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

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Articles should be sent to the Editor, African Union/Interafriican Bureau for Animal Resources, E-mail: bahpa@au-ibar.org.

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An article submitted for publication implies that its content has not been published elsewhere and that it is subject to editorial revision.

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- Short Communications.
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Every line on the text should be numbered.
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Abstract not exceeding 200 words giving a synopsis of the findings presented and the conclusion(s) reached.

Introduction stating the purpose of the work.

Materials and Methods used.

Results presented concisely.

Discussion of significance.

Acknowledgements.

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Surname and initials of authors(s), year of publication (in parentheses), World List abbreviation of title of periodical (underlined), volume number (arabic numerals), first page number. The title of the articles should not be included.

2. Books
Surname and initials of author(s), year of publication (in parentheses), the exact title (underlined), town of publication, publisher, first page number.

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Name of country, year of reference, followed by the name of the department or organisation, first page number.

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Tables should be limited and number of headings restricted. A massive table is difficult to read even if it can be reproduced. Tables and figures should be numbered consecutively. Table 1 etc., or Fig. 1 etc., respectively, and attached at the end of the text. References to tables and figures in the text should be by number and not to “table below” or “figure below”. Coloured illustrations are reproduced only at the author(s) expense.

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# BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

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EVIDENCE OF IMPROPER USAGE OF VETERINARY DRUGS IN CATTLE IN MAASAILAND, KENYA

P. Irungu1,3*, B. Bett2,3, S.G. Mbohog1, S.O. Nyamwaro2, G.A. Murilla2 and T. Randolph3

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PREUVE DE L’USAGE INCONSIDERE DES MEDICAMENTS VETERINAIRES POUR LE TRAITEMENT DES BOVINS EN TERRE MAASAI AU KENYA

Résumé

Cent treize éleveurs dans les localités d’Olkiramatian et Siana au Kenya étaient interrogés entre octobre 2005 et février 2006. L’objet de l’entretien était de collecter des informations sur l’usage de médicament vétérinaire à la ferme. La technique de statistique descriptive et la technique de régression étaient utilisées pour analyser les données. Les éleveurs utilisaient des doses plus faibles que celles recommandées pour tous les trypanocides disponibles chez tous les groupes d’âge de bétail, à l’exception des taureaux adultes à qui on a administré une surdose d’acéturate de diminazène (Veriben®, Ceva Santé Animale, Libourne, France), de chlorure d’homidium (Novidium®, Merial, Lyon, France) et de diacéturate de diminazène (Tryzan®, Cooper, Nairobi, Kenya). L’hydrochlorure d’oxytétracycline (Adamycine®, Assia, Nairobi, Kenya), l’antibiotique le plus largement utilisé dans les deux sites d’études, était sous-dosé dans toutes les concentrations chez tous les groupes d’âge de bétail, sauf pour l’hydrochlorure d’oxytétracycline à 20% utilisé pour les taureaux adultes. A part le chlorure d’homidium, que les éleveurs ont dissous correctement, ils ont mis moins d’eau que ce qui est recommandé pour préparer les trypanocides. De même, la concentration d’acaricides était plus faible que celle recommandée pour le contrôle des tiques, sauf pour l’alphacyperméthrine (Dominex®, FMC, Philadelphia, Etats-Unis) ; et les éleveurs pulvérisaient plus de bétail que ce qui a été recommandé à chaque concentration. La tendance à utiliser correctement les médicaments vétérinaires était positivement associée à l’âge de l’éleveur et à son lieu d’origine (P <0,1), mais avait une corrélation négative avec le niveau d’éducation (souvent faible) du chef de ménage (p <0,05). On a formulé des recommandations à la lumière des conclusions de l’étude.

Mots-clés: Préparation du médicament, traitement de la maladie, bain antiparasitaire, éleveurs Maasai.

Summary

One hundred and thirteen farmers in Olkiramatian and Siana Locations of Kenya were interviewed between October 2005 and February 2006. The aim was to gather information on farmers’ veterinary drug use practices at the farm level. Descriptive statistical and regression techniques were used to analyse the data. Farmers used

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lower than recommended doses of all available trypanocides in all classes of cattle except in adult bulls where they overdosed with diminazene aceturate (Veriben®, Ceva Sante Animale, Libourne, France), homidium chloride (Novidium®, Merial, Lyon, France) and diminazene diacetate (Tryzan®, Cooper, Nairobi, Kenya). Oxytetracycline hydrochloride (Adamycin®, Assia, Nairobi, Kenya), the most commonly used antibiotic in the two study sites, was under-dosed at all concentrations in all classes of cattle except for oxytetracycline hydrochloride 20% used on adult bulls. Except for homidium chloride which farmers dissolved correctly, farmers used less than the recommended volume of water to prepare trypanocides. They also used less than the recommended concentration of acaricides for tick control, except for alphacypermethrin (Dominex®, FMC, Philadelphia, USA), and sprayed more cattle at each concentration than recommended. The propensity to use veterinary drugs correctly was positively associated with farmer’s age and Location of origin (p<0.1) but negatively correlated with years of formal education of the household head (p<0.05).

Keywords: Drug-preparation, Disease-treatment, Dipping, Maasai farmers

Introduction

The use of veterinary drugs is one of the major strategies for alleviating the incessant disease constraint in livestock production systems. With regard to trypanosomosis, for instance, it has been noted that more animals are currently protected by trypanocidal drugs than by all the other methods of trypanosomosis control put together¹. If used properly, veterinary drugs can permit higher levels of livestock production, improve animal welfare and safeguard the livelihood assets on which over 700 million poor farmers in developing countries depend². The adoption of economic reforms by developing countries in the early 1990s reduced farmers’ access to professional veterinary services which were hitherto publicly provided³, thereby placing the task of drug procurement and administration in the hands of untrained farmers⁴. Although this practice runs contrary to the existing legal and policy frameworks of most countries including Kenya⁵, it has nonetheless gained popularity amongst low income livestock keepers owing to their inadequate access to professional veterinary services particularly in the marginal and remote rural areas of Africa. Because of high illiteracy among livestock keepers in developing countries, their ability to make correct diagnosis and administer drugs is highly compromised, implying that consumers of livestock products in these countries are exposed to varying degrees of human health risk associated with improper use of veterinary drugs.

In this paper, improper drug use (IDU) refers to the actual or intended use of any drug in an animal in a manner that is inconsistent with the approved label or package insert of the drug, including the use of a drug in a species for which it is not approved⁶. IDU has two major implications on public health. Firstly, IDU can lead to violative levels of drug residues in animal-based human food products which may affect human health. Secondly, IDU has the potential of inducing the development, persistence and spread of antimicrobial resistance which ultimately affects human health through the food chain⁷. Apart from
its negative human health impacts, IDU can limit a country's access to international livestock markets thereby reducing its capacity to earn the much needed foreign exchange. In addition, a drug that is used improperly can potentially pollute the environment, for example by the excreta spreading or infiltrating into the environment.

In Kenya, non-adherence to withdrawal period, itself an IDU issue, has been blamed for the presence of high tetracycline residue levels in beef samples collected from Nairobi slaughterhouses. Another study reported that beef in Nairobi and the surrounding areas had violative levels of antibiotics and significant amounts of trypanocides. The authors attributed their findings to misuse of veterinary drugs by livestock producers, particularly pastoralists.

Most drug labels and package inserts usually contain information on the range of diseases that the drug is supposed to treat as well as the dosage, drug preparation procedures and the route of administration. Precautionary information is sometimes also provided including the withdrawal period and the animal's immediate reaction to treatment. It is worth noting that drug labels or package inserts are in most cases meant for professionals, who in Kenya consist of veterinarians and pharmacists. The existing legal provisions prohibit the use of veterinary drugs by non-professionals. Drug labels and package inserts are often printed in major languages particularly English perhaps as they are meant for use by professionals.

Previous studies have mainly been concerned about issues related to drug residues in animal-based products from Kenya. Other issues such as the correct use of drugs by farmers in the right doses and for the indicated diseases, drug preparation and the route of drug administration as well as drug mixing have largely been ignored. Therefore, our ability to quantitatively assess the risks associated with IDU at the farm level as well as to predict the future impacts of IDU on animal, human and environmental health is greatly constrained by lack of information on IDU. This study expects to contribute to the bridge information gaps specifically by assessing the extent of IDU at the farm-level in Olkiramatian and Siana Locations of Kajiado and Narok Districts respectively. The two Districts constitute the major sources of red meat supplied to the city of Nairobi and its environs.

This study focuses on issues related to IDU particularly those pertaining to the use of drugs for specific diseases, dosage, drug preparation and the route of administration for both trypanocides and antibiotics in cattle. It also examines the extent of drug mixing by farmers. Further, the study assesses farmers' acaricide use practices for tick control in cattle. The assessment of farm-level IDU practices is important firstly for identifying the cause(s) and the magnitude of the problem, and secondly for creating awareness among drug manufacturers and policy makers about the nature and extent of the problem for policy redress. In addition, the knowledge of farmers' drug use practices could be used to design extension messages that promote rational use of veterinary drugs among livestock keepers to improve the safety of animal-based foods.

**Materials and methods**

This study was part of a longitudinal tsetse fly repellent evaluation project conducted in Olkiramatian Location of Kajiado District and Siana Location of Narok.
District between August 2004 and February 2006. The two study sites are inhabited by Maasai pastoralists who mainly rely on livestock keeping for their livelihood. Both Districts are arid to semi-arid; rainfall is bimodal and highly variable both spatially and temporally. The average annual rainfall for Olkiramatian Location varies between 400 and 600mm. The mean annual temperature ranges between 28 and 34°C. In Siana Location, the mean annual rainfall varies between 600 and 800mm, while the mean annual temperature ranges between 18 and 30°C.

One hundred and thirteen livestock keepers were interviewed using a pre-tested questionnaire between October 2005 and February 2006. The questionnaire gathered information on farmers’ personal characteristics (age, education level, household size, income sources), diseases of cattle that were common in their locality, farmers’ knowledge with regard to disease identification and treatment, drug preparation and administration, and sources of information on livestock health care. The data gathered by the questionnaire were analyzed in SAS. Descriptive statistics were carried out to characterize farmers’ disease management practices. The proportion of respondents that used various disease management practices were compared using a $\chi^2$ test while sample means were compared using a Tukey’s test. Simple regression analysis was used to identify factors that influenced farmers to use veterinary drugs correctly. The deviation from the recommended volume of water used to dissolve a one gram sachet of diminazene aceturate (Veriben®, Ceva Sante Animale, Libourne, France) was used as an indicator of improper drug use. This was regressed against farmer’s age, his level of formal education, participation in past research, whether or not he had previously attended an animal health seminar, ownership of a working radio, number of children resident in the household with six or more years of formal education, and farmer’s Location of origin. These factors were hypothesized to be positively associated with farmers’ ability to use drugs correctly.

It is worth noting that the data used in this study are based on farmers’ self-reports extracted during the interview as opposed to direct observation of farmers’ practices by the authors. While this approach has the potential of introducing some bias associated with exaggerated farmer responses, the findings of this study are generally in agreement with those of farmers’ drug use monitoring reported elsewhere.

**Results**

During the interview, farmers were asked to name the type of drugs they used in the treatment of the most common diseases of cattle in their locality. Three common diseases were mentioned; trypanosomosis, East Coast Fever (ECF) and Foot and Mouth Disease (FMD). The results are shown in Table 1. The Table shows that although most of the respondents used the right drugs for indicated diseases, some used them on non-indicated diseases.

