 66.2 ± 0.02 and 62.2 ± 0.08% were obtained for the goats on HPLE, MPME and LPHE diets, respectively. Goats that received the MPME diet had the highest apparent organic matter digestibility. The goats on the HPLE diet had close apparent OMD with those on the LPHE diet. Apparent digestibility coefficients of crude fibre, organic matter, nitrogen free extract and total digestible nutrients were however, higher (P<0.05) in the MPME diet.

### Discussion

The nutrient value of the cassava flour used in the experimental diets corresponds with values reported by Gohl\textsuperscript{11} and Aregheore\textsuperscript{12}. Also, nutrient values of brewers' dried grains and guinea grass used in this trial are similar to values reported by Susumu\textsuperscript{13} and Cawa\textsuperscript{14} respectively. The analyzed nutrient contents of the experimental diets especially, energy and proteins were similar to calculated values.
Table 4: Apparent digestibility coefficients of nutrients and energy values of diets

<table>
<thead>
<tr>
<th>Nutrients (%)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLE</td>
</tr>
<tr>
<td>Dry matter</td>
<td>69.2 ± 0.22a</td>
</tr>
<tr>
<td>Crude protein</td>
<td>75.5 ± 0.05a</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>68.1 ± 0.04a</td>
</tr>
<tr>
<td>Organic matter</td>
<td>63.8 ± 0.04a</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>46.3 ± 0.16b</td>
</tr>
<tr>
<td>Total digestible nutrients</td>
<td>60.1 ± 0.06a</td>
</tr>
<tr>
<td>Gross energy</td>
<td>66.2 ± 0.03b</td>
</tr>
</tbody>
</table>

a,b,c figures in the same row not marked by the same prefix are significantly different from one another (P<0.05)

± (standard error of mean)

Total feed intake (concentrate + forage) decreased with increase in the levels of energy and not with protein levels. As a result of this, the goats on the MPME and LPHE diets therefore consumed smaller amounts of concentrates to meet their requirements for growth. It has been reported that animals tend to consume less if a diet is high in energy before meeting their energy requirements for growth. On the other hand, animals tend to eat more of a diet if it is low in energy. Therefore, the high concentrate intake observed in the goats fed HPLE compared to MPME and LPHE could be due to its low energy content. It is well known that animals on ad libitum feeding will tend to equalise their digestible energy consumption. It was observed that the voluntary concentrate intake of the goats in this experiment was influenced by the concentration of the diets’ energy content (Table 3).

Live weight gains of the goats fed the three diets are within the values reported by Susumu, but higher than the values of Solomon. For the same breed and age of goats used in this trial. Results obtained on goats fed the HPLE and LPHE diets demonstrated that high energy and high protein fed separately have an effect on growth rate of crossbred Anglo-Nubian goats.

It was observed that the diets had similar organic matter contents. However, due to the composition of the main nutrient, i.e. energy and protein levels apparent organic matter digestibility was highly influenced. The microbial population in the rumen is responsible for digestion of feed consumed and fermentation results in the production of volatile fatty acids, the major source of energy for ruminants and this is represented by digestible organic matter. The diet with the higher digestible organic matter is expected to provide more energy and, therefore, more production, i.e. high weight gain. The goats on the MPME had the highest digestible organic matter among the experimental diets and the corresponding weight gain was higher in goats that received the diet, showing the superiority of MPME diet over LPHE and HPLE diets, respectively. The results obtained, therefore, demonstrate that dietary energy and protein should be in equilibrium at a particular level as in MPME diet to meet requirements for growth of crossbred Anglo-Nubian goats in the tropical environment of Samoa.

Apparent digestibility coefficients of nutrients reflected nutrient components of the experimental diets, their level and efficiency of utilization. Increased dietary protein had been observed to cause depression in crude fibre digestibility. Depression in crude fibre digestibility in the HPLE diet demonstrated resultant implication of high protein intake. The results obtained on crude protein and gross energy digestibility indicated that the goats responded to the utilization of the two critical nutrients for growth.

In conclusion, the estimated dietary level of protein and energy for optimum growth in this study is 13.4% CP plus 13.4 MJ GE/kg BW. Although the National Academy of Sciences
(NAS)\(^{19}\) suggested that 11 to 12\% CP concentration was adequate to meet requirements for moderate gains in goats, it was observed in this experiment that the goats offered the LPHE diet containing 12.4\% CP had the least growth rate. Therefore, it could be suggested that breed, environmental conditions and the ecological zone might be implicated in the requirements for protein and energy. This further suggested that the breed of goat should be considered when nutrient requirements of growing goats are estimated. This trial therefore recommends that for maximum performance in terms of optimum growth rate a concentrate diet that contains 13.4\% CP and 13.4 MJ GE/kg BW best meets crossbred Anglo-Nubian goats requirements and a MPME dietary treatment is recommended in the tropical environment of Samoa, South Pacific region.

Acknowledgements

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References


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CROSS-IMMUNITY STUDIES BETWEEN COWDRIA RUMINANTII ISOLATES FROM THE
SAVANNA AND FOREST VEGETATIONAL ZONES OF NIGERIA

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ÉTUDES SUR L'IMMUNITÉ CROISSE ENTRE LES SOUCHE DE COWDRIA RUMINANTII
PROVENANT DES ZONES DE SAVANE ET DE FORET DU NIGERIA

Résumé
Deux souches de Cowdria ruminantium obtenues des zones de savane et de forêt du Nigeria ont été utilisées pour entreprendre des études sur l'immunité/croisée chez les chèvres de savane roussettes locales. Les expériences consistaient en une infection primaire des animaux répartis en deux groupes de 4 animaux/groupe avec l'une des souches, suivie d'une inoculation d'épreuve secondaire homologue et tertiaire hétérologue à trois semaines d'intervalle. L'absence de réactions cliniques aux inoculations d'épreuve homologue et hétérologue a permis de confirmer l'immunité et l'immunité croisée respectivement. Il a été constaté que les deux souches conféraient l'immunité homologue aux animaux infectés. Cependant, alors que les animaux immunisés contre la souche de savane étaient protégés contre l'épreuve d'infection par la souche de forêt, celle-ci n'a pas pu protéger tous les animaux immunisés contre la souche de savane plus pathogène.

Abstract
Two Cowdria ruminantium isolates obtained from savanna and forest vegetational zones of Nigeria were used in immunity and cross-immunity studies in local savanna brown goats. The experiments involved primary infection of the animals divided into two groups of four animals per group with either of the isolates followed by secondary homologous and tertiary heterologous challenges, three weeks apart. Lack of clinical reactions to homologous and heterologous challenges was used to confirm immunity and cross-immunity respectively. It was observed that both isolates conferred homologous immunity on the infected animals. However, while the animals immune to the savanna isolate were protected against challenges by the forest isolate, the latter failed to protect all the animals, immune to it, against the more pathogenic savanna isolate.

Introduction
Previous reports indicated antigenic similarities among Cowdria ruminantium isolates from different geographical areas. The belief changed when a murinotrophic strain of C. ruminantium, the Kumm strain, was isolated. Antigenic differences have been shown between the Kumm stock and the Ball 3 for which there was only unilateral cross-immunity, incomplete cross-immunity between the Kwanyanga (another mice pathogenic strain) and Ball 3, complete lack of cross-immunity between Kwanyanga and Kumm strain and also between Ball 3 and the Senegal stocks.

The different stocks of C. ruminantium were tested, eight from South Africa, one from Mali and one from the Comoro Islands. Only two (Ball 3 and Germishuy) showed antigenic similarities among the eight South African stocks tested.

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out. The studies reported here were therefore undertaken to assess the protection /cross protection ability of *C. ruminantium* isolates from sedimentary herds in the forest (Akure) and savanna (Shika) zones of Nigeria. This is important because of the likelihood of future heartwater control strategies that may involve the use of vaccines in all parts of Nigeria.

**Materials and Methods**

**Isolate of *C. ruminantium***

The savanna (Shika) isolate was obtained from twenty five *Amblyomma variegatum* ticks collected from sedimentary Zebu cattle in Shika, Zaria, in the savanna zone of Nigeria. The forest isolate originated from Akure in the forest zone and was also isolated from twenty engorged *A. variegatum* ticks collected from sedimentary Zebu/ N'Dama cattle in Akure. The supernatant of the ticks from each zone initiated heartwater in goats.