Farmers were also asked about the sources of water that they used to prepare trypanocidal drugs as well as how they prepared the water before mixing with the drug. The results are shown in Table 2. Most (54%) of the farmers obtained water from the river. In addition, a sizeable proportion (31%) of respondents used the water directly from the source without any special preparation. Information was also sought
Table 1. Frequency of veterinary drug use in the treatment of three most common diseases of cattle in Olkiramatian and Siana Locations, Kenya.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indicated for</th>
<th>Frequency of farmer responses (%)†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ECF</td>
</tr>
<tr>
<td>Diminazene aceturate</td>
<td>Trypanosomosis</td>
<td>28.42</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>Trypanosomosis</td>
<td>3.83</td>
</tr>
<tr>
<td>Isometamidium chloride</td>
<td>Trypanosomosis</td>
<td>-</td>
</tr>
<tr>
<td>Diminazene aceturate B.Vet.C</td>
<td>Trypanosomosis</td>
<td>-</td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>Trypanosomosis</td>
<td>-</td>
</tr>
<tr>
<td>Dibenzimidane diacetate</td>
<td>Trypanosomosis</td>
<td>-</td>
</tr>
<tr>
<td>Oxytetracycline hydrochloride (short-acting)</td>
<td>ECF</td>
<td>56.83</td>
</tr>
<tr>
<td>Dihydrostreptomycin Sulphate BP (Penstreps, Norbrook, Newry Northern Ireland)</td>
<td>Bacterial infections</td>
<td>7.10</td>
</tr>
<tr>
<td>Buparvaquone (Butalex®, Cooper, Nairobi, Kenya)</td>
<td>ECF</td>
<td>0.55</td>
</tr>
<tr>
<td>Parvaquone (Parvexon®, Twiga, Nairobi, Kenya)</td>
<td>ECF</td>
<td>2.73</td>
</tr>
<tr>
<td>Parvaquone (Clexon®, Wellcome, Nairobi, Kenya)</td>
<td>ECF</td>
<td>0.55</td>
</tr>
<tr>
<td>FMD Vaccine</td>
<td>FMD</td>
<td>45.06</td>
</tr>
</tbody>
</table>

†‡Restricted to the three diseases reported by farmers
††Represents number of responses rather than number of respondents

from farmers regarding the volume of water used to reconstitute trypanocidal drugs. Farmers' responses were compared with the manufacturers' recommendations given on drug labels. The results show that farmers used an equal volume of water to dissolve diminazene aceturate and homidium chloride (Novidium®, Merial, Lyon, France) (Table 3). In all cases except for homidium chloride, farmers used less water than recommended on the drug label.

On drug dosages, an IDU dose was taken to be any dose 2mls above or below the rate recommended by the manufacturers. As shown in Table 4, farmers under-dosed at all concentrations of oxytetracycline hydrochloride (Adamycin®, Assia, Nairobi, Kenya) in all classes of cattle except for oxytetracycline hydrochloride 20% used on adult bulls. Furthermore, farmers over-dosed adult bulls with diminazene aceturate, homidium chloride and diminazene diaceturate (Tryzan®, Cooper, Nairobi, Kenya) but under-dosed them with isometamidium chloride (Samorin®, Merial, Lyon, France) and dibenzimidane diacetate (Norotryp®, Norbrook, Newry, Northern Ireland). Adult cows, immatures and calves
Table 2: Number of farmers who reported using different sources of water for mixing injectable drugs classified by method of preparation

<table>
<thead>
<tr>
<th>Water source</th>
<th>Boil only</th>
<th>Boil and sieve</th>
<th>Warm</th>
<th>None</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shop (mineral water)</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>19 (13.8)</td>
</tr>
<tr>
<td>Tap</td>
<td>17</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>40 (29)</td>
</tr>
<tr>
<td>River</td>
<td>30</td>
<td>18</td>
<td>5</td>
<td>22</td>
<td>75 (54.3)</td>
</tr>
<tr>
<td>Dam/Rain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>54 (39.1)</strong></td>
<td><strong>29 (21)</strong></td>
<td><strong>12 (8.7)</strong></td>
<td><strong>43 (31.2)</strong></td>
<td><strong>138†</strong></td>
</tr>
</tbody>
</table>

Numbers in brackets represent percentages
†Number is greater than 113 because some farmers used more than one method

Table 3: Mean volume of water used by farmers to dilute various trypanocidal drugs in Olkiramatian and Siana Locations, Kenya, and the recommended rate for each drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean volume of water (ml)</th>
<th>Used by farmers</th>
<th>Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diminazene aceturate</td>
<td>10.03(0.25)</td>
<td>15/2.36g sachet</td>
<td></td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>10.03(0.21)</td>
<td>10/tablet</td>
<td></td>
</tr>
<tr>
<td>Isometamidium chloride</td>
<td>40.93(4.38)</td>
<td>50/1g sachet</td>
<td></td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>9.65(0.43)</td>
<td>15/2.36g sachet</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in brackets represent standard error

were under-dosed with isometamidium chloride, diminazene aceturate (Dimaze®, Wockhardt, Mumbai, India) and dibenzamide diacetate. In all cases with a significant difference, farmers in Siana Location used lower doses compared to those in Olkiramatian Location (p<0.05).

Farmers' drug administration practices are summarized in Table 5. An almost similar proportion of respondents injected the drug intravenously (51%) and intramuscularly (49%) at the neck region. Calves were mainly injected through the ribs. Most (73%) of those who used the intravenous route reported that they did so in order to treat resistant trypanosomosis which they referred to as “emunywa” in Maasai. The other 16% and 11% of the respondents used the intravenous route to treat ECF and normal trypanosomosis respectively.

Although drug combination is not an established IDU issue, the prevalence of this practice was assessed among the respondents. About 92% of the farmers reported that they mixed different drugs. The proportion of those who mixed drugs was
Table 4. Mean dosage of oxytetracycline hydrochloride and trypanocidal drugs administered by farmers and their recommended rates for different classes of cattle in Olkiramatian and Siana Locations, Kenya

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recommended dose (mg/kg)</th>
<th>Mean of farmers' dose (mg/kg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adult bull</td>
<td>Adult cow</td>
<td>Immature</td>
<td>Calf</td>
</tr>
<tr>
<td>Oxytetracycline hydrochloride (5%)</td>
<td>10</td>
<td>4.5'</td>
<td>3.2'</td>
<td>2.5'</td>
<td>2.5'</td>
</tr>
<tr>
<td>Oxytetracycline hydrochloride (10%)</td>
<td>10</td>
<td>7.8</td>
<td>5.5</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Oxytetracycline hydrochloride (20%)</td>
<td>10</td>
<td>11.1</td>
<td>7.9'</td>
<td>5.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Diminazene aceturate</td>
<td>3.5</td>
<td>3.7'</td>
<td>2.4'</td>
<td>1.8'</td>
<td>1.0'</td>
</tr>
<tr>
<td>Homidium chloride</td>
<td>1.4'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isometamidium chloride</td>
<td>0.5</td>
<td>9.2</td>
<td>6.1</td>
<td>4.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>3.5</td>
<td>2.8'</td>
<td>1.8'</td>
<td>1.4'</td>
<td>0.8'</td>
</tr>
<tr>
<td>B.Vet.C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>3.5</td>
<td>2.8'</td>
<td>1.9'</td>
<td>1.5'</td>
<td>0.9'</td>
</tr>
<tr>
<td>Dibenzamidine diactuate</td>
<td>3.5</td>
<td>1.9</td>
<td>1.8</td>
<td>0.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Means are significantly different between Olkiramatian and Siana Locations (p<0.05)
Source: Survey data

Figure 1. Proportion of respondents who used different acaricides in Kajiado and Narok districts, Kenya
Table 5. Number of respondents who used different routes to administer injectable drugs on cattle in Olkiramatian and Siana Locations, Kenya

<table>
<thead>
<tr>
<th>Injection region</th>
<th>Intravenous</th>
<th>Intramuscular</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>60</td>
<td>57</td>
<td>117</td>
</tr>
<tr>
<td>Bottom</td>
<td>4</td>
<td>44</td>
<td>48</td>
</tr>
<tr>
<td>Ribs</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>107</td>
<td>171</td>
</tr>
</tbody>
</table>

†Number is greater than 113 because some farmers reported more than one route
Source: Survey data

similar in the two Locations (p>0.05). The drugs most frequently mixed were diminazene aceturate and oxytetracycline hydrochloride (in 38% of cases), diminazene aceturate and homidium chloride (33% of cases) and isometamidium chloride and diminazene aceturate (8% of cases). Some respondents mixed together up to three drugs. For instance, diminazene aceturate, oxytetracycline hydrochloride and homidium chloride were mixed in 2% of the cases; diminazene aceturate, isometamidium chloride and oxytetracycline hydrochloride in 1% of the cases, while diminazene aceturate, oxytetracycline hydrochloride and homidium chloride were mixed in 0.4% of the cases. In all cases where oxytetracycline hydrochloride was used, it substituted water as a diluent for trypanocides. With regard to acaricides, 30 (29%) farmers reported that they combined different acaricides before use. This practice was most prevalent amongst farmers in Siana Location who accounted for 23 (77%) of all those who mixed acaricides. Amitraz (Bovitraz®, Bayer E.A., Nairobi, Kenya) and Diazol® and Stelladone® and Bovitraz® were mixed in 20%, 16% and 13% of the cases respectively.

Stelladone® and Bovitraz® were the most popular acaricides used in the two study areas (Figure 1). The average spraying interval by farmers for different acaricides is shown in Table 6. The spraying interval for amitraz (Triatix®, Cooper, Nairobi, Kenya) and amitraz (Taktic®, Intervet BV, The Netherlands) was significantly higher among farmers in Siana Location than in Olkiramatian Location (p<0.05). IDU was also assessed with regard to the strength of acaricides used as well as the number of cattle sprayed at each concentration. The results are presented in Table 7. Overall, farmers used less than the recommended acaricide strength in all cases except for alphacypermethrin (Dominex®, FMC, Philadelphia, USA) which they overdosed slightly. In addition, farmers sprayed more cattle at each concentration than the number recommended by the manufacturers. In all cases with a significant difference, farmers in Siana Location used lower acaricide concentration compared to those in
Table 6. Mean spraying intervals for various acaricides practiced by farmers in Olkiramatian and Siana Locations, Kenya

<table>
<thead>
<tr>
<th>Acaricide¹</th>
<th>Mean spraying interval (weeks)²</th>
<th>Range (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triatix®</td>
<td>1.69(0.10)</td>
<td>1-4</td>
</tr>
<tr>
<td>Amitraz (Almatix®, Hichem, Nairobi, Kenya)</td>
<td>1.75(0.12)</td>
<td>1-3</td>
</tr>
<tr>
<td>Dominex®</td>
<td>2.11(0.12)</td>
<td>1-4</td>
</tr>
<tr>
<td>Taktic®</td>
<td>1.67(0.12)</td>
<td>1-4</td>
</tr>
<tr>
<td>Amitraz (Mostraz®, Assia, Nairobi, Kenya)</td>
<td>2.13(0.27)</td>
<td>1-4</td>
</tr>
<tr>
<td>Bovitraz®</td>
<td>1.50(0.09)</td>
<td>1-4</td>
</tr>
<tr>
<td>Amitraz (Tixfix®, Twiga, Nairobi, Kenya)</td>
<td>1.74(0.14)</td>
<td>1-4</td>
</tr>
<tr>
<td>Stelladone®</td>
<td>1.41(0.08)</td>
<td>1-4</td>
</tr>
<tr>
<td>Cypermethrin high-cis (Ectomin®, Norvatis Animal)</td>
<td>2.33(0.88)</td>
<td>1-4</td>
</tr>
</tbody>
</table>

Health, Basle, Switzerland

*Numbers in brackets represent standard error
††See other chemical names in the text
Source: Survey data

Table 7. Mean acaricide strength, recommended strength and number of cattle sprayed at each concentration by farmers in Olkiramatian and Siana Locations, Kenya.

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>Concentration (ml/L)</th>
<th>Number of cattle sprayed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farmers¹</td>
<td>Recommended</td>
</tr>
<tr>
<td>Triatix®</td>
<td>0.98(0.08)</td>
<td>2.0</td>
</tr>
<tr>
<td>Almatix®</td>
<td>1.07(0.11)</td>
<td>2.0</td>
</tr>
<tr>
<td>Dominex®</td>
<td>1.01(0.16)</td>
<td>0.5</td>
</tr>
<tr>
<td>Taktic®</td>
<td>1.34(0.23)</td>
<td>2.0</td>
</tr>
<tr>
<td>Mostraz®</td>
<td>0.95(0.16)</td>
<td>2.0</td>
</tr>
<tr>
<td>Bovitraz®</td>
<td>1.15(0.09)</td>
<td>2.0</td>
</tr>
<tr>
<td>Tixfix®</td>
<td>1.02(0.09)</td>
<td>2.0</td>
</tr>
<tr>
<td>Stelladone®</td>
<td>1.24(0.09)</td>
<td>1.75</td>
</tr>
<tr>
<td>Ectomin®</td>
<td>0.57(0.29)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Numbers in brackets represent standard error
*Means are significantly different between Olkiramatian and Siana Locations (p<0.05)
Source: Survey data
Table 8. Factors influencing farmers’ propensity to use veterinary drugs correctly in Olkiramatian and Siana Locations, Kenya

<table>
<thead>
<tr>
<th>Variable</th>
<th>β estimate</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.78***</td>
<td>0.97</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of resident children in class 6 and above (number)</td>
<td>-0.26</td>
<td>0.21</td>
</tr>
<tr>
<td>Formal education of household head (years)</td>
<td>-0.17**</td>
<td>0.08</td>
</tr>
<tr>
<td>Participation in previous research (dummy: 1=yes; 0=no)</td>
<td>0.16</td>
<td>0.65</td>
</tr>
<tr>
<td>Number of animal health seminars attended in previous year (number)</td>
<td>-0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>Possession of a working radio (dummy: 1=yes; 0=no)</td>
<td>-0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>Location of origin (dummy: 1=Olkiramatian; 0=Siana)</td>
<td>0.93*</td>
<td>0.56</td>
</tr>
</tbody>
</table>

***, **, * significant at 1%, 5% and 10% levels respectively

Olkiramatian Location. There was no difference in the number of cattle sprayed at each strength in the two Locations (p>0.05).

The results of the regression analysis are shown in Table 8. The adjusted $\chi^2$ was 11%, indicating a low fit of the model onto the data. Nevertheless, such a low fit is expected for cross-sectional questionnaire data. The deviation from the recommended volume of water needed to dissolve a one gram sachet of diminazene aceturate was positively associated with farmer’s age and the Location of origin (p<0.1). The same variable was negatively correlated with the number of years of formal education of the household head (p<0.05).

Discussion

Improper use of veterinary drugs is a public health issue of international concern. Incorrect use of veterinary drugs could lead to the accumulation of drugs in animal products which poses danger to human health. This study found some evidence of IDU in cattle among the Maasai pastoralists in Olkiramatian and Siana Locations of Kenya. For instance, some farmers used some drugs for non-indicated diseases. A case in point is the use of diminazene aceturate and homidium chloride to treat ECF and homidium chloride to treat FMD. A number of farmers also treated trypanosomosis using antibiotics. This corroborates a study in South Africa which found that farmers in Madikwe District used drugs for the wrong indication. Use of drugs to treat non-indicated diseases not only puts the life of the animal at risk but also leads to wastage of scarce financial resources associated with drug misuse.

A sizeable number of farmers reported that they obtained water from various sources but did not subject it to any treatment before use. Lack of tapped water and the high cost of industrially treated ‘mineral water’ in the study areas could be
some of the factors that entrenched this practice. Use of unsterilised water not only exposes the animal to infectious microorganisms but may also lead to slowed absorption of the drug. In some cases that we examined, this practice resulted in abscesses at the site of injection. This finding reflects some degree of lack of knowhow on the part of farmers about drug preparation.

Except for homidium chloride which farmers dissolved correctly, most respondents in the two study sites used less water than recommended by the manufacturers to prepare trypanocidal drugs. Under-dilution could raise the concentration of the drug thereby interfering with its pharmacokinetics and bio-availability. In addition, it could also lead to wastage of the drug thus increasing the cost of treatment. However, most trypanocidal drugs have a large therapeutic index particularly in cattle. This means that they can be tolerated at high dosages without causing adverse systemic toxicity. However, this characteristic may encourage overdosing with the effect of increasing drug resistance.

Farmers used more than the recommended doses of diminazene aceturate, homidium chloride and diminazene diaceturate and less than the recommended doses of isometamidium chloride and dibenzamidine diactuate on adult bulls. Adult cows, immatures and calves received less than recommended doses of isometamidium chloride, diminazene aceturate and dibenzamidine diactuate. With regard to oxytetracycline hydrochloride, farmers administered less than recommended doses at all concentrations (5%, 10% and 20%) in all classes of cattle except for 20% used on adult bulls. Although it is difficult to explain this observation, one plausible reason why farmers used improper dosages could be due to their inability to estimate the body weight of the animal. In addition, we observed that the Maasai pastoralists have high preference for bulls and lactating cows which they treat indiscriminately, even when there are no obvious clinical signs of disease.

Farmers in Olkiramatian Location have been reported to overdose their cattle with diminazene aceturate where they used twice the standard recommended dose of 3.5mg/kg live weight. Another study carried out in the Eastern Province of Zambia found that farmers there under-dosed with both diminazene aceturate and isometamidium chloride. In Burkina Faso, farmers overdosed with diminazene aceturate but under-dosed with isometamidium chloride. These studies suggest that the problem of IDU could be widespread in Africa. Under- and/or over-dosing of drugs has been associated with inadequate responses, risk of toxicity, accumulation of unused medication in animal tissues, increased cost of treatment and risk of emergence of antimicrobial resistance.

The route of drug administration plays a key role in the drug’s pharmacokinetic properties. As such, a drug that is intended for intramuscular use should not be given intravenously. Administering a drug via a route different from the intended one can result in adverse outcomes including death. Although the majority (63%) of farmers used the intramuscular route, the rest (37%) administered injectable drugs intravenously contrary to the instructions on the drug label or package insert. This finding tallies with that in a South African study that found farmers in Madikwe District administered
drugs via the wrong route. They attributed this to farmers' failure to interpret the package insert correctly. It is recommended that diminazene aceturate, homidium chloride and isometamidium chloride should be administered intramuscularly. Furthermore, antibiotics such as oxytetracycline hydrochloride and dihydrostreptomycin sulphate used by farmers in the two study areas should be administered intramuscularly.

A significant proportion (92%) of survey farmers mixed trypanocidal drugs. Others used oxytetracycline hydrochloride to dissolve trypanocidal drugs. Another study also found similar practices for farmers in Olkiramatian Location. In West Africa, farmers mixed together isometamidium chloride and diminazene aceturate and also used antibiotics to dilute trypanocides. The effect on the efficacy of mixing trypanocides and antibiotics is not fully known. However, such mixing is likely to increase the risk of drug toxicity and provide conditions for multiple drug resistance.

The results of regression analysis suggest that advancement in age increased farmers' propensity to misuse drugs while advancement in formal education of the household head reduced this tendency. It is instructive to note that 73% of the survey farmers did not have any formal education. Of these, 58% were over 40 years old. Illiteracy has been associated with improper drug use. Formal education raises the farmers' literally ability needed to read the drug label or package insert thereby reducing their tendency to misuse drugs. Being a resident of Siana Location elevated a farmer's propensity to misuse drugs compared to being a resident of Olkiramatian Location. This could be attributed to the general lack of access to animal health information by farmers in Siana Location, which is about 80km away from Narok town. In fact, out of the 53 farmers interviewed in Siana Location, only five had attended an animal health seminar; two had been involved in previous research and 11 had a working radio. The comparative figures for the Olkiramatian Location were, 30, 22 and 30, respectively, out of the 60 farmers interviewed.

Farmers used less than the recommended strength of all acaricides except alphacypermethrin for which they slightly overdosed. In addition, they sprayed more cattle at each strength of acaricide than recommended by the manufacturers. Furthermore, farmers mixed different acaricides. Our finding corroborates that of another study where farmers in northern Tanzania over-/under-dosed with both Stelladone® and amitraz (Tixfix®, Twiga, Nairobi, Kenya). Use of low acaricide doses could expose ticks to sub-lethal acaricide concentrations thereby reducing the risk of tick resistance to available acaricides. This author argues that at concentrations below the recommended strength, more ticks with a greater variety of resistance states survive and total resistance develops more slowly such that the acaricide has a potentially longer effective life. One plausible explanation for the observed improper acaricide use could be farmers' failure or inability to read the instructions on the drug labels or package inserts which clearly give the correct dosages as well as the number of animals to treat at each dilution. This was perhaps because most of the farmers were illiterate and could therefore not read the drug labels or package inserts, which as noted earlier, are meant for professional users. Another reason could be due to lack of an appropriate
method to measure both the acaricide and the diluent.

**Policy options**

This study found substantial evidence of IDU among the Maasai pastoralists in Olkiriamatian and Siana Locations of Kajiado and Narok Districts respectively. This evidence gives credence to earlier studies that found violative drug residues in cattle meat sold in Nairobi and its environs. Notably, Kajiado and Narok Districts constitute the major sources of red meat supplied to Nairobi and its environs. Our study further established that livestock farmers in Olkiriamatian and Siana Locations routinely treat their own animals. While this practice is contrary to existing legal provisions, evidence elsewhere suggests that it is widespread in other pastoral and agro-pastoral production systems of Kenya. It is therefore imperative to address the following policy issues in order to curb the problem of IDU at the farm level.

Firstly, deliberate efforts should be made to avail professional veterinary services to farmers living in the marginal areas of Kenya. This would not only increase farmers' access to quality veterinary services but also reduce their tendency to treat their own livestock. Privatization of veterinary services in these areas has failed to attract a significant number of qualified veterinary service providers due to farmers' inability to pay and high transactions costs and low rate of return on investment in private animal health service. The high incidence of poverty in the marginal areas of Kenya could justify the provision of subsidized veterinary services in these areas in line with the government's equity and poverty reduction goals.

Secondly, given the huge capital outlays required to provide subsidized professional veterinary services in the marginal areas, Kenya should consider adopting the relatively low-cost and more flexible community-based animal health worker model in the short term as a framework for engendering community-led private sector-driven rural animal health care. Existing evidence suggests that this model has a great promise in rural areas of Africa. However, the adoption of this model would require the review of existing legal and policy frameworks in animal health. Mechanisms of how to go about it have been suggested. Such mechanisms include the legal recognition, registration and regulation of community-based animal health workers as well as the review of existing legislation that prohibits non-professionals from handling veterinary drugs.