**Infective materials**

The infective material used was the supernatant of the tick from the Savanna and forest zones, prepared according to Anon\(^1\) and Burrige et al.\(^2\) with some modifications. These included ten minutes of three times washing of the ticks in chilled 70% ethanol and thric in chilled sterile distilled water before being finally rinsed once in chilled phosphate buffered saline (PBS, pH 7.4). The washed ticks were ground-up using sterile pestle and mortar with addition of PBS at 1.8ml per tick. The ground material was allowed to settle for an hour under the laboratory conditions and the supernatant pipetted into sterile test tubes. The supernatant was cryopreserved with the addition of dimethylsphoxide (DMSO) to a concentration of 10% as cryopreservant and snap-frozen in liquid nitrogen in labelled 2ml vials as stabilates. Just before use, the stabilates were thawed rapidly in lukewarm water and 2ml of the material was inoculated intravenously into each animal.

**Experimental animals**

Twelve local savanna brown goats aged between ten months to one year were used. These breeds are highly susceptible to heartwater and mortality of one hundred per cent has been recorded in untreated infections. The animals were negative for blood and intestinal parasites on pre-infection evaluation using parasitological techniques. The animals were preconditioned for three weeks in arthropod-free pens before use and were fed grass hay and cotton seed cake. Salt lick and water were provided *ad libitum.*

**Immunity and Cross-immunity tests**

The animals were divided at random into three groups of four animals per group. Eight animals were infected (primary infection) while four identified as (118, 5258, NNM, 5259) were left as uninfected controls. Four of the infected group identified as (5252, 5253, 5254, 5257) were infected intravenously with 2ml of cryopreserved stabilate of savanna (Shika) isolate while four (5256, 52550271, NN11) were similarly infected with the forest (Akure) isolate of *C. ruminantium.* The rectal temperatures were monitored early in the morning (7 a.m.) in addition to physical inspections of the animals. Ensuing clinical reactions (fever) were treated using oxytetracycline at a dosage of 20mg/Kg (Terramycin LA; Pfizer) on the second day of the febrile reaction. The animals infected with the forest isolate were not treated as spontaneous recovery has been reported after some days of pyrexia (Lawal, unpublished observation). However, 5256 was sacrificed two days post-reaction and Giemsa brain squash smears were prepared to further confirm the viability of the stabilate.

All the surviving animals were challenged with the homologous stabilate using the same dose three weeks after the primary infection. The animals were also monitored by taking the rectal temperatures at 7 a.m. in the morning in addition to physical inspections. Three weeks after the homologous challenge, the animals were challenged heterologously and monitored. Febrile reactions of 40°C or more were taken as indicators of clinical reaction. Giemsa stained brain squash smear were made of animals that died and examined for *Cowdria ruminantium.*

**Results**

The febrile responses of the animals to primary infections with the savanna and forest isolates are shown in Figures 1 and 2 respectively. The
Figure 1: Temperature profile of savanna brown goats infected with savanna isolate of *Cowdria ruminantium* (C = Control). (Arrows show points of reactions)
Figure 2: Temperature profile of savanna brown goats infected with forest isolate of *Cowdria ruminantium* (C = Control). (Arrows show points of reactions)
Cross-Immunity Studies Between Cowdria Ruminantium Isolates From The Savanna And Forest Vegetational Zones Of Nigeria

Figure 3: Temperature pattern following homologous and heterologous challenges of savanna isolate infected goats (5257, 5254, and forest isolate infected goats (5255, 0271).
animals infected with the savanna isolate exhibited exceptionally short incubation periods of two to four days prior to the onset of fever which lasted two to three days with a maximum temperature of 40.8°C. The other clinical signs in addition to pyrexia were reduced appetite and weakness. The animals 5253 and 5254 that were not treated died on days 16 and 18 respectively after infection. Scanty colonies of C. ruminantium were detected in the brain capillaries of these animals.

Three of the four animals infected with the forest isolate showed febrile responses after a relatively longer incubation period of 13 to 19 days. Fever lasted two to four days and the maximum temperature attained was 40.5°C. The animals recovered without treatment. The brain of the sacrificed animal (5256) revealed large and extensive colonies of C. ruminantium.

There was no febrile reaction following the homologous and heterologous challenges (Fig. 3). However one of the forest isolate immune animal (0271) which was also heterologously challenged with the savanna isolate, collapsed and died in the morning of the third week and was positive for Cowdria ruminantium particles which resembled the Shika (savanna) type.

Discussion

The infectivity of both isolates was confirmed by the detection of C. ruminantium in the brain squash smears of the dead/sacrificed animals. The two isolates were able to initiate homologous immunity by the absence of clinical reaction to challenge infection. One of the features of the savanna (Shika) isolate is its virulence which was exemplified by the short incubation period. The much studied D225 of C. ruminantium isolate from a research farm adjacent to a sedentary farm where the savanna (Shika) isolate originated, gave an incubation period of 4 to 12 days when infection was introduced by whole blood. The shorter incubation period in the present study might be due to the use of tick derived-inoculum which is known to contain much more infective antigens than the infected blood. Variations in the ages of experimental animals might also be a contributing factor in the discrepancy noticed in the incubation periods recorded by the different authors. The mildness of the forest (Akure isolate) in savanna goats is noteworthy as none of the several isolates of Nigerian origin has been associated with these features.

It is indicative in this study that the animals which are immune to the Shika isolate do not succumb to a challenge by the forest (Akure) isolate. The Akure isolate also protected most of the animals against the Shika one with the exception of animal 0271. The Akure isolate was only able to give partial protection to the animal against the pathogenic Shika isolate by the absence of febrile reaction. However, the organism succeeded in colonizing the brain of the goat which died eventually. Although it is difficult to speculate that there are antigenic differences between the two isolates because only one animal succumbed to heterologous challenge, there are certainly glaring differences in their morphology and pathogenicity. It is paradoxical that the Shika isolate which produces small and scanty colonies in the brain is more pathogenic than the Akure isolate which has long and extensive colonies almost transversing the entire microscopic field. Probably, little or no mechanical damage is associated with the presence of C. ruminantium in the brain of infected animals as reported previously. Thus toxins produced by the parasite have been incriminated in the pathogenic effects of C. ruminantium. Although the conclusion from this study may be limited, it may not be epidemiologically safe to assume that our local isolates of C. ruminantium are antigenically similar as reported earlier. More investigations on the antigenic characteristics of Nigerian isolates are therefore required using in vitro techniques which will enable more detailed studies than the in vivo experiments.

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ACUTE TOXICITY STUDIES ON AQUEOUS EXTRACT OF STEM BARK OF *BUTYROSPERMUM PARADOXUM* IN RATS

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ETUDE DE LA TOXICITE AIGUE SUR L’EXTRAIT AQUEUX DE L’ECORCE DE *BUTYROSPERMUM PARADOXUM* CHEZ LES RATS

Résumé

L’extrait aqueux de l’écorce de *Butyropernum paradoxum* a été analysé pour déterminer sa toxicité aigue chez les rats. On a noté que DL50 par voie intrapéritonéale de l’extrait était de 240 mg/kg. L’apparition des signes cliniques dépendait de la dose. Avec de fortes doses, tous les rats étaient très déshydratés, faibles, déprimés, anorexiques, avaient des difficultés pour respirer avant de mourir. Tandis qu’avec de faibles doses, ils avaient une légère dépression, une difficulté respiratoire et l’anorexie. La néphrite avec la dégénerescence tubulaire et la nécrose était observée dans les reins de tous les rats morts, tandis que la dégénérescence grasseuse avec la nécrose, la congestion pulmonaire et l’œdème était notée dans le foie et les poumons respectivement. Ces résultats montrent que l’écorce de *B. paradoxum* est fortement toxique et doit être utilisée avec précaution en médecine traditionnelle.

Mots-clés : *Butyropernum paradoxum*, écorce, toxicité, rats, médecine traditionnelle.

Abstract

Aqueous extract of stem bark *Butyropernum paradoxum* was tested for acute toxicity in rats; the intraperitoneal LD50 of the extract was found to be 240 mg/kg. The manifestation of clinical signs was dose related. At high doses all the rats became markedly dehydrated, weak, depressed, anorexic and had difficulty in respiration before death; while at low doses they developed mild depression, respiratory difficulty and anorexia. Nephritis with tubular degeneration and necrosis were noticed in the kidneys of all dead rats, while fatty degeneration with necrosis and pulmonary congestion and oedema were noticed in the liver and lungs respectively. These findings indicated that the stem bark of *B. paradoxum* is highly toxic and should be used with caution in folkloric medicine.

Key words : *Butyropernum paradoxum*, stem bark, toxicity, rats, folklore medicine.