Thirdly, the prevailing situation of inadequate access to qualified veterinary service providers in the marginal areas of Kenya calls for the provision of information to farmers to enhance the proper use of veterinary drugs at the farm level. While this approach may seem to go against the existing legal and policy frameworks that regulate drug use by non-professionals, it would serve to reduce the incidence of drug misuse in the short term. In the medium term, subsidized professional veterinary services could be provided in affected areas. In the longer term, well coordinated public-private sector partnerships could be initiated and fostered for improved animal health and welfare of low income livestock keepers in the marginal areas of Kenya. One strategy through which information could be availed to farmers would be to revive the existing public animal health extension services,
which have the advantage of a nation-wide outreach. Another strategy would be to adopt the Livestock Farmer Field School methodology at the community level where farmers access information at low cost by creating linkages with local extension service providers and research organizations. Nevertheless, farmers should be encouraged to meet part of the cost of providing such services. In addition, suitable partners could be identified to assist in defraying part of the initial set up costs of Farmer Field Schools which could be relatively high. Alternatively, the revived Kenya Meat Commission and the Livestock Marketing Council as major stakeholders in the livestock industry should come up with novel livestock farmer training programs such as those provided by crop-marketing boards (e.g. the Tea Board of Kenya, the Pyrethrum Board of Kenya, the Kenya Planters Cooperative Union) and veterinary drug companies to promote their interests. Farmer field days, workshops, field demonstrations and seminars as well as radio and written materials are possible ways of conveying relevant information to farmers.

This study has established that more educated farmers had a lower propensity to misuse drugs, probably because they were able to follow instructions provided on the drug label or package insert. While we appreciate that most drugs had labels and/or package inserts, most were written in English, which means that they were of little or no use to the majority of livestock keepers in the two study sites owing to their low literacy. Drug companies should consider having labels written in Kiswahili, and if possible, in some of the major vernacular languages to enable farmers fully benefit from the information provided. Formularies and handbooks describing important livestock diseases and their treatment in a pictorial format could go a long way to helping illiterate farmers prepare and administer drugs correctly. Furthermore, products should be appropriately labelled and supplied with product literature that is understandable to the farmer. This would be most appropriate particularly if the current situation of poor access to professional veterinary services in the marginal areas of Kenya prevails.

Finally, given the importance of formal education in making it possible to read and understand instructions on proper drug use, livestock keepers and particularly pastoralists, should be encouraged to send their children to school, especially now that the government is implementing a free-primary education program and has announced plans to provide free secondary education from next year. This will not only equip them with literate knowledge to be able to understand and utilize livestock disease control technologies but will also build their capacity to better cope with a rapidly changing global economy.

Conclusions

This study focused on farm-level drug use practices of Maasai pastoralists in Olkiramatian and Siana Locations of Kajiado and Narok Districts respectively. The objective was to document the extent of IDU among the farmers, an issue with food safety and public health implications. The study found substantial evidence of IDU in the two study sites. In particular, farmers used drugs for non-indicated diseases. There was also evidence of over-/under-dosing with both trypanocides and acaricides. Drug mixing was also widely practiced in the two study
areas. The propensity to use drugs incorrectly was positively correlated with farmer's age but negatively associated with his level of formal education. In order to reduce the incidence of IDU, there is need to improve the provision of professional veterinary services in the marginal areas of Kenya such as the two study sites. If that is not immediately possible, particularly taking cognisance of budgetary limitations, then the provision of drug use information to farmers would enhance their capacity to administer drugs to their livestock in the short term in order to reduce the incidence of IDU.

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References

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MULTIPLE ANTHELMINTIC RESISTANCE ON A SHEEP FARM IN KENYA AND ITS IMPLICATION FOR HELMINTH CONTROL

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RESISTANCE ANTHELMINTIQUE MULTIPLE DANS UNE FERME OVINE AU KENYA ET SES CONSEQUENCES SUR LE CONTROLE DES HELMINTHES

Résumé
Les cas de résistance anthelmintique chez les nématodes gastro-intestinaux dans une ferme ovine à Kabete au Kenya ont fait l'objet d'enquête entre octobre 2005 et mars 2006 lorsqu'un foyer d' helmintiase aigue et mortelle est apparu malgré l'usage de l'ivermectine pour le contrôle des vers. L'efficacité des anthelmintiques disponibles sur place, à savoir : l'ivermectine, le levamisole, l'association levamisole/rafoxanide et l'albendazole a été évaluée en tenant compte du taux de réduction du nombre d'œufs dans les fèces (% FECR), du signe clinique de l' helmintiase et du nombre de vers à la necropsie (NV). Tous les médicaments testés avaient une efficacité réduite avec des %FECR de 44.2% ; 77% ; 66.9% et 42.3% pour l'ivermectine, le levamisole, l'association levamisole/rafoxanide et l'albendazole respectivement. Pendant la période de test, 18 cas cliniques ont été observés, parmi lesquels il y a eu dix morts. Le NV moyen chez les animaux morts baissait peu à peu, allant de 8905 avant le traitement à 1545, 1529, 618 et 111 après les traitements à l'ivermectine, au levamisole, à l'association levamisole/rafoxanide et à l'albendazole respectivement. Haemonchus contortus et Trichuris ovis étaient résistants à tous les médicaments testés. Les espèces Trichostrongylus étaient résistantes à l'ivermectine et au levamisole, mais sensibles à l'albendazole, ce qui a rendu nécessaire le traitement de tout le troupeau à l'albendazole et le déplacement vers de nouveaux pâturages et parcs à moutons. Aucun cas clinique d' helmintiase ou de mortalité n'a été enregistré un mois après le déplacement. Cette étude a montré la nécessité de résistance anthelmintique multiple dans la ferme comme signalé ailleurs dans le pays. A la lumière de ces conclusions, il est nécessaire de donner l'instruction aux éleveurs pour ce qui est de l'adaptation de la méthode d'approche intégrée au contrôle de l' helminthe avec l'usage judicieux des anthelmintiques.

Mots-clés: Moutons, résistance anthelmintique.

Summary
The occurrence of anthelmintic resistance in gastrointestinal nematodes in sheep on a farm in Kabete, Kenya was investigated between October 2005 and March 2006 when an outbreak of acute and fatal helminthosis occurred despite the use of ivermectin in worm control. The efficacies of locally available anthelmintics, namely, ivermectin, levamisole, levamisole-rafoxanide combination and albendazole were evaluated based on faecal egg count reduction percentages (FECR%), clinical manifestation of helminthosis and post-mortem worm count (WC). All drugs tested showed low efficacies with FECR% of 44.2%, 77.0%, 66.9% and 42.3% for ivermectin, levamisole, levamisole-rafoxanide combination and albendazole respectively. During the test period, 18 clinical cases were observed out of which 10 died. The mean WC in the dead animals declined gradually from 8905 in the pre-treatment period to 1545, 1529, 618, and 111 following ivermectin, levamisole, levamisole-rafoxanide combination and albendazole treatments respectively. Haemonchus contortus and Trichuris ovis were resistant to all drugs tested. Trichostrongylus species were resistant to ivermectin and levamisole, but susceptible to albendazole. This necessitated treatment of the entire flock with albendazole and movement to new pastures and pens. No clinical case of helminthosis or mortality was recorded one month after the movement. This study indicated presence of multiple anthelmintic resistance on the farm as reported elsewhere in the country. Based on these findings, it is necessary to educate farmers on adapting integrated approach to helminth control with appropriate use of anthelmintics.

Key Words: Sheep, anthelmintic resistance

*Corresponding Author:
Introduction

Gastrointestinal nematode parasitism has been recognized as a major factor limiting small ruminant livestock production in Kenya\textsuperscript{1,2}. Infections have primarily been controlled by extensive use of anthelmintics and grazing management. This is particularly so on large commercial farms that can afford the high cost of drugs. In recent times, there have been increasing reports from many parts of the world on the occurrence of resistance to anthelmintics in trichostrongyloid nematodes especially in small ruminants\textsuperscript{3,4,5}. This resistance may involve all groups of anthelmintics, including the most recently introduced ivermectin / milbemycin group\textsuperscript{6}. In Kenya, resistance has mostly been reported in institutional farms, which unfortunately supply breeding stocks to other farms with the potential danger of spreading this problem\textsuperscript{7,8}. This paper reports on the occurrence of multiple anthelmintic resistance on a sheep rearing farm in Kabete, Kenya.

Materials and Methods

Study site and background

The study was carried out on a farm in Kabete, about 20 Km west of Nairobi. The sheep enterprise on the farm consisted of about 100 Dorpers at the onset of the study. The sheep were kept permanently on pastures and grazed the same paddock over the years. During the last 20 years, thiabendazole (Thibenzole\textsuperscript{®}, Merck, New Jersey, USA), fenbendazole (Panacur\textsuperscript{®}, Hoechst, Munich, Germany), albendazole (Valbazen\textsuperscript{®}, SmithKline Beecham, UK), thiophanate (Nemafax\textsuperscript{®}, May and Baker), levamisole (Nilverm\textsuperscript{®}, Wellcome Kenya Ltd, Nairobi), rafoxanide (Ranide\textsuperscript{®}, Merck, New Jersey, USA) and ivermectin (Noromectin\textsuperscript{®}, Norbrook Laboratories Ltd, Northern Ireland) have been used haphazardly for helminth control on this farm. In 1995 a strain of Haemonchus contortus that was resistant to benzimidazoles, levamisole and ivermectin was isolated\textsuperscript{9}. Since then animals were only treated based on clinical helminthosis. In October 2005, three adult sheep died of helminthosis despite ivermectin treatment.

Experimental animals and anthelmintic treatments

In November 2005, fifteen animals of mixed ages were randomly selected from the flock and assigned to three treatment groups (A, B and C) with equal members. Animals in Group A were treated with ivermectin (Noromectin\textsuperscript{®}, Norbrook Laboratories Ltd, Northern Ireland) at a dose rate of 0.2 mg Kg\textsuperscript{-1} body weight. Those in group B were treated with levamisole (Nilverm\textsuperscript{®}, Coopers Kenya Ltd, Nairobi) at a dose rate of 7.5 mg Kg\textsuperscript{-1} body weight as was the rest of the flock, while those in group C remained as the un-treated controls. Rectal faecal samples were taken on the day of treatment (Day 0) and 14 days post-treatment. On day 14 all control group animals were treated with levamisole as above. A modified McMaster technique as described in the MAFF manual\textsuperscript{10} was used for determination of eggs per gram of faeces (EPG).

In December 2005, ten other animals were selected and assigned to two treatment groups (A and B) with equal members. Animals in Group A were treated with levamisole – rafoxanide combination (Multidose, Univet Ltd., Tullyvin, Cavan, Ireland) at a dose rate of 7.5 mg levamisole Kg\textsuperscript{-1} and 11.25 mg rafoxanide Kg-1 body
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-treatment (day 0)</th>
<th>Post-treatment (day 14)</th>
<th>FECR%</th>
<th>95% CI</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td>Ivermectin</td>
<td>3600</td>
<td>2720</td>
<td>3833</td>
<td>2140</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>(1800 - 7000)</td>
<td>(800 - 7200)</td>
<td>(2400 - 5600)</td>
<td>(200 - 5200)</td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>3600</td>
<td>1480</td>
<td>3833</td>
<td>880</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>(1800 - 7000)</td>
<td>(200 - 2400)</td>
<td>(2400 - 5600)</td>
<td>(0 - 1700)</td>
<td></td>
</tr>
<tr>
<td>Levamisole / Rfoxanide</td>
<td>1260</td>
<td>3320</td>
<td>6820</td>
<td>2260</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>(100 - 4000)</td>
<td>(1800 - 6300)</td>
<td>(800 - 16200)</td>
<td>(500 - 4600)</td>
<td></td>
</tr>
<tr>
<td>Albendazole</td>
<td>1060</td>
<td>850</td>
<td>2340</td>
<td>1350</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>(100 - 5100)</td>
<td>(100 - 4000)</td>
<td>(500 - 7400)</td>
<td>(0 - 7700)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1: The Faecal Egg Count Reduction Percentage (FECR%) and its Lower Confidence Interval (CI) calculated according to the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP) developed by Coles et al., (1992).

<table>
<thead>
<tr>
<th>Nematode species</th>
<th>Untreated (c = 6)</th>
<th>Ivermectin (c = 2)</th>
<th>Levamisole (c = 5)</th>
<th>Levamisole / Rfoxanide (c = 9)</th>
<th>Albendazole (c = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemonchus contortus</td>
<td>7997</td>
<td>995 (88)</td>
<td>852 (89)</td>
<td>96 (88)</td>
<td>87 (9)</td>
</tr>
<tr>
<td>Trichostrongylus axei</td>
<td>40</td>
<td>20 (50)</td>
<td>23 (43)</td>
<td>12 (43)</td>
<td>0 (100)</td>
</tr>
<tr>
<td>Trichostrongylus colubriformis</td>
<td>863</td>
<td>518 (60)</td>
<td>644 (75)</td>
<td>503 (42)</td>
<td>12 (98)</td>
</tr>
<tr>
<td>Oesophagostomum columbianum</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichuris ovis</td>
<td>5</td>
<td>12 (-140)</td>
<td>10 (-100)</td>
<td>7 (30)</td>
<td>12 (-71)</td>
</tr>
<tr>
<td>Tapeworms</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
<tr>
<td>Mean nematode worm count</td>
<td>8905</td>
<td>1545 (83)</td>
<td>1529 (83)</td>
<td>618 (60)</td>
<td>111 (82)</td>
</tr>
</tbody>
</table>

TABLE 2: The total and differential worm counts in sheep that died during the treatment period between November 2005 and March 2006. Figures in brackets represent the percentage worm cont reduction from the preceding treatment levels and the letters c stands for the number of clinical cases and d stands for the number of animals dead during the drug treatment periods.
weight as was the rest of the flock. Those in group B remained as the un-treated controls. Rectal faecal samples were collected and processed for EPG determination as above. On day 14 all control group animals were treated as in Group A.

In January 2006, twenty other animals were selected, and assigned to two treatment groups (A and B) with equal members. Animals in Group A were treated with albendazole (Valbazen®, Ultravetis East Africa, Pfizer Inc. Nairobi, Kenya) at a dose rate of 7.5 mg Kg⁻¹ body weight as was the rest of the flock. Those in group B remained as the un-treated controls during which period rectal faecal samples were collected and processed for EPG determination. On day 14, all control group animals were treated as in Group A.

Post-mortem examination was done on all animals that died during the study period and worms recovered from their guts and counted as described in the MAFF manual. In February, 2006, faecal samples were taken from 20 sheep before treating the entire flock with albendazole and moving them to new pastures and pens (not previously grazed by sheep). Their faecal egg output was monitored a month after (March, 2006).

Anthelmintic efficacy tests

The anthelmintic efficacies were determined through faecal egg count (FECR %) and post-mortem worm count (WC) reduction percentages. The FECR % and the 95% confidence limit (CL) for the reduction were calculated according to the method described in the World Association for the Advancement of Veterinary Parasitology (WAVP) recommendations for the detection of anthelmintic resistance and the worm burden estimated.

Anthelmintic resistance was declared when the FECR % was <95% and the lower 95% CL was <90% and when the worm counts declined by less than 90%. The animals were monitored for clinical helminthosis by close observation of typical signs such as anaemia, "bottle jaw"and or diarrhea.

Results

The results of the FECR% and their corresponding 95% CL are shown in Table 1. FECR% of 44.2%, 77%, 66.7% and 42.3% were recorded for ivermectin, levamisole, levamisole – rafaxanide combination and albendazole, respectively. The WCs are shown in Table 2. Haemonchus contortus and Trichuris ovis were resistant to all drugs tested. Trichostrongylus species were resistant to ivermectin and levamisole, but susceptible to albendazole. The mean pre albendazole faecal egg counts during the month of February 2006 was 1684 compared to 321 in March 2006 (80.7% reduction) after treatment and movement to new pastures.

Discussion

The results of the present study clearly indicate that gastrointestinal nematodes in sheep on the study farm were strongly resistant to ivermectin, levamisole, levamisole – rafaxanide combination and albendazole. Similar multiple anthelmintic resistance has been reported in other parts of the country. Multiple anthelmintic resistance develops as a result of selection pressure exerted by regular use of anthelmintics from different classes at the same time without alternation as has been recommended. This is likely to have been the case on the study farm where different classes of anthelmintics were used over the
past 20 years.

In an earlier study conducted on animals from the same farm, a strain of *H. contortus* that showed simultaneous anthelmintic resistance to albendazoles, levamisole and ivermectin was isolated⁶. More than ten years later, the present study confirms the existence of strains of *H. contortus* and *T. ovis* with simultaneous resistance to the three drugs as earlier reported and, in addition, rafoxanide whose efficacy had not been tested earlier. The results further indicate the presence of *Trichostrongylus* species that were resistant to ivermectin and levamisole but susceptible to albendazole. The results indicate that, not only was the spectrum of the ineffective anthelmintics increasing, but the number of resistant nematode species too.

In the present study, sheep on the farm were permanently on pasture and grazed the same paddock over the years. This practice is likely to have resulted in high levels of pasture contamination necessitating frequent use of anthelmintics. In the presence of resistance as indicated above, the occurrence of clinical helminthosis and mortalities were therefore not surprising. However, when the sheep were drenched with albendazole and moved to "cleaner" pastures and pens, the incidence of clinical helminthosis and related mortalities were drastically reduced. Although the "dose and move" method of helminth control as adapted here may lead to solid resistance to anthelmintics, it was necessary as a salvage measure. However, in order to effectively control helminthosis and to preserve the effectiveness of anthelmintics, there is need to alternate between anthelmintic classes on an annual basis and to combine treatments with appropriate pasture rotation that reduces contamination¹⁵.

**Acknowledgements**

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**References**


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PREVALENCE OF SOLE HAEMORRHAGES AND ITS CORRELATION WITH SUBCLINICAL AND CHRONIC LAMINITIS IN DAIRY COWS

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PREVALENCE DE L’HEMORRAGIE DE LA SOLE ET SA CORRELATION AVEC LA FOURBURE AIGUE INFRACLINIQUE ET CHRONIQUE CHEZ DES VACHES LAITIERES

Résumé
La prévalence et les facteurs de risque de la fourbure aigue étaient déterminés chez 300 vaches laitières de 29 fermes en zéro-pâturage et de 3 ferme en pâturage à Nairobi et dans les districts périurbains. La boîterie était estimée à l'aide d'un système d'évaluation de la locomotion universellement reconnu. Les ongîons étaient examinés et une couche de 1-2mm d'épaisseur de la corne de la sole était rognerée pour exposer davantage les lésions qui étaient en-dessous. La localisation des lésions sous les ongîons était enregistrée selon les 6 zones universellement reconnus. La prévalence de la fourbure aigue infraclinique et chronique était de 49,3% et 21% respectivement. Alors que l’hémorragie de la sole n'était observée que chez 82% sur les 70,3% des vaches souffrant de fourbure aigue (à la fois infraclinique et chronique), elle a en revanche été notée chez toutes les 49,3% des vaches en phase infraclinique de fourbure aigue. Les hémorragies étaient réparties en 5 catégories universellement reconnues. Les hémorragies dans la zone 4 en-dessous de l'onglon étaient souvent associées à la fourbure aigue infraclinique, tandis que celles dans les zones 2, 3 et 6 étaient surtout associées à la fourbure aigue chronique. Les hémorragies « faible à modérée » (point 1 et 2) avaient une forte corrélation ($\chi^2 = 18,01$ ; $p<0,0001$) avec la fourbure aigue infraclinique. Les hémorragies « modérée à grave » (point 2 et 3) avaient une forte corrélation ($\chi^2 = 33,08, p<0,0001$) avec la fourbure aigue chronique. L'hémorragie de la sole que l'on observe beaucoup plus à travers le rognage de la corne peut très bien être utilisée pour le diagnostic de la fourbure aigue.

Summary
Prevalence and risk factors of laminitis were determined in 300 dairy cows from 29 zero-grazed and 3 pasture-grazed dairy farms in Nairobi and the peri-urban districts. Lameness was assessed using a universally recognized locomotion scoring system. Claws were examined and 1-2mm thick layer of the horn of the sole was trimmed-off to further expose underlying lesions. Location of lesions on the under-side of the claws was recorded corresponding to 6 universally recognized zones. Prevalence of subclinical and chronic laminitis was 49.3% and 21% respectively. While sole haemorrhages were recorded in 82% of the 70.3% of the cows with laminitis (both subclinical and chronic), it was nevertheless present in all the 49.3% of the cows with subclinical phase of laminitis. Haemorrhages were categorized into 5 universally recognized scores. Haemorrhages in zone 4 of the claw under-side were frequently associated with subclinical laminitis while those in zones 2, 3 and 6 were frequently associated with chronic laminitis. Slight to moderate (score 1 and 2) haemorrhages were significantly correlated ($\chi^2 = 18.01, p<0.0001$) with subclinical laminitis. Moderate to severe (score 2 and 3) haemorrhages were significantly correlated ($\chi^2 = 33.08, p<0.0001$) with chronic laminitis. Sole haemorrhage which is made clearer through horn trimming can reliably be used to diagnose laminitis.
Introduction

Cattle lameness in most parts of the world ranks as the third most important disease causing economic losses after infertility and mastitis. It has also been identified as an important cattle welfare determinant. Depending on the type of risk factors within specific dairy production systems, claw horn lesions account for 23% to 60% of lameness in dairy cows. Among these claw horn lesions are subclinical and chronic laminitis. In Europe, 90% of lameness in cattle is localized in the claws and laminitis contributes a high proportion of it. The economic importance of laminitis is that it is considered as a major predisposing factor to other claw lesions.

Laminitis is a diffuse aseptic inflammation of the corium, whose microvasculature is progressively damaged to the extent that production of good quality claw horn is compromised. It has also been referred to as "claw horn disruption" (CHD), a term that more appropriately describes its main effect on the claw. Laminitis not only affects the claw but represents a syndrome that includes a systemic disease affecting the general condition of the animal. The disease can be subclinical, acute or chronic. Acute laminitis is related to acute rumen overload with grain feeds, while subclinical and chronic laminitis are related to subacute ruminal acidosis (SARA) and may result into irreversible degenerative changes that affect the sensitive and the horny lamina of the claws.

Subclinical laminitis is the most important form of laminitis in cattle since it causes physiological changes in the claws while posture and gait of the animal remains deceptively normal. Diagnosis of subclinical laminitis is therefore difficult to determine. Its significance is further exemplified by its contribution to development of chronic laminitis and other laminitis-related claw lesions such as sole ulcers, heel erosions and separation of the white line. Therefore, a reliable method of making a positive diagnosis of this early phase of laminitis is important for prompt remedial intervention before it progresses to lesions that could irreversibly damage the corium and the horn of the claws.

In laminitis, the main effect on the corium is breakdown of the microvasculature which eventually leads to haemorrhage and exudation of serum from capillary beds into the sole corium. Subsequently, there appears red or yellow discoloration of the horn in different zones of the sole and particularly the white line zone. Moreover, this seepage from the microvasculature also causes oedema, pressure, ischaemia and hypoxia of the corium with consequent occurrence of epidermal damage and extensive arterio-venous shunts. The frequency of occurrence of haemorrhages and the haemorrhage score have been found to differ at different zones of the sole. The hind claws manifest more severe haemorrhage than the fore claws; and the lateral claws of the hind feet manifest more severe haemorrhages than the medial claws. Primiparous cows are reported to be more prone to severe sole haemorrhages than multiparous cows. It has been suggested that cows fed on high plane level of concentrates and housed on concrete floors have high haemorrhage scores; the latter however contributes a greater effect than the former. Factors related to peri-parturient period were found to enhance development of sole haemorrhages and laminitis. These factors are related to the effects of the hormone...
"relaxin" that is present at calving and which also causes weakening of the suspensory connective tissues between the hoof and the pedal bone resulting in claw lesions\textsuperscript{16,17}.