Introduction

Toxicological problems that would be encountered in range and sown pasture may be mainly due to poisonous plants used either as feed or as medicinal plants. Conditions which favour the occurrence of plant poisoning in livestock are prevalent in Nigeria especially in the arid and semi-arid regions1. During the long annual dry season and particularly during the periodic droughts that occur in the semi-arid and arid areas, animals may be forced to move from one place to another looking for pasture and water. This movement of animals to a new environment usually exposes them to poisonous plants which they are not familiar with, and which they may consume resulting in poisoning. Quite a number of plant poisoning has been reported in Nigeria1,2,3. Poisoning has also been known to occur from herbal treatment of animals4 especially as herdsmen, village elders and others who keep animals claim experience in diagnosing and treating animal diseases5. However, only a few of these plants used by traditionalists have been properly identified and documented6,7,8. Of these, only a very small percentage has been subjected to scientific analysis, hence their safety and efficacy are questionable.

Recently, there has been an upsurge in the use of the decoction from stem bark of *Butyropernum paradoxum* sub spp. Parkii (G. Don) Hepper for treatment of animals suspected to be suffering from trypanosomosis by herdsmen in Borno State, Nigeria. A description of the plant and its distribution is available9 and was previously documented to have efficacy against gastrointestinal helminths10. When macerated with the bark of *Ceiba pentandra* (L) GAETRN and Salt bark infusions of *B. paradoxum* have been used to treat cattle with worms in Senegal and Guinea11. Stem bark infusions of the plant have been used in the
treatment of leprosy in Guinea Bissau and for gastric problems as well as for diarrhoea and dysentery. A bark decoction of the plant is used in Côte d’Ivoire in baths and sitz-baths to facilitate delivery in labour and is drunk to encourage lactation after delivery. The bark infusion is also used as an eyewash to neutralize the venom of the spitting cobra, and also as foot bath to help extract jiggers. The stem bark of *B. paradoxum* is so popular in African folklore medicine that Greenwood noted that the tripping of the bark for medicinal purposes may have a severe impact on the health of the tree and may even be fatal. However, no report of the toxicity of *B. paradoxum* is found in the literature. The purpose of this study was to investigate the acute toxicity of the stem bark of *B. paradoxum* using rats as models.

**Materials and Methods**

**Plant collection and identification**

The plant *Butyrospermum paradoxum* was collected in November 1998 and identified by a botanist in the Department of Biological Sciences, University of Maiduguri. Part of the stem bark of the plant has been deposited at the Department of Veterinary Physiology and Pharmacology, University of Maiduguri for reference.

**Extract preparation**

The stem bark of the plant was air-dried in the laboratory for two weeks and ground up into powder with the help of a mortar. A hundred grams of the powdered stem bark was exhaustively extracted in water (700 mls) for eight hours at 100°C using soxhlet extractor (Quickfit, England). The extract was concentrated in a vacuum rotary evaporator. The concentrated extract was stored at 4°C until used.

**Experimental design**

Twenty albino rats of both sexes weighing 92 to 245.5 gm were randomly separated into groups (A,B,C,D) of five rats each. The rats were obtained from Biochemistry Department of the University of Maiduguri and kept in clean plastic rat cages in the Department of Veterinary Physiology and Pharmacology, where they were given water and feed (Growers Mash, ECWA, Nigeria PLC) *ad libitum*. They were allowed to adjust to the laboratory environment for a period of two weeks before the commencement of the experiment.

The animals in all the groups were intraperitoneally treated with single doses (100, 200, 400, 800 mg/kg body weight) of the water extract of the stem bark of *B. paradoxum*.

**Figure 1**: The severity of clinical signs in rats given different dose of *Butyrospermum paradoxum* stem bark extract.

![Figure 1: The severity of clinical signs in rats given different dose of *Butyrospermum paradoxum* stem bark extract.](image)

**Keys for the scores:**

- 1 Depression; 2 Anorexia; 3 Starry hair coat; 4 Incoordination; 5 Dehydration; 6 Weakness; 7 Respiratory difficulty; 8 Recumbency/coma; 9 Death
respectively. The rats were observed for a period of 24 hours for clinical signs and death. The LD$_{50}$ of the water extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude$^{18}$. Postmortem examination of dead animals was performed and samples collected and placed in 10% formal saline, embedded in paraffin wax, and cut 5u thick before staining with haematoxylin and eosin (H&E).

Results

Clinical signs

Following the administration of the water extract of *B. paradoxum* stem bark to rats intraperitoneally, the animals showed clinical signs of anorexia, starry haircoat, depression, weakness of the hindlimbs which progressed to the forelimbs, sternal recumbency, difficulty in respiration, dehydration, coma and death. The anorexia and depression were noticed immediately after extract administration, the other signs appeared after three hours and they continued until death.

The dose of the extract that produced 100% mortality was 400mg/kg, while the dose that produced 0% mortality was 100mg/kg. Scores were allocated for each of the above signs; and these scores are summed up in Figure 1. The severity of the signs were dose related, at higher doses (200, 400, 800mg/kg) coma and death were observed.

Gross lesions

The carcasses were markedly dehydrated and there was congestion and oedema of the lungs and kidneys. There was hepatomegaly with focal necrosis. The liver was also pale and fragile. These lesions were dose related and were observed to be more severe with the highest dose.

Histopathology

The liver showed fatty change, bile duct hyperplasia, and mild periportal necrosis (Figure 2). The portal triad exhibited mononuclear cell infiltration. In the kidney there was severe necrosis, degeneration and atrophy of renal epithelial cells and dilatation of some tubules. Also there was mild mononuclear cell infiltration into the interstitium of the kidney (Figure 3).

![Figure 2: Liver of rats showing fatty change and bile duct hyperplasia (H & E x 400)](image-url)
Figure 3: Kidney showing tubular necrosis, degeneration and mild leukocytic infiltration and dilatation of tubules in rats given water extract of Butyrospermum paradoxum at a dose of 400 mg/kg (H & E x 400).

Discussion

From the results obtained in the acute toxicity study, the LD_{50} (IP) of the water extract of Butyrospermum paradoxum stem bark in rats is 240mg/kg showing that the extract is very toxic. According to Clarke and Clarke any substance whose LD_{50} in rats falls between 50-500mg/kg is regarded as very toxic.

The administration of the extract to rats resulted in pathological changes in the liver, kidney and lungs. The lesions observed in the liver were mainly fatty change and bile duct hyperplasia suggesting that the toxic or active principle of the extract interfered with lipid metabolism and is irritating to the bile ducts. These lesions are, however, not specific to the extract of B. paradoxum as many other toxins are known to induce fatty change in the liver. The lesions observed in the liver may have occurred due to the metabolism of the toxic principle in the liver, which is the primary organ of biotransformation. The kidney was also adversely affected by the extract. The necrotic/degenerative changes observed in tubular epithelia with attendant leukocytic infiltration suggest that the toxic principle may be excreted through the kidney and is toxic to tubular epithelial cells. The renal tubular damage might have been responsible for the dehydration, starry haircoat and depression since such damage can cause increased loss of water in tubular urine. Pulmonary congestion and oedema were observed in this study indicating wide distribution of the toxic principle to the various organs and tissues in the body. The observed respiratory difficulty in the treated rats was probably due to the respiratory lesions. Indeed in northern Nigeria the stem bark extract of B. paradoxum is suspected to be toxic.

In conclusion, this study has shown that the aqueous extract of the stem bark of B. paradoxum can cause damage to the liver, kidney and lungs with resultant production of clinical signs such as depression, weakness, anorexia, recumbency, dehydration, starry haircoat, respiratory difficulty and death; if
consumed in high amount. Therefore, the aqueous extract of the stem bark of *B. paradoxum* should be used with caution in African folklore medicine.

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AN EVALUATION OF AN IgG ELISA FOR THE DIAGNOSIS OF BOVINE LEPTOSPIROSIS
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UNE EVALUATION DE L'ELISA - IgG POUR LE DIAGNOSTIC DE LA LEPTOSPIROSE BOVINE

Résumé
Cinq cent quatre-vingt-dix-sept bovins de boucherie ont été examinés pour le dépistage d'anticorps anti leptospires à l'aide du Test d'agglutination microscopique (MAT) et du Titrage avec immunoadsorbant lié à une enzyme (Test ELISA).
Cent quarante (23,45%) des animaux échantillonnés étaient positifs avec la technique ELISA, tandis que 107 (17,92%) réagissaient au Test MAT. Toutefois, il n'y avait pas de différence significative (P>0,01, χ² = 5,56) quant au nombre d'animaux qui ont réagi aux deux tests. L'ELISA avait une sensibilité de 95% et une spécificité de 93,27%. En utilisant le test MAT, 25 (23,37%) des animaux séropositifs ont réagi à l'antigène Leptospira hardjo, alors que 20 (18,69%) et 18 (16,82%) étaient séronegatifs à L. pomona et L. interrogans, respectivement.

Abstract
Five hundred and ninety seven abattoir cattle were tested for evidence of leptospiral antibodies using the Microscopic Agglutination Test (MAT) and the Enzyme-Linked Immunosorbent Assay (ELISA) test. One hundred and forty (23.45 percent) of the sampled animals were positive by ELISA technique while 107 (17.92 percent) were reactive in the MAT. However, there was no significant difference (p>0.01, χ² = 5.56) in the number of reactors by both tests. The sensitivity of the ELISA was found to be 95 percent while the specificity was 93.27 percent. One MAT, twenty five (23.37 percent) of the seropositive animals reacted with the Leptospira hardjo antigen, while 20 (18.69 percent) and 18 (16.82 percent) were seronegative to L. pomona and L. interrogans, respectively.

Introduction
Bovine leptospirosis is a worldwide zoonotic spirochetal disease with major economic implications in the cattle industry. A major limiting factor to leptospirosis research is the Modus Operandi of the conventional Microscopic Agglutination Test (MAT) for leptospirosis. By comparison with ELISA, the MAT is tedious, time consuming, requiring a darkfield microscope and may be associated with a human health risk. Some investigators have evaluated the ELISA technique as an alternative to the MAT for the diagnosis of leptospirosis in naturally and experimentally infected cattle. However, there is controversy on the suitability of the ELISA as a screening test for leptospirosis.

At present, there is a paucity of information on bovine leptospirosis in Africa. Serological prevalences to leptospirosis in Nigerian cattle have been shown to vary from 72 percent to 11.9 percent in northern Nigeria. The objectives of this work were to determine the suitability of an ELISA technique as a screening test for leptospirosis in cattle and the leptospiral serovars associated with seropositive cattle in Ibadan, Nigeria.

Materials and Methods
Leptospiral Strains and Antigens Used
Eleven live leptospiral serovars obtained from the Veterinary Research Laboratories in Stormont, Northern Ireland were used for the MAT. These were: L. bratislava (Jez bratislava), L. ballum (S102), L. canicola (Hond Utrecht IV), L. ceyonoperi (3522C), L. grippoyphs (Moska V), L. hammoni (hampton), L. hardjo (hardjo prajtino), L. interrogans (RGA), L. javanica (Veldarat bataviae), L. pomona (pomona), L. tarassovi (perepelicin). They were grown in Ellinghausen media modified by Johnson and Harris. For the ELISA Sodium dedocyl sulphate (SDS) solubilized antigens of L. grippoyphs hamtoni, L. hardjo and L. pomona were used.

Blood Collection and Processing
Blood samples were obtained from a total of 597 randomly selected cattle at the Bodija abattoir in Ibadan, Nigeria. The blood samples were collected aseptically via the jugular vein at slaughter. About 15ml of blood was collected from each animal into sterile plain bottles and allowed to clot. The approximate age, breed, sex of animals were determined and recorded. The pregnancy status of female animals were also...
recorded. After clotting of the blood, the sera was separated by centrifuging at 1,000 xg for ten minutes. The sera from the 597 cattle were divided into two aliquots of about 3 ml each and stored frozen at –20°C until required.

**Preparation of ELISA Antigen**

Sodium dodecyl sulphate (SDS) solubilized antigens of *L. grippotyphosa*, *L. hamptoni*, *L. hardjo* and *L. pomona* were prepared as earlier described and modified with a few variations. Briefly, each of the test organism was grown aseptically in two litres of EMJH media for six days. The leptospiras were harvested and washed thrice with Phosphate Buffered Saline (PBS pH 7.2) using a Sorvall® superspeed RC 2B automated centrifuge. 50 ml of 1M NaCl was added to the sedimented leptospiras. The mixture was incubated at 37°C for 30 minutes. Spherical salt altered leptospiras were looked for under darkfield microscope. After at least 90 percent of the leptospiras had converted to spherical or cylindrical forms the mixture was centrifuged at 31,000 xg for 30 minutes and the sediment diluted with 5 ml distilled water. 50 ml of a 0.02 percent (W/V) SDS was added and the sample incubated at room temperature for 30 minutes. The mixture was recentrifuged at 31,000 xg for 30 minutes at 4°C using a Beckman® L8 – 80 ultracentrifuge. The concentration of protein was determined in the detergent solubilized fraction. The antigen concentration was adjusted to 500 μg/ml of protein.

Elimination of SDS from the detergent solubilized fraction was done by ion exchange, with a few changes using freshly prepared extraction solvent. This was prepared using a mixture of anhydrous acetone: triethylamine: acetic acid: water (ratio 85:5:5:5; V/V). The detergent solubilized antigen was added to freshly prepared extraction solvent at a ratio of 1:1 (V/V). The mixture was incubated at 4°C overnight and then centrifuged at 7,000 xg at 4°C for 10 minutes. The supernatant was removed leaving a whitish precipitate. This precipitate was washed twice with anhydrous acetone and left in a vacuum desiccator for two hours to remove excess acetone. The antigen was resuspended in 2 ml of carbonate-bicarbonate buffer (pH 9.6). The protein concentration was again determined and stored in aliquots of 500 μl each at –20°C until wanted for the ELISA.

**The Enzyme-Linked Immunosorbent Assay (ELISA)**

The ELISA was carried out as previously described. The ELISA plates were coated overnight with the optimum concentration of a pool of the detergent solubilized antigen of the four test serovars. After washing of the plates with Tris buffered saline with 0.05 percent Tween (TBS-Tween, pH 7.4), a two-fold serial dilution of the test sera was made with negative and positive sera reference wells. Subsequently the plates were incubated at 37°C for one hour and washing repeated.

Horseradish peroxidase conjugated goat antibovine IgG and O-Phenelenediamine (OPD) (Sigma Laboratories, USA) were used as label antibodies and chromogen respectively.

**The Microscopic Agglutination Test (MAT)**

The 589 bovine serum samples were tested for antibodies to the eleven live leptospiras using the MAT as described by Galton et al. and modified. The leptospiras were subcultured in EMJH media. Six days old cultures containing approximately 5 x 10⁸ leptospiras per ml were used for the test. A titre of 1/100 and above was considered significant.

**Analysis of the Data**

The effect of age and sex on the seropositivity status were analyzed using the Chi-square test with Yates correction for continuity.

**Results**

**Sampled Animals**

Five hundred and ninety seven slaughter cattle were tested for evidence of antibody to leptospiras using an antibody capture ELISA technique and the MAT. Out of these, 376 (62.98 percent) were females while the rest were males.

**Seroology Results**

One hundred and forty of the sampled cattle were positive to leptospiral antigen by the ELISA. This represented a 23.45% reactor rate. Of the seropositive cattle, 69% were females while the rest were males.
One hundred and seven animals were positive to nine out of the eleven screening leptospiras at a titre of 1/100 and above, representing a 17.92% seropositivity rate. Twenty five (23.37%) of the animals reacted with L. hardjo antigen while 20 (18.69%) and 18 (16.82%) were seropositive to L. pomona and L. icterohaemorrhagiae respectively. There was no reactor to L. shermani and L. cynopteri antigen.

Comparative Results of the ELISA and MAT

A total of 140 (23.45%) of the sampled animals were positive by the ELISA technique while 107 (17.92%) were reactive by the MAT (see Table 1). However, there was no significant difference (P>0.01, = 5.56) in the number of animals seropositive by both techniques. Using the MAT as the reference test, the sensitivity of the ELISA was found to be 95% while the specificity was 93.27%.

Discussion

In this work, our data showed that 23.49% and 17.93% of the sampled cattle were positive by the ELISA and the MAT respectively, although the results were not significantly different. Also, the sensitivity and specificity of the ELISA are high (95 and 93.27% respectively). This makes the test suitable for routine screening of leptospirosis in Nigerian cattle where routine vaccination of cattle against the disease is not done. High sensitivity and specificity are desirable in a diagnostic test if the disease has serious economic and public health implications. Similarly high sensitivities have been reported by some workers although others could find no agreement between the ELISA and the MAT.