Another key clinical symptom in laminitis is the excessive softening of the horn of claws rendering the sole prone to physical injuries, hence leading to sepsis\textsuperscript{6,11}. In chronic laminitis, the shape of claws is altered and becomes difficult to clinically distinguish from regular claw deformities that have no association with laminitis\textsuperscript{10}.

Zero-grazing, concentrate feeding and long periods of confinement on hard concrete floors are some of the production systems that are gaining fast adoption in the Kenyan dairy farming. These are some of the risk factors that have been incriminated as predisposing to lameness caused by claw lesions particularly laminitis\textsuperscript{18,19}. Moreover, laminitis is insidious in nature and therefore it is not as easy to notice the affected cattle as it is with infective conditions of the claws\textsuperscript{6}. Furthermore, diagnosis and prevalence of laminitis in these Kenyan dairy farms have not been determined. It was therefore deemed necessary to carry out farm survey to establish the extent of laminitis in "Nairobi and its environs" as well as define an easy and practical method of making positive diagnosis of laminitis.

Materials and methods

Study animals and farms

A total of 300 dairy cows were included in the study of claw lesions. Out of the 300 cows, Friesians were 76% (228), Ayrshires 20% (60) and the remaining 4% (12) were a mixture of Guernseys and Jerseys. A majority (83%) of them were zero-grazed and 17% were pasture-grazed. The cows that were included in the study were those that had calved at least once. The proportion of those with 3rd and 4th parities was 60% and those in the 1st and 2nd parities were 40%. Both lame and non-lame cows were included in the study.

These dairy cows were sampled from 32 farms purposively selected from Nairobi area, Kiambu, Kajiado and Thika districts. The farms included were\textsuperscript{6,12,13}, and \textsuperscript{1} from Nairobi, Kiambu, Kajiado and Thika respectively. Those under zero-grazed production system were 29 each having 5-20 cows and those under pasture-grazed production system were 3 each having more than 60 cows. Purposive selection was adopted for logistic reasons based on the farmers' willingness to allow their cows to be used in the study. The district or division based veterinarians and animal health assistants with whom farmers were acquainted, facilitated in locating the farms and convincing farmers to participate in the study.

Sampling method

It was considered inappropriate to use farm records for sampling the cows due to improper record keeping in most farms. In each farm, 50% of the cows that met the selection criteria were recruited into the study group using systematic sampling method\textsuperscript{20}.

The sampling formula adopted was \(k = N/n\), where \(N\) is the population size, \(n\) is sample size and \(k\) is the 1st sample (1st cow in every farm). In each farm, cows that met the study criteria were temporarily tagged with serial numbers 1, 2, 3, to \(n\), where \(n\) was the serial number of the last cow being selected in the farm. To avoid biased numbering, assigning of serial numbers was randomly done by an independent farm worker. Systematic
selection of individual cows from the serialized groups was done by picking every other serial number. For example, in the serial n1 to n10, if the first cow selected was n1, the next serially selected would be n3, n5, n7 and n9. The serial number of the first cow selected was alternated between odd and even numbers from farm to farm, until all the farms were covered. Therefore, the cows selected in any farm were those with either “odd” or “even” serial numbers.

**Examination of cows**

Each cow was examined for general lameness using a previously recommended locomotion scoring system\(^{21}\) as shown in table 1. Due to the nature of physical restraint structures available in the farms, it was difficult to examine the fore claws and therefore, it was decided that closer examination of the claws should be done only on the hind feet. The hind claws of all selected cows (whether lame or non-lame) in each of the farms were examined. Each cow was manually restrained in standing position in a crush and each hind limb, one at a time, was tied and lifted above the ground with a rope fastened to an overhead wooden or metal bar. The claws were thoroughly washed to remove all dung and manure especially from the sole to expose possible lesions. A thin layer (1 to 2mm) of the horn of the weight-bearing surface of each claw was trimmed-off. This was considered an important part of claw examination because lesions underneath the horn of the

<table>
<thead>
<tr>
<th>Locomotion score</th>
<th>Clinical description</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>• Stands and walks normally.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• All feet placed with purpose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture standing: flat.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture walking: flat.</td>
</tr>
<tr>
<td>2</td>
<td>Mildly lame</td>
<td>• Stands with flat back, but arches when walking.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Gait is slightly abnormal.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture standing: flat.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture walking: arched.</td>
</tr>
<tr>
<td>3</td>
<td>Moderately lame</td>
<td>• Stands and walks with an arched back.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Short strides with one or more legs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture standing: arched.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture walking: arched.</td>
</tr>
<tr>
<td>4</td>
<td>Lame</td>
<td>• One or more limbs favoured but partially weight bearing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture standing: arched.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture walking: arched.</td>
</tr>
<tr>
<td>5</td>
<td>Severely lame</td>
<td>• Refuses to bear weight on one limb.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May refuse or have great difficulty moving from lying position.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture standing: arched.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Back posture walking: arched.</td>
</tr>
</tbody>
</table>
claws are not discernible externally until exposed by trimming of the horn. The handling and trimming of the claws did not involve the corium and therefore no pain was elicited to the cows. However, for the cases that had exposed corium or had infective claw lesions, foot anaesthesia was done in order to prevent pain during lesion manipulations. It was done by administering 2% lignocaine hydrochloride into digital veins distal to a tourniquet placed round the metatarsus.

The sole was examined closely for any lesions and particularly those related to laminitis such as sole ulcer, sole erosion (bruising), heel erosion, double (underrun) sole, white line separation, claw deformities and haemorrhagic (red/yellow) discolorations. Each claw was photographed using a digital camera (Sony DSC-S80, 4.1Mega Pixels) for the purpose of recording lesions for later comparison with each other. Other claw lesions not related to laminitis were also noted and recorded. For the purpose of description and analysis, the weight-bearing surface of the claw was divided into 6 universally recognized zones as shown in figure 1.2 Haemorrhagic discolorations were divided into 5 universally recognized scores as shown in table 2.2 Haemorrhages involving only one zone of the weight-bearing surface of the claw were categorized as slight (score 1), haemorrhages involving two zones were categorized as moderate (score 2) and haemorrhages involving more than two zones were categorized as severe (score 3).

### Diagnosis of laminitis

The claws that manifested any haemorrhage score were considered to be laminitic. Concurrent occurrence of any lesions such as white line separation, sole ulcers, double soles, heel erosion, sole bruising, or claw deformities with sole haemorrhages was also considered as an indicator of laminitis. When two or more such lesions were present on the claws even without sole haemorrhages, the claws were considered to also have laminitis. However, whenever only one such lesion occurred without sole haemorrhages it was concluded that the claw did not have laminitis but instead the diagnosis was that of the specific lesion seen grossly.

Statistical analysis was done for comparison of the findings between lateral and medial claws in the individual cows and through the entire study population. The data collected was analyzed using GENSTAT for Windows Discovery Edition 2 (VSN International). Descriptive statistics were computed.

---

**Figure 1**: the six zones of the bovine weight-bearing surface and the key showing names of the zones (Greenough and Vermut, 1991).

<table>
<thead>
<tr>
<th>Key</th>
<th>Zone</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>White zone at the toe</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Abaxial white zone</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Abaxial wall-bulb junction</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Sole-bulb junction</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Apex of the sole</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>The bulb</td>
</tr>
</tbody>
</table>
Results

It was found that out of the 300 dairy cows examined, 88% had at least a claw lesion. Among these, 69% did not show any sign of lameness but had score 1 on the locomotion score scale. The prevalence of subclinical laminitis was 49.3%, while that of chronic laminitis was 21%. Laminitis was found to occur independently or together with other lesions and disorders such as white line separation, sole ulcers, double soles, heel erosion, sole bruising and claw deformities. All these disorders are usually considered to be related to some phase of laminitis although some of them occurred independently.

Sole haemorrhages were present in 52% of the cows and out of this, 44.8% were slight to moderate (score 1 and 2) while 7.0% showed severe (score 3 and 4) haemorrhages (Fig. 2), which included exposed corium through sole ulceration (Fig. 3 B-E). Haemorrhages were highly prevalent in zone 4 (34.7%), moderately prevalent in zones 3 (14.3%), 6 (14.1%) and 2 (13.6%), and least prevalent in zones 5 and 1 at 8.7% and 0.7% respectively (Fig. 4).

Haemorrhage score and the number of haemorrhagic zones had a strong positive correlation ($r = 0.546$) with subclinical laminitis, and slightly weaker positive correlation ($r = 0.41$) with chronic laminitis. These correlations were found to be significant for both subclinical ($\chi^2 = 89.45$, $p < 0.05$) and chronic ($\chi^2 = 50.16$, $p < 0.05$) laminitis. Subclinical laminitis was positively but weakly correlated ($r = 0.245$) with slight and moderate (scores 1 and 2) sole haemorrhages. However, chi-square test
Figure 3: Haemorrhage scores: A-Normal (no haemorrhage), B-Slight (double-headed arrow), C-Moderate (curved arrows along abaxial white line), D-Severe (double headed arrow), and E-Exposed corium or sole ulcer (notched arrow).

indicated that this correlation with subclinical laminitis was significant ($\chi^2 = 18.01$, $p < 0.0001$). Chronic laminitis was also positively but weakly correlated ($r = 0.334$) with moderate and severe (scores 2 and 3) sole haemorrhages. Chi-square test showed this correlation with chronic laminitis to be also significant ($\chi^2 = 33.08$, $p < 0.0001$). Correlation coefficient indicated that haemorrhages in zone 4 of the weight-bearing surface of the claw were positively but weakly correlated ($r = 0.276$) with subclinical laminitis, and this correlation was significant ($\chi^2 = 22.83$, $p < 0.05$). Chronic laminitis was positively but weakly correlated with haemorrhages in zone 3 ($r = 0.28$), zone 2 ($r = 0.247$) and occasionally but very weakly with zone 6 ($r = 0.122$). However,
these correlations with zone 3 ($\chi^2 = 23.45$), zone 2 ($\chi^2 = 18.23$) and zone 6 ($\chi^2 = 4.48$) were all significant ($p < 0.05$).

In this study, 82% of the cows with subclinical and chronic laminitis had sole haemorrhages and only 18% did not show any haemorrhage. However, all the cows with sole haemorrhages had either subclinical or chronic laminitis. Occurrence of sole haemorrhages was significantly ($\chi^2 = 86.5$, $p < 0.001$) higher in the hind lateral claws than the medial claws. This trend was also similar between the hind lateral and medial claws with regard to the other claw lesions. The hind lateral claws had lesions in 82.7% of the cows and the medial claws in 46.3% of the cows.

**Discussion**

The results of this study showed that a high number of cows had claw lesions, most of which did not manifest clinical lameness. This means that most of the claw lesions affecting dairy cows are subclinical and therefore making of diagnosis is not possible unless a closer scrutiny is done. The results also indicated that a high proportion (about 70%) of the cows had laminitis, most (about 50%) of which was subclinical. It has previously been cited by others that subclinical laminitis predisposes cattle to other claw lesions particularly those related to laminitis including chronic laminitis. Although subclinical laminitis does not cause clinical lameness, it contributes to the occurrence of lesions that cause not only lameness, but also severe and sometimes irreversible damage to the claws. This therefore necessitates early diagnosis of subclinical laminitis for prompt management of the condition before it advances to the destructive phases. A method of positively making such a diagnosis is required.