The slightly greater prevalence rate reported for the ELISA in this work may be of significance in that the MAT is a serogroup specific test. If all the serogroups prevalent in an area are not used in the agglutination test, there might be an under-estimation of the actual prevalence rate. At present there are about 23 serogroups of pathogenic leptospiras recognized worldwide and information on leptospirosis in African livestock is scarce.

The overall reactor rate to leptospiras reported in this work using the MAT was higher than the 14.4% infection rate earlier reported but lower than the 48% and the 72% infection rates earlier reported in cattle in northern Nigeria. These earlier reported prevalence to leptospiras in northern Nigeria and the results of the present work indicate that leptospirosis is endemic in Nigeria.

Although only an IgG ELISA was used in this work, it is known that both IgG and IgM classes of antibodies are involved in agglutination in leptospirosis. The high prevalence rate reported for the ELISA may be due to the production of IgG as well as IgM antibodies early in the course of leptospiral infection. It has been observed that both IgM and IgG antibodies were produced at about the same time in experimentally infected cattle, but the IgG antibodies persisted much longer in infected animals. In this work, it is possible that IgM

Table 1: Comparative Leptospiral Titres in Bovine Sera Measured by an ELISA Technique and the MAT

<table>
<thead>
<tr>
<th>MAT Titres</th>
<th>ELISA TITRES</th>
<th>1:50</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>1:800</th>
<th>1:1,600</th>
<th>1:3,200</th>
<th>1:6,400</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>457</td>
<td>18</td>
<td>15</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>1:100</td>
<td>11 (17)</td>
<td>9 (11)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>1:200</td>
<td>1 (2)</td>
<td>5 (B)</td>
<td>27</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1:400</td>
<td>1 (3)</td>
<td>1</td>
<td>3</td>
<td>22</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1:800</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>1:1,600</td>
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<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>1:3,200</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>1:6,400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>457</td>
<td>31</td>
<td>30</td>
<td>29</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>597</td>
<td></td>
</tr>
</tbody>
</table>

( ) 14 multiple reactions seen in MAT
antibodies were present in the sampled animals. The ELISA technique reported in this work was faster than the agglutination test requiring a maximum of three hours for completion. In comparison, the MAT required at least five hours. The ELISA was also less subjective with no human health risk involved in the handling of the antigen prepared. The ELISA appears well suited for the routine screening of bovine leptospirosis in Nigeria where vaccination against the disease is not a routine procedure.

Acknowledgements
We are grateful to Prof. W.A. Ellis and Mr. John Montgomery of the Veterinary Research Laboratories, Stormont, Northern Ireland for the supply of live leptospirae and some reagents. The first author was on a Fulbright fellowship at the University of Georgia, Athens, USA when the SDS solubilized antigen were prepared. We are grateful to the Fulbright organization for this award.

References

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THE EFFECT OF COCCIDIAL INFECTION ON THE REACTIVITY OF THE SMOOTH MUSCLE OF THE CAECA OF DOMESTIC CHICKENS (GALLUS DOMESTICUS) TO HISTAMINE AND CARBACHOL

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EFFET DE L’INFECTION COCCIDIENNE SUR LA REACTIVITE DU MUSCLE LISSE DES CAECUMS DES POULETS DOMESTIQUES (GALLUS DOMESTICUS) A L’HISTAMINE ET AU CARBACHOL

Résumé

Les réactions contractiles des segments du caecum des poulets domestiques en bonne santé et de ceux infectés par Eimeria tenella, exposés au carbachol et à l’histamine exogènes, ont été étudiées. Les concentrations molaires progressives des agonistes étaient introduites dans des suspensions des segments dans la solution de Tyrode à 37°C et les réactions étaient enregistrées. Les segments chez les poulets en bonne santé et infectés étaient sensibles aux agonistes. Même si les segments chez les poulets infectés avaient une plus forte sensibilité aux agonistes, ils produisaient des réactions maximales beaucoup plus faibles (P<0,05) dans tous les cas. Alors que les segments chez les poulets en bonne santé laissaient apparaître une courbe de réaction globale avec une forme sigmoïde, cela n’était pas le cas avec les segments chez les poulets infectés. Il est donc conclu que l’infection coccidienne des poulets a considérablement changé la réaction contractile des segments du caecum au carbachol et à l’histamine.

Abstract

The contractile responses of caecal segments from healthy and domestic chickens infected with Eimeria tenella on exposure to exogenous carbachol and histamine were studied. Graded molar concentrations of the agonists were introduced into suspensions of the segments in Tyrode’s solution at 37°C and the responses recorded. Both healthy and infected segments were sensitive to the agonists. Although the infected segments showed greater sensitivity to the agonists, they produced significantly (P<0.05) lower maximum responses in all cases. While the healthy segments elicited an overall response curve with a sigmoid shape, that of the infected segments was non-sigmoid. It is concluded that coccidial infection of chickens significantly altered the contractile response of caecal segments to carbachol and histamine.

Key words: Histamine, carbachol, caecal segments, coccidial infection.

Introduction

Agonists such as histamine and carbachol have attracted a great deal of attention from many investigators of the pharmacology of the autonomous nervous system. Histamine (P-imidazolyl-ethylamine) is a powerful and consistent stimulant of smooth muscle1.

In the intestinal longitudinal muscle of chicks, histamine produced relaxations that were relatively resistant to mepyramine and seemed to result from the release of adrenaline or noradrenaline2. In high concentrations, histamine produces tonic contraction and depolarization3. Apart from being a powerful gastric secretagogue1, histamine causes contraction of the smooth muscles of the intestine4,5.

Carbachol with close structural relationship to acetylcholine was observed to produce an increase in the tone and amplitude of contraction in isolated guinea pig ileum6. This action is mediated by the muscarinic cholinergic receptors and could be blocked by atropine. Changes in receptor population7, and responsiveness of tissues to agonists have been reported in some disease conditions8,9. Although much work has been done on the pharmacological effects of agonists such as histamine and carbachol and their antagonists in various animal species6,10,11,12, there is no information on their effects on isolated smooth muscle preparation in coccidial infected birds. The reactivity of the smooth muscles of the caeca of coccidial infected domestic chicken to histamine and carbachol was therefore studied.
Materials and Methods

Experimental birds

Ten (10) male domestic chickens aged 12 to 14 weeks old obtained from the Monday market, Maiduguri, Nigeria were used. The birds were housed in cages and fed water and grower’s marsh ad libitum. The birds were allowed two weeks acclimatization prior to use.

Drugs

The pharmacological agonists used were histamine acid phosphate (British Drug House Biochemicals, Poole, UK) and carbachol (Carbamylcholine chloride) (Sigma Chemical Compound, USA). All the drugs were dissolved in sterile distilled water and a stock solution of $10^{-2}$ prepared. Further dilutions were made from the stock as desired.

Coccidia organisms

*Eimeria tenella* oocysts used were obtained from infected chicken using direct techniques. The oocysts collected were cultured using freshly prepared 2.0% potassium dichromate for six days for sporulation to take place. Harvested coccidial organisms were administered orally to the chickens. Each bird received 35,550 oocysts. Seventy two hours after infection, the birds were sacrificed and the caeca removed.

Experimental groups

The chickens were divided into two equal groups: one consisted of healthy birds while the other was infected with *E. tenella* oocysts.

Figure 1: Mean ± SD contractile response of caecal segments from healthy (○) and coccidial infected (●) chicken exposed to graded concentrations of carbachol.
**Figure 2:** Mean ± SD contractile response of caecal segments from healthy (○) and coccidial infected (●) chicken exposed to graded concentrations of histamine acid phosphate.

### Tissue preparation

The birds were killed and exsanguinated by cutting the jugular vein and carotid artery in the neck. They were thereafter dissected and the caeca removed. Segments of the caeca, about 2-3 cm in length, were cleaned of faecal materials with Tyrode's solution (NaCl, 8.0; KCl, 0.2; CaCl₂, 0.2; MgCl₂, 0.01; NaH₂PO₄, 0.05; NaHCO₃, 1.0; and glucose, 1.0 g/l). The caecal tissue preparations were then set up in a 50 ml organ bath containing Tyrode's solution, maintained at 39°C and aerated with air using an aeration pump. Each segment was connected to an isotonic myograph transducer and the changes in muscle length were recorded on a biograph recorder (Desk Model Harvard Apparatus 2120).

Varying concentrations of the agonists (histamine and carbachol) were added to the organ bath after an equilibration time of 30 minutes. A contact time of 60 seconds was allowed for the tissue response. Thereafter, the tissue was washed and fluid in the bath replaced to allow the tissue to retain its original tone. The procedure was repeated for each concentration of agonist and the amplitude of contractions measured by standard methods and the degree of agonist effect expressed in percentage.

### Statistical analysis

Data obtained were analysed statistically using unpaired Student's t-test. Significant differences between the coccidial infected and healthy caecal segments were detected at 95% confidence limits.
Results

The mean responses of both normal and infected caecal segments to graded concentrations of the agonists are shown in Figures 1 and 2. The caecal segments (healthy and infected) were sensitive to histamine and carbachol. In all cases the dose response curves of the segments from infected birds shifted to the left, while the peak responses were significantly (P<0.05) lower than those of the healthy caecal segments. The infected segments appeared to respond to the agonist in an irregular manner, while the healthy segments produced curves which fit the sigmoid shape.

Discussion

The results of the present study showed that carbachol and histamine elicited contractile responses in isolated caecal segments from both healthy and coccidial infected chickens. Similar findings have been reported with guinea pig ileum, cattle pulmonary and bronchial artery\(^ {1,5,6}\). However, it was remarkable that maximum contractions of the caecal segments were obtained at lower concentrations of the agonists than in the case of the ileum and strips of arteries.

Coccidial infection was observed to significantly alter the responses of the caecal smooth muscle to the agonists. There were quantitative and qualitative differences between the healthy and infected caecal segment responses to the same concentration of agonists. The infected segments demonstrated threshold responses to lower concentrations of agonists with resultant shifting of the dose responses curves to the left. Also, the peak responses of the infected segments were significantly lower than those of the healthy segments. Similar findings have been observed with ilial and jejunal segments in trypanosom-infected guinea pigs and rabbits, respectively\(^ {8,9}\). It appears that coccidial infection renders the tissues more sensitive but weaker in response to carbachol and histamine. It has been observed that in disease condition there are changes in receptor population, hence, the decreased contractile responses recorded in coccidia infected chickens may have resulted in changes in receptor population. *Eimeria tenella* is known to infect the caecum producing pathological changes that may result in lowered receptor population. Furthermore, the decreased responsiveness to the agonist by the infected tissues may also be due to changes in metabolic activity of the infected tissue cells\(^ {9}\).

In conclusion, coccidial infection of chickens resulted in altered caecal responses to both carbachol and histamine.

Acknowledgements

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References


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SHORT COMMUNICATION

SERO-PREVALENCE OF BRUCELLOSIS IN TRADE CATTLE SLAUGHTERED IN IBADAN, NIGERIA

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Brucellosis is a contagious systemic bacterial disease primarily of ruminants characterised by inflammation of the genital organs and fetal membranes, abortion, sterility and formation of lesions in the lymphatic system and joints. It is a zoonotic disease characterised by undulant fever in man and is associated with considerable economic losses.

In Nigeria, bovine brucellosis is endemic with a pattern of low to high infection rates in specific areas. Evidence of infection has been reported in trade cattle in and from the northern states of Nigeria and also from across the northern borders with Chad and Niger from where most of the cattle slaughtered in southern Nigeria including Ibadan usually originate. Sero-prevalence rates of 6.3% (SAT) and 6.79% were found in cattle tested at slaughter in Ibadan in 1974 and 1994, respectively.

The disease being economically important in Nigeria and an occupational hazard thus requires periodic determination of its prevalence to serve as an index of success or otherwise of its control. This paper reports on the prevalence of brucellosis in slaughtered cattle in Ibadan and the possibility of breed susceptibility to the infection.

Blood samples were collected from the 398 cattle (consisting of 111 bulls and 287 cows) slaughtered at the Bodija Municipal Abattoir in Ibadan. These were mainly trade cattle brought from the northern part of the country and the neighbouring countries like Niger and Chad. Blood from the severed jugular vein of each animal was collected into universal bottles and allowed to clot. Serum was separated from each blood sample and decanted after centrifugation at 3000 rpm for 15 minutes. The sex and breed of cattle were recorded.

Serum samples were then tested for evidence of brucellosis using RBPT as a screening and SAT as a confirmatory test. Weybridge acidified Rose Bengal stained Brucella abortus S19 standard antigen was used for the RBPT while SAT antigen of B. abortus obtained from the National Veterinary Research Institute (NVRI) was used for the SAT. The RBPT and SAT tests were conducted as described by Morgan et al. and Alton et al., respectively.

Table 1: Brucella Abortus in cattle slaughtered at the municipal abattoir, Ibadan

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of sera tested</th>
<th>No. positive for RBPT</th>
<th>Serum Agglutination (SAT) IU/ml*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>titres</td>
</tr>
<tr>
<td>Bunaji</td>
<td>179</td>
<td>11</td>
<td>6.15</td>
</tr>
<tr>
<td>Keteku</td>
<td>114</td>
<td>5</td>
<td>4.39</td>
</tr>
<tr>
<td>Rahaji</td>
<td>64</td>
<td>5</td>
<td>7.81</td>
</tr>
<tr>
<td>Muturu</td>
<td>27</td>
<td>2</td>
<td>7.41</td>
</tr>
<tr>
<td>Sokoto Gudali</td>
<td>14</td>
<td>2</td>
<td>14.28</td>
</tr>
<tr>
<td>Total</td>
<td>398</td>
<td>25</td>
<td>6.28</td>
</tr>
</tbody>
</table>

RBPT = Rose Bengal Plate Test
SAT = Serum Agglutination Test.
IU/ml = Titres in International Units per ml.
Table 2: Brucella Abortus in cattle slaughtered at the municipal abattoir, Ibadan

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. Tested</th>
<th>No. of reactors</th>
<th>Males Number positive</th>
<th>Infection rate (%)</th>
<th>Number tested</th>
<th>No. of reactors</th>
<th>Females No. positive</th>
<th>Infection rate (%)</th>
<th>No. of sera tested</th>
<th>Overall Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunaji</td>
<td>41</td>
<td>2</td>
<td>1</td>
<td>2.44</td>
<td>138</td>
<td>9</td>
<td>6</td>
<td>4.35</td>
<td>179</td>
<td>3.91</td>
</tr>
<tr>
<td>Keteku</td>
<td>42</td>
<td>2</td>
<td>1</td>
<td>2.38</td>
<td>72</td>
<td>3</td>
<td>3</td>
<td>4.17</td>
<td>114</td>
<td>3.51</td>
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<tr>
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<td>11</td>
<td>2</td>
<td>1</td>
<td>9.09</td>
<td>53</td>
<td>3</td>
<td>3</td>
<td>5.56</td>
<td>64</td>
<td>6.25</td>
</tr>
<tr>
<td>Muturu</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>11.11</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>3.70</td>
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<tr>
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<td>1</td>
<td>12.50</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>7.14</td>
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<tr>
<td>Total</td>
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<td>287</td>
<td>17</td>
<td>12</td>
<td>4.18</td>
<td>398</td>
<td>4.27</td>
</tr>
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</table>

RBPT = Rose Bengal Plate Test
SAT = Serum Agglutination Test

Presence of agglutination was recorded as positive for RBPT and such sera were further tested using the SAT. Titres of 50 IU/ml and above were regarded as positive. The prevalence rates across the breeds and between sexes were compared statistically using the Chi-square method and differences were considered statistically significant at the 5% level.

Out of the 398 sera tested, 25 (6.28%) tested positive to the RBPT while only 17 (4.27%) were positive with the SAT. Sero-prevalence rates of 7.14% and 6.25% were obtained for Sokoto Gudali and Rahaji breeds while prevalence rates of 3.91%, 3.70% and 3.51% were obtained, respectively for Bunaji, Muturu and Keteku breeds (Table 1). There were no significant differences in the prevalence rates across the breeds (Chi-square 1.135, P>0.05).

From the 17 sera samples that tested positive to both the RBPT and SAT, 12 (4.18%) and 5 (4.50%) were females and males, respectively (Table 2). There was no significant difference between the males and females (Chi-square 0.021, P>0.05).

The higher prevalence (6.28%) found with RBPT compared to 4.27% found with the SAT is in agreement with previous studies and shows that the RBPT is more sensitive than the SAT. Hence, RBPT should be regarded as a screening test only while SAT can be used as a confirmatory test for field surveillance programmes in localities where facilities for other more accurate tests are not readily available. The presence of positive results confirm the presence of brucellosis in this region and thus human beings, especially those with occupational exposure, are at risk of contracting the disease from cattle.