The results show that all cows that had sole haemorrhages had one form of laminitis. This could be attributed to the fact that the initial phase in the pathogenesis of laminitis
involves changes in the pododermal microvasculature, which results in extravascular seepage of serum with subsequent signs of haemorrhage in the horn of the sole as has been suggested previously\textsuperscript{6,11}. The high prevalence of sole haemorrhages seen in association with laminitis indicated that this was the main feature that appeared on the horn of the soles of claws that had laminitis. Such observations have been made previously\textsuperscript{13}. Therefore, sole haemorrhage can be used as a reliable key indicator of presence of laminitis on the claws of dairy cows. It is particularly useful in diagnosis of subclinical laminitis which may not manifest any other symptoms. However, while sole haemorrhages are visible on the surface of the claw-horn in some severe cases of chronic laminitis, in others, trimming of the horn is necessary to expose, confirm and reveal the distribution of the haemorrhages. In addition to sole haemorrhages, it has been shown that the chronic phase of laminitis can also be diagnosed by changes that occur in the shapes of the claws\textsuperscript{10}.

The varying distribution of haemorrhages among different zones of the weight-bearing surface of the claws has been reported previously\textsuperscript{12}. However, the frequency of distribution of the haemorrhages among the zones has not been documented previously. The current study showed the prevalence of haemorrhages to be high in zone 4 and moderate in zones 2, 3 and 6 while low in zones 1 and 5. This pattern of haemorrhage distribution can be attributed to the fact that zone 4 is the sole-bulb junction which anatomically bears most weight\textsuperscript{15,24}, while zones 3 and 6 are closer to the bulb and therefore have softer horn that easily succumbs to pressure. Zone 2 is on the abaxial white line that is closer to the weight-bearing wall of the claw\textsuperscript{23,24}. However, zones 1 and 5 being closer to the toe of the claw, bear least weight\textsuperscript{24}.

Furthermore, during laminitis, the horn of the claw becomes excessively softer and weaker\textsuperscript{10}. This softening and weakening of the horn makes the corium under the zones that naturally bear most weight (zones 2 and 4) and those closer to the bulb (zones 3 and 6) more vulnerable to ground pressure insult. Moreover, in conditions such as chronic laminitis that cause claw overgrowth and shape alterations, affected cattle shift weight-bearing to the more proximal zones (zones 3, 4 and 6)\textsuperscript{25}. The shifted weight, in addition to softened horn during chronic laminitis, then allows transmission of immense pressure to the underlying corium resulting in more injury to its microvasculature. This explains the reason for association of more severe haemorrhages with chronic laminitis and less severe haemorrhages with subclinical laminitis. Subclinical laminitis is not accompanied by claw shape alterations. The corium microvasculature breaks more in chronic laminitis and results in more severe haemorrhages than in subclinical laminitis\textsuperscript{6,22}.

A high association between subclinical laminitis and haemorrhages in zone 4 can be explained by the occurrence of an early pododermal microvasculature that results in an initial escape of blood components into the tubules of the horn of the sole and bulb\textsuperscript{22}. Furthermore, haemorrhages from the underlying corium appear on the surface of zone 4 earlier (during subclinical laminitis) due to its thinner horn compared to the rest of the sole with thicker horn in which haemorrhages appear later (during chronic laminitis). On the other hand, the frequent association of haemorrhages in zones 2, 3
and 6 with chronic laminitis could be related to claw shape alterations and more weight distribution to these zones during this phase of laminitis.

The finding of more sole haemorrhages and other laminitic claw lesions in the hind lateral claws than the medial claws is consistent with observations made by others and is related to anatomic weight distribution in which the lateral claws in the hind limbs of cattle bear more weight than the medial claws.

This study indicates that chronic laminitis and other lesions related to laminitis can be avoided by preventive management at subclinical laminitis phase. It is therefore concluded from the results of this study that sole haemorrhage can positively be used to diagnose laminitis and it is particularly useful at the subclinical stage when no other signs are manifested. The study has also shown that subclinical laminitis can be diagnosed early by regular deliberate (at least once or twice a year) claw trimming which is likely to reveal presence or absence of haemorrhage in the claws. This study shows that there is a high prevalence of laminitis, particularly subclinical laminitis, in the zero-grazed smallholder dairy herds in “Nairobi and its environs”. This could possibly be attributed to the varied housing and confinement environment of cows in the smallholder dairy units. Further experimental studies may be necessary in other parts of the country in order to validate a conclusion on laminitis and other claw lesions for the Kenyan zero-grazed smallholder dairy production system.

Regular herd health programmes are recommended for diagnosis of claw lesions so that remedial management can be instituted at the subclinical phase before severe and irreversible claw damage occurs.

References


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THE DISTRIBUTION OF BOVINE LEUKEMIA VIRUS GENOTypes IN CATTLE FROM JAPAN AND DIAGNOSTIC PERFORMANCE OF AGAR GEL IMMUNODIFFUSION (AGID) AND POLYMERASE CHAIN REACTION (PCR) TESTS

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REPARTITION DES GENOTYPES DE VIRUS DE LA LEUCEMIE BOVINE CHEZ DES BOVINS DU JAPON ET PERFORMANCE DES TESTS DE DIAGNOSTIC D’IMMUNODIFFUSION EN GELOSE (AGID) ET D’AMPLIFICATION EN CHAINE PAR POLYMERASE (PCR)

Résumé
Une étude pour enquéter sur les types et la répartition des génotypes de virus de la leucémie bovine (VLB) et pour évaluer la performance du test de diagnostic d’immunodiffusion en gélose (AGID) a été menée sur 807 bovins issus de 68 fermes dans 16 préfectures au Japon pendant la période allant de juin 2002 à décembre 2003. On a effectué le test AGID sur des prélèvements de sérum et le test PCR pour détecter le provirus VLB à partir des lymphocytes du sang périphérique. En vue d’identifier les génotypes, le test RFLP a été effectué sur le fragment 444 bp du gène enV sur le produit amplifié par PCR en utilisant les endonucléases Bcl I, Hae III, PVu II, BamH I et Bgl I. Dans la présente étude, six génotypes étaient identifiés, à savoir: les génotypes 1, 2, 3 et 5. Deux de ces génotypes étaient peu communs dans la mesure où ils exposaient une fragmentation de bande unique ; nous avons appelé ces génotypes 2-3 et 3-4. Les génotypes 1, 3 et 5 sont les types prédominants au Japon que l’on a relevés chez 48,3%; 32,7% et 16,9% des bovins testés respectivement. La sensibilité et la spécificité de l’AGID étaient de 89,3% et 58,8% respectivement. La concordance entre les tests AGID et PCR était de 0,48%.

Mots-clés : Virus de la leucémie bovine (VLB), bovins, génotype, immunodiffusion en gélose (AGID), amplification en chaîne par polymérase (PCR), sensibilité, spécificité, concordance de test.

Summary
A study to investigate the types and distribution of bovine leukemia virus (BLV) genotypes and to estimate diagnostic test performance of agar gel immunodiffusion (AGID) was conducted on 807 cattle drawn from 68 farms found in 16 prefectures in Japan from June 2002 to December 2003. AGID test on serum samples and polymerase chain reaction (PCR), to detect BLV provirus, from peripheral blood lymphocytes (PBL) were performed. To identify genotypes, restriction fragment length polymorphism (RFLP) was done on the 444 bp fragment of enV gene on PCR amplified product using endonucleasases Bcl I, Hae III, PVu II, BamH I, and Bgl I. In this study, six genotypes were identified. These are genotypes 1, 2, 3, and 5. Two of the genotypes were unusual in that they exhibited unique band fragmentation; we named these genotypes 2-3 and 3-4. Genotypes 1, 3, and 5 are the most dominant types in Japan, which are recorded in 48.3%, 32.7%, and 16.9% of tested cattle, respectively. The sensitivity and specificity of AGID was found to be 89.3% and 58.8%, respectively. The test agreement between AGID and PCR was 0.48.

Key words: bovine leukemia virus (BLV), cattle, genotype, agar gel immunodiffusion (AGID), polymerase chain reaction (PCR), sensitivity, specificity, test agreement
Introduction

Bovine Leukemia virus (BLV) is the causative agent of enzootic bovine leukosis (EBL), a neoplasm of lymphatic tissue in the bovine species. BLV is classified under the genus *Deltaretrovirus* family *Retroviridae*. BLV infects B lymphocytes in cattle. The majority of infected animals remain healthy and show no economic down performances, but a significant size of BLV carriers develop a form of the disease known as persistent lymphocytosis (PL), and only a small percentage of BLV infected animals develop lymphoid tumors.

BLV infection has a worldwide distribution and sero-epidemiological studies indicated a higher prevalence in different countries. BLV infection was found to be high in some farms in Japan.

The BLV envelope comprises of two glycoproteins: gp 35 (30), a transmembrane protein, and gp 51, an associated surface molecule. Both are derived by post-translational proteolytic cleavage of a common precursor – gpr 72- encoded by the env gene. Glycoproteins contain the recognition site of the cell surface receptor required for virus adsorption and entry. Glycoproteins (gp 51) elicit immune responses. Based on restriction enzyme analysis and nucleotide and amino acid sequence comparisons, it was demonstrated that different BLV variants are found in different geographical regions. The previously sequenced BLV isolates demonstrated that BamHI I, Bcl I, Hae III, Bgl I, and Pvu II restriction sites on BLV fragment are good markers to differentiate BLV variants.

Most cattle develop a strong permanent antibody response to the BLV envelope glycoprotein antigen some weeks after BLV infection and keep the provirus in their lymphocytes. Diagnosis of BLV infection using Agar gel Immunodiffusion (AGID) test on gp51 is widely used outside sub-Saharan Africa. In the last few years, BLV infections with low or transient, or even absence of any detectable BLV antibodies, even if, provirus is integrated have been described. This makes elimination of BLV infection difficult using only serological tests. Serology can be complemented by genomic diagnosis, like PCR. The use of PCR in the diagnosis of BLV has increased in recent years. The existence of different types of BLV is suspected in Japan. The knowledge regarding characteristics of AGID serological test performance is inadequate and the experience needs to be shared into the African situation where the disease is believed to occur.

The objectives of this study were to investigate the types of BLV genotypes, estimate the sensitivity and specificity of AGID, and know the level of agreement between AGID and PCR tests.

Materials and Methods

Study area and animals

The study was carried on a total of 807 cattle drawn from 68 farms found in 16 prefectures in Japan from June 2002 to December 2003. Samples from across all islands and corners of Japan were collected to identify the existence and classify BLV genotypes.

The blood samples, collected with the help of livestock hygiene service center workers, were of two types; one type was used for serum separation, and the other was used for DNA extraction and total
leukocyte count. Serum separation and AGID test, leukocyte count, and DNA extraction were carried upon receipt without delay.

Ager gel immunodiffusion

Ager gel immunodiffusion (AGID) was performed on all the 807 serum samples collected from cattle. The gel consisted of 1% noble agar, 8.5% NaCl, and 0.6% Tris. The pH was adjusted to 8.6. A 5mm diameter one central and six peripheral wells were made. The peripherally located wells were 3mm far from the central well. The antigen was placed in the central well. Each of the reference positive sera and test samples were put in the six peripheral wells alternatively – reference positive sera on the three wells and test samples on the remaining three. The gel plate was allowed to stand at room temperature for 48 hrs before reading precipitation lines.

Leukocyte count

Leukocyte counting was done using a Coulter Counter (Model ZF, Coulter Electronics Inc.). Cattle that had peripheral blood lymphocyte (PBL) numbers of more than 20,000 /µl were considered as having persistent lymphocytosis (PL). However, granulocytes and monocytes are included as the total white blood cell count and not differential count was conducted in this study.