The prevalence of 4.27% (SAT) obtained in this study is relatively lower than earlier reports of 6.3% and 6.79% recorded in trade cattle tested at slaughter in Ibadan. This confirms the prevailing endemic status of the disease but at a lower level. This low level of brucella infection could be due to the fact that the majority of the trade cattle tested were reared by nomads who are constantly on the move thereby limiting the chances of accumulating infection or spreading it among the animals.

The prevalence rates observed for the different breeds of cattle show the probable infection rates in the areas from which the breeds originate. Since there were no significant differences in the prevalence rates across the breeds or between the sexes, it showed that the organism can infect any breed or any sex as long as the conditions are favourable for its survival. Infection rates have been found to be equally distributed among varying breeds. In view of the economic and public health importance of brucellosis which has maintained an endemic status in Nigeria for some time, there is need to institute a national control programme.

**Acknowledgement**

We are grateful to Professor F.O. Ayanwale for his immense contributions to the success of this study.

**References**

Dermatophilosis caused by * Corynebacterium bovis* is a chronic, progressive and sometimes fatal exudative dermatitis. The disease affects a wide host range which include cattle, sheep, goat, horses, pigs and a variety of wild species such as antelope, deer, giraffe, zebras, snakes, and lizards. It causes severe weight loss, decrease in milk yield during lactation (in dairy animals), death, and histologically it causes boilages, incontinence of basal lamina and destruction of the collagen fibril with epithelial separation in the gran and consequently resulting in poor leather quality.

Dermatophilosis is worldwide in distribution though earlier reports were from the tropical and sub-tropical countries. Prevalence rates reported in previous studies were 11.28% in herds in northern Nigeria and 32.8% in abattoir samples in Ibadan. It has been reported to be the most important skin disease of cattle in the tropics and is considered by the FAO as one of the four major bacterial diseases of cattle in the tropics. The disease has been observed to be more prevalent among Fulani herds during the wet and dry seasons of the year.

This paper reports the prevalence of dermatophilosis in trade cattle slaughtered in Oyo State between 1990 and 1997. This is with a view to updating existing knowledge on the disease.

Data on dermatophilosis cases diagnosed at the abattoirs in Oyo State were collected. Meat inspection in these abattoirs was conducted by staff of the Veterinary Department of the Ministry of Agriculture. Since cases in different breeds and ages were not included in the abattoir records, a special survey was conducted between January 1995 and December 1996 to

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SHORT COMMUNICATION

THE PREVALENCE OF DERMATOLOGIȘIS IN TRADE CATTLE SLAUGHTERED IN OYO STATE, NIGERIA

V., ADETUNJI, G.A.T OGUNDIPE* and A. ADEGBOLA

Department of Veterinary, Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria

Dermatophilosis caused by *Dermatophilus congolensis*, a gram positive pleomorphic bacterium is characterised by an acute or chronic, local or progressive and sometimes fatal exudative dermatitis¹. The disease has a wide host range which include cattle, sheep, goat, horses, pigs and a variety of wild species such as antelope, deer, giraffe, zebra, snakes, and lizards². It causes severe weight loss, decrease in milk yield during lactation (in dairy animals), death, and histologically it causes breakages in continuity of basal laminae and destruction of the collagen fibrillar with spatial disorientation in the grain and consequently resulting in poor leather quality³.

Dermatophilosis is worldwide in distribution though earlier reports were from the tropical and sub-tropical countries². Prevalence rates reported in previous studies were 11.98% in herds in northern Nigeria⁴ and 1.28% in abattoir samples in Ibadan⁵. It has been reported to be the most important skin disease of cattle in northern and western Nigeria⁴,⁵,⁶ and is considered by the FAO as one of the four major bacterial diseases of cattle in the tropics⁷. The disease has been observed to be more prevalent among Fulani herds during the wet than dry seasons of the year⁵,⁷.

This paper reports the prevalence of the dermatophilosis in trade cattle slaughtered in Oyo State between 1990 and 1997. This is with a view to updating existing knowledge on the disease.

Data on dermatophilosis cases diagnosed at the abattoirs in Oyo State were collected. Meat inspection in these abattoirs was conducted by staff of the Veterinary Department of the Ministry of Agriculture. Since cases in different breeds and ages were not included in the abattoir records, a special survey was conducted between January 1995 and December 1996 to obtain them. During the survey, the authors participated in meat inspection at Bodija and Moniya abattoirs on Tuesdays and Thursdays. The ages of cattle slaughtered were determined using dentition as described by Dyce and Wensing⁸.

The mean monthly rainfall data for the state were obtained from the Department of Geography, University of Ibadan. The seasonal prevalence was determined through the analysis of correlation between monthly infection rates and the volume of rains in each month.

A total of 7,825 or 1.48% of the 528,707 cattle slaughtered in Oyo State between 1990 and 1997 were infected with *D. congolensis*. The annual infection rates were on the increase (Table 1). The highest infection rate of 2.13% was recorded during the peak rainy season, followed by 2.03% during the pre-dry season, and 0.89% during the pre-rain period and lastly 0.47% during the peak dry season (Table 2; Figure 1). There is a significant positive correlation (r=0.68, P<0.05) between monthly rainfall and infection rates.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Cattle Slaughtered</th>
<th>Number Infected</th>
<th>As % of Cattle Slaughtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>86,262</td>
<td>899</td>
<td>1.04</td>
</tr>
<tr>
<td>1991</td>
<td>97,662</td>
<td>901</td>
<td>0.92</td>
</tr>
<tr>
<td>1992</td>
<td>27,766</td>
<td>269</td>
<td>0.97</td>
</tr>
<tr>
<td>1993</td>
<td>20,313</td>
<td>289</td>
<td>1.38</td>
</tr>
<tr>
<td>1994</td>
<td>42,594</td>
<td>736</td>
<td>1.73</td>
</tr>
<tr>
<td>1995</td>
<td>76,684</td>
<td>1,579</td>
<td>2.06</td>
</tr>
<tr>
<td>1996</td>
<td>93,677</td>
<td>1,561</td>
<td>1.67</td>
</tr>
<tr>
<td>1997</td>
<td>83,749</td>
<td>1,599</td>
<td>1.91</td>
</tr>
<tr>
<td>Mean</td>
<td>66,088</td>
<td>978</td>
<td>1.48</td>
</tr>
<tr>
<td>Total</td>
<td>528,707</td>
<td>7,825</td>
<td>1.48</td>
</tr>
</tbody>
</table>
Table 2: Seasonal prevalence of dermatophilosis in trade cattle slaughtered in Oyo State (1990-97)

<table>
<thead>
<tr>
<th>Season</th>
<th>No. slaughtered</th>
<th>No. Infected</th>
<th>% Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dry (Oct-Nov)</td>
<td>91,915</td>
<td>2,093</td>
<td>2.28</td>
</tr>
<tr>
<td>Dry (Dec-Feb)</td>
<td>133,934</td>
<td>626</td>
<td>0.47</td>
</tr>
<tr>
<td>Pre-rain (Mar-April)</td>
<td>90,122</td>
<td>801</td>
<td>0.89</td>
</tr>
<tr>
<td>Rain (May-Sept)</td>
<td>212,736</td>
<td>4,305</td>
<td>2.02</td>
</tr>
<tr>
<td>Total</td>
<td>528,707</td>
<td>7,825</td>
<td>1.48</td>
</tr>
</tbody>
</table>

The overall prevalence rate of 1.48% obtained in this study was slightly higher than the 1.28% obtained in previous studies in Ibadan abattoirs. The gradual increase in the infection rates may be due to the absence of any concrete nationally coordinated efforts to control the disease. The prevalence rate of 1.48% that we obtained in this study could be considered very low when compared to the 11.98% reported by Kelley and Bida and 6.16% - 10.44% by Oduye and Lloyd in some northern states of Nigeria. The higher prevalence recorded during the rainy months of the year was similar to previous findings. This has been attributed mainly to the usual high prevalence of blood sucking flies (Lyperosa minuta) and ticks especially Amblyomma variegatum during the rainy seasons. The wounds they cause provide easy entrance for bacterial infection. Furthermore, the prolonged moistness of the hide during this period often dissolve the water-soluble bacteriostatic lipid film which occupies the disjunct part of the horny layer thereby allowing D. congolensis to proliferate and precipitate clinical dermatophilosis.

Figure 1: Infection rates of bovine dermatophilosis nd rainfall pattern in Oyo state.

Breed specific infection rates were 12.20% for Keteku, 11.77% for Janli, 5.17% for Sokoto gudali, 3.82% for Bunaji, 2.37% for Rahaji, and 0.45% for Kuri (Table 3). Infection rates in the different ages were 2.83% in = five years, followed by 2.70% in both = four years and = seven years, 2.43% in = two years and zero percent in = one year (Table 4). The differences in the infection rates for the age groups were not significant. Of the 160 cases examined during the special survey, 134 or 83.75% had more of the characteristic lesions (scab and crust formation) on the upper parts of the body (mainly the hump, back, neck and head), 16 or 10.00% on the under parts of the belly and 11 or 6.88% on the legs.
Table 3: Prevalence of dermatophilosis in different breeds of cattle slaughtered in two sampled abattoirs in Oyo State.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Description</th>
<th>Source</th>
<th>Total cattle examined</th>
<th>Infected Number</th>
<th>Breed specific infection, rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunaji</td>
<td>Humped, coat is grey-white with black extremities, has long horns</td>
<td>Widespread in Nigeria</td>
<td>2381</td>
<td>91</td>
<td>3.82</td>
</tr>
<tr>
<td>Sokoto gudali</td>
<td>Humped, coat is white or fawn and has medium-sized horns</td>
<td>From north-western part of Nigeria</td>
<td>116</td>
<td>6</td>
<td>5.17</td>
</tr>
<tr>
<td>Keteku</td>
<td>Humpless, coat is white, black grey or light red and has short horns</td>
<td>Widespread in South-western Nigeria</td>
<td>41</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Kuri</td>
<td>Humpless, coat is light coloured, and with long bulbous horns</td>
<td>From Lake Chad area in the north-eastern part of Nigeria</td>
<td>1,103</td>
<td>5</td>
<td>0.45</td>
</tr>
<tr>
<td>Rahaji</td>
<td>Humped, coat is dark red and has long horns</td>
<td>Widespread in northern Nigeria</td>
<td>2,148</td>
<td>51</td>
<td>2.37</td>
</tr>
<tr>
<td>Janli</td>
<td>Humped, coat is grey or black and has long lyre-shaped horns</td>
<td>From outside Nigeria and found in the north-western part of the country</td>
<td>17</td>
<td>2</td>
<td>11.77</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>5,806</td>
<td>160</td>
<td>2.76</td>
</tr>
</tbody>
</table>

Table 4: Prevalence of dermatophilosis in different ages of cattle slaughtered in two sampled abattoirs in Oyo State

<table>
<thead>
<tr>
<th>Estimated minimum age in years</th>
<th>Number examined</th>
<th>Infected Number</th>
<th>As % of number examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>123</td>
<td>3</td>
<td>2.43</td>
</tr>
<tr>
<td>4</td>
<td>1,965</td>
<td>53</td>
<td>2.70</td>
</tr>
<tr>
<td>5</td>
<td>2,894</td>
<td>82</td>
<td>2.83</td>
</tr>
<tr>
<td>7</td>
<td>815</td>
<td>22</td>
<td>2.70</td>
</tr>
<tr>
<td>Total</td>
<td>5,806</td>
<td>160</td>
<td>2.76</td>
</tr>
</tbody>
</table>

The variations in the incidences reported in different breeds may be due to hereditary predisposition and resistance, the basis of which is not clearly understood. On the other hand, Koney et al. has attributed some assumed breed resistance to the fewer tick infestation in such breeds. The similarities in the infection rates in different ages imply that they are equally vulnerable to infection.

The manner in which lesions were distributed on the bodies of animals was suggestive of mechanical mode of transmission. The upper parts of the body (the hump, shoulder, flanks and head) where the lesions were more...
pronounced have been identified as the favourite biting sites of the blood-sucking flies and ticks while lesions around feet and mouth were suggestive of the effects of spiny plants. In view of the prime locations of lesions in as high as 83.23% of the infected animals which is indicative of the enormous losses resulting from the down-grading of the cattle hides due to this disease and the reported increases in the annual prevalence, we strongly recommend that routine measures to control ecto-parasites infestations especially during rainy seasons and proper education of cattle rearers on appropriate preventive measures should be put in place. There is also the need to fund studies on the efficacy of such promising medicaments as Oxytetracycline (long acting), Penicillin with Streptomycin, 'Crozogal', 'Echaka' ointment developed by Morphy and Lamstreptocide A & B, with a view to developing a drug of choice for dermatophiosis.

Acknowledgements

We acknowledge the assistance rendered by Dr. Segun Oriade and Mr. G.O. Olafimihan of Veterinary Control post and Bodija abattoir in Ibadan, for their assistance in data collection. We also thank the University of Ibadan for the grant to carry out this study.

References

SHORT COMMUNICATION

THE OCCURRENCE OF AFLATOXIN IN POULTRY TISSUES COLLECTED IN NAIROBI, KENYA

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Aflatoxins are a group of fungal toxins produced as secondary metabolites by certain strains of the ubiquitous mould species Aspergillus flavus and A. parasiticus. They are acutely toxic, carcinogenic, mutagenic, teratogenic and immunosuppressive. Aflatoxin B1 is the most prevalent and most toxic metabolite.

The occurrence of aflatoxins in human food and animal feeds in Kenya is well documented. This occurrence has been associated with clinical and fatal outbreaks of aflatoxicosis in human beings and animals. In poultry, aflatoxin intoxication causes acute deaths, an acute or chronic disease, reduced growth, lowered productivity and interferes with immunity against diseases.

Chicken fed on aflatoxin contaminated feeds retain aflatoxin residues in their tissues. These residues are highest in the liver, kidney and gizzard. The determination of these toxic residues is important for the clinical diagnosis of aflatoxicosis and for epidemiological investigations. As human beings are indirectly exposed to aflatoxins through consumption of meat and eggs from chicken fed on aflatoxin contaminated diets, the determination of these residues is also important in evaluating the total aflatoxin exposure to man.

In a study designed to assess the extent of aflatoxin B1 contamination in poultry liver tissues, 106 chicken liver samples were collected over a period of one month in October 1997. Eighty samples were from a poultry slaughterhouse and 26 from sick birds brought to the poultry diseases diagnostic laboratory of the University of Nairobi. Analysis for aflatoxin B1 was done according to the 2-dimensional TLC method for aflatoxin B1 and M1 in beef and liver of the Association of Official Analytical Chemists. Individual livers (ca. 20-30g) were analysed and the above method scaled down to use proportionally reduced volumes of extraction solvents.

Final extract cleanup and quantification of aflatoxins was achieved by a 2-dimensional thin layer chromatography (TLC) and the plates read under longwave (365nM) U.V. light. Aflatoxin B1 was quantified visually by comparison with aflatoxin B1 standard spots and confirmation of aflatoxin B1 positive samples was done by spraying the plates with hexane-trifluoroacetic acid (4+1).

None of the 80 samples collected from the poultry slaughterhouse had any aflatoxin B1 residues. Three out of the 26 samples collected at the university laboratory were contaminated with aflatoxin B1: one at 4 ng/g tissue and the other two at trace (<0.5ng/g) levels. None of the birds examined at the university clinic had gross lesion suggestive of aflatoxicosis.

The absence of aflatoxins in most liver samples is probably due to the absence of these toxins in feeds during the time of this study or due to the high ratio of aflatoxin in feed to that in tissues. This ratio has been variedly estimated at 1200:1 and 12100:1. Similar studies carried out in other parts of the world have found no or low level aflatoxin residues in animal tissues. In Brazil, no aflatoxin residues were found in poultry liver tissues while in Egypt, only 4 out of 150 samples of fresh meats were found to be contaminated. A survey of aflatoxins in tissues of pigs grown in areas that had a high incidence of aflatoxin contamination in corn found only low level (0.04-0.06ppb) contamination. The results of the present study are in agreement with the widely held view that consumption of meat is not an important route of exposure of human beings to aflatoxins.

In contrast to livers tissues sampled from the slaughterhouse that showed no aflatoxin residues, three (11.5%) samples of the liver
tissues collected from sick birds at the university laboratory were contaminated with aflatoxin. Low level aflatoxin intake, not adequate to cause clinically overt aflatoxicosis, has been shown to be experimentally and epidemiologically associated with increased disease susceptibility and vaccination failures in poultry flocks. These findings and the observations findings of Wolzak et al., that there exists a high individual birds variation in aflatoxin susceptibility, in which highly susceptible birds accumulate higher levels of aflatoxin residues and for longer durations of time after exposure, probably explain the relatively high incidence of aflatoxin residues in sick birds. There is need to carry out research work on possible epidemiological associations between aflatoxin intake, aflatoxin residues and disease occurrence in poultry flocks in Kenya.

Acknowledgement

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References


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