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed on all 807 samples. Primers chosen for this study were designed on the basis of published sequence data\textsuperscript{16}. The primers were reported previously for BLV provirus detection by PCR\textsuperscript{18}. These primers amplified the 444 bp (base pair) DNA fragment in the gp51 region of the env gene. A nested – PCR was carried out using the following forward and reverse 22-mer primers (ESPEC Oligo Service Corp.)

forward primers:

\begin{align*}
\text{env}_{5032} & (5' - \text{TCTTGCCAAGTCTCCAGATA} - 3') \\
\text{env}_{5099} & (5' - \text{CACAAGGGCGCGCCTGT} - 3') \\
\end{align*}

reverse primers:

\begin{align*}
\text{env}_{5531} & (5' - \text{GCGAGGCCGTTCCAGAGCTTG} - 3') \\
\text{env}_{5606} & (5' - \text{AAACAAAATCTGGGAAGGT} - 3') \\
\end{align*}

A reaction mixture was prepared in 50 µl aliquots (1µl sample and 49 µl reaction mixture). External primers – \text{env}_{5032}/\text{env}_{5606} – resulted in the amplification of a 598 bp DNA fragment, and internal primers – \text{env} 5099/\text{env} 5521 – amplified a 444 bp DNA fragment. The amplification reactions were performed by PE 9700 DNA Thermal Cycler (Applied Biosystems). At first, an initial incubation at 95°C for 9 minutes was carried out followed by 40 cycles, each consisting of denaturation at 95°C for 30 seconds, annealing at 62°C (external primers) or at 70°C (internal primers) in both cases for 30 seconds, and extension at 72°C for 4 minutes. This was followed by a final extension at 72°C for 4 minutes. For the second round of PCR 1µl of product was taken from the first amplification and reamplified. The PCR products were placed on a gel plate prepared using 1.5% agarose. After electrophoresis in 100v, the gel plate was stained by ethidium bromide. The amplified PCR products were observed using UV light.

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) was performed on PCR products from 391 infected bovine
leukocytes. The 444 bp DNA fragment of the env gene of the BLV provirus was amplified by nested-PCR. The amplified products were digested with five different restriction endonucleases in the method of RELP (Table 1). Hence, each sample was digested with 10U of Bam H I, 9U of Bgl I, 10U of Hae III, 12U of Bcl I, and 9U of Pvu II restriction endonucleases at 37°C for 2 hours and 30 minutes (all restriction endonucleases were obtained from Toyobo Co. LTD.). The digested products were subjected to electrophoresis at 50v in 2% agarose gels. They were stained in ethidium bromide and the bands observed using UV light. The sizes of the resulting bands were compared with 0.5mg of 100bp DNA ladder.

Data analysis

Data were analyzed using descriptive statistics, percentages, and sensitivity and specificity as well as kappa statistic was calculated following established procedures\textsuperscript{19,20}.

**Results**

**BLV genotypes of Japan**

Restriction sites for Bam H I and Bgl I endonucleases are common among the DNAs and did not help in the differentiation of BLV genotypes. However, the other endonucleases, namely, Bcl I, Hae III, and Pvu II were used in the differentiation of BLV genotypes. Bam H I cleaved the 444 bp DNA

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Bcl I</th>
<th>Hae III</th>
<th>Pvu II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>225,220</td>
<td>200,100,85</td>
<td>444</td>
</tr>
<tr>
<td>2</td>
<td>220,120,105</td>
<td>315,95</td>
<td>444</td>
</tr>
<tr>
<td>3</td>
<td>220,120,105</td>
<td>285,95</td>
<td>444</td>
</tr>
<tr>
<td>4</td>
<td>220,120,105</td>
<td>200,100,85</td>
<td>444</td>
</tr>
<tr>
<td>5</td>
<td>225,220</td>
<td>285,95</td>
<td>444</td>
</tr>
<tr>
<td>6</td>
<td>225,220</td>
<td>200,100,85</td>
<td>280,165</td>
</tr>
</tbody>
</table>
Table 2: Percentage of the different BLV genotypes found in Japan

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of prefectures</th>
<th>Number of farms with</th>
<th>Number of animals with (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>31</td>
<td>189 (48.3)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>18</td>
<td>128 (32.7)</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5</td>
<td>66 (16.9)</td>
</tr>
<tr>
<td>2-3</td>
<td>2</td>
<td>2</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>3-4</td>
<td>1</td>
<td>1</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

fragment into fragments of 315 and 130 bp in all animals. Similarly, Bgl I cleaved the 444 bp DNA fragment into fragments of 330 and 115 bp in all animals.

Based on BLV provirus amplification using nested-PCR and afterwards restriction fragment length polymorphism (RFLP) six genotypes were identified in Japan (Table 2). These are genotypes 1, 2, 3, 5 and two other unusual types. In the first unusual type, all proviral DNA fragments typical of genotype 2 and genotype 3 are detected in one sample; we call this type genotype 2-3. Similarly, in the other type we observed all DNA fragments typical of genotype 3 and genotype 4 in one sample; we call this type genotype 3-4. The former was detected in Miyagi and Shiga prefectures, and the latter in Shimane Prefecture, Japan.

Genotype 1 was recorded in 48.3% of the samples. Next in importance were genotype 3 and genotype 5, recorded in 32.7% and 16.9% of the samples, respectively. Genotypes 1, 3, and 5 were

Fig 1. In the pictures wells 1, 3, and 5 are samples: wells 2, 4, and 6 are positive control sera; and the central well contains BLV antigen. In picture A all samples (wells 1, 3, and 5) are positive; in picture B wells 1 and 3 are positive while well 5 is negative.
also the most widely distributed. Unusual genotypes – genotypes 2-3 and genotypes 3-4 – were rare; each genotype was recorded only once from 391 samples.

No special genotypes were detected in cattle with persistent lymphocytosis (PL) and weak positive reactors. PL was observed only in 7% of cattle which were positive by AGID; however, this figure included monocytes and granulocytes.

Fig 1. In the pictures wells 1, 3, and 5 are samples: wells 2, 4, and 6 are positive control sera; and the central well contains BLV antigen. In picture A all samples (wells 1, 3, and 5) are positive; in picture B wells 1 and 3 are positive while well 5 is negative.

**Sensitivity and specificity of AGID**

Taking the PCR as a gold standard, the sensitivity of AGID was found to be 89.3%. However, the specificity of AGID was found to be very low (58.8%). When nested-PCR was taken as a gold standard the sensitivity and specificity of AGID was not much different (Table 3).

**Table 3. Sensitivity and specificity of AGID, and kappa value for test agreement**

<table>
<thead>
<tr>
<th>PCR</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>300</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>277</td>
</tr>
<tr>
<td>AGID</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>=336</td>
<td>=471</td>
</tr>
</tbody>
</table>

**Fig. 2.** Picture A shows Hae III enzyme cleavage products of 285 and 95 base pair DNA fragments; the genotype is either 3 or 5 (to say exactly what genotype it is we need to do RFLP again using Bcl I enzyme). Picture B shows PCR positive samples in Lanes 8, 13, and 15; Lane 16 is positive control; Lane 17 is the DNA ladder (magnified to show the size of the DNA pairs).
Discussion

A reliable diagnostic test should be used to establish a correct diagnosis whenever possible. Agar gel immunodiffusion (AGID) is a widely used serological test for the diagnosis of viral diseases. However, BLV infections with lack of detectable BLV antibodies have been observed in recent years\(^\text{15, 14, 16}\). This makes eradication of BLV infection difficult using serology alone. Polymerase chain reaction (PCR) is being increasingly used in the diagnosis of BLV infections\(^\text{14, 15, 16, 17}\). Fechner et al.\(^\text{15}\) detected almost 17.0% more positive animals by PCR than by AGID and 10% more by PCR than with ELISA. In this study, more samples were detected by AGID than PCR. However, the specificity of AGID was found to be low (high false positive result), that is, it classifies non-infected animals as test positive. The BLV antigen used for AGID test was prepared from FLK-BLV (Fetal Lamb Kidney - BLV) tissue culture fluid that is persistently infected with BLV type 1. About half of the samples in this study are not type 1. Hence, there might be a difference in the reactions of the AGID test between homologous and heterologous strains of BLV. On the other hand, production of BLV antibody detectable in the AGID test was not influenced by genomic heterogeneity\(^\text{8}\). Further study is required to determine the nature of association between heterologous strains and BLV antibody production.

Six types of genomic heterogeneity of BLV in the gp51 gene were identified in this study. Genotype 1 is recorded in half of the cattle tested in eleven prefectures out of the sixteen. Thus, this is the predominant genotype in Japan. Genotypes 3 and 5 are also common in Japan. Genotypes 4 and 6 are confirmed to exist in Japan\(^\text{8}\). Hence, genotypes so far identified in Japan totals to eight.

In this study, two types of unusual genotypes with a similar nature were identified. At present we do not know the explanation for this observation. However, one of the following hypotheses may hold true: that one animal may be infected by two types, due to mutations, or different type of genotypes with their own unique restriction sites. There are evidences that the BLV genome is quite susceptible to genetic mutation\(^\text{8}\). On the other hand, the genetic variation of the BLV genus appears to be minimal\(^\text{9}\). Further study to solve this dilemma is required.

BLV infection was found in all prefectures; and in almost all farms in this study. The prevalence of BLV infection in Japanese cattle is quite high. There is free movement of cattle among prefectures. Ferrer and Piper\(^\text{21}\) indicated that 3-20% of calves from infected dams are BLV infected at birth thereby maintaining the infection from one generation to the next. This is one reason why BLV infection is still high even in those herds where animals are not introduced for a long time.

About ten percent of infections, confirmed to be positive by PCR, were not detected by AGID. Other authors have reported similar results\(^\text{8, 14, 16}\). One reason is genetic heterogeneity as indicated above. Another reason for the observed false negative result and reduced test agreement is that cattle are sometimes transiently seronegative under certain circumstances such as early infection\(^\text{22}\), exposure to small inocula, impending parturition or the immediate postparturient state\(^\text{22}\), prolonged latency and coinfection with bovine virus
diarrhoea^{24}. Furthermore, if the infected leukocytes are low, the sensitivity of PCR could be reduced^{14}. In cattle with a low gp antibody titre, BLV virus isolation required many lymphocytes^{25}.

Our study demonstrated the presence of multiple BLV provirus genotypes. Two unusual genotypes were also detected. To find out the effect of different genotypes on AGID serological test performance, further a study is required. We also recommend additional study to elucidate the nature of unusual genotypes observed in this study. AGID is by far a cheap test requiring only minimal reagents and facilities compared with PCR. Its sensitivity – measure of disease detection ability - is close to ninety percent. The test’s only limitation, according to this study, is its high false positive result; this however doesn’t affect disease detection. Hence, agar gel immunodiffusion (AGID) can be used in Africa for the diagnosis of bovine leukemia infections and conduct epidemiological studies on BLV across the continent.

Acknowledgement

We thank Livestock Hygiene Service Center workers for their cooperation in providing blood samples. This study was funded by the Japan International Cooperation Agency (JICA).

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The distribution of bovine leukemia virus genotypes in cattle from Japan and diagnostic performance of agar gel immunodiffusion (AGID) and polymerase chain reaction (PCR) tests.


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A REVIEW OF RISK-BASED APPROACHES FOR EMERGING DISEASES ASSOCIATED WITH ANIMAL SOURCE-FOODS

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EXAMEN DES METHODES D’APPROCHE A BASE DE RISQUE POUR LES MALADIES EMERGENTES ASSOCIEES AUX ALIMENTS D’ORIGINE ANIMALE

Résumé

L’urbanisation rapide, la mondialisation et l’intensification de la production animale dans les pays en développement ont entraîné de profonds changements quant à la production, à l’approvisionnement et à la consommation alimentaires avec des possibilités d’offrir des opportunités sans précédent aux paysans démunis, aux spécialistes de la transformation et aux commerçants. Toutefois, le changement des systèmes de production et des écosystèmes entraînent aussi l’apparition de maladies émergentes et ré-émergentes. L’aliment d’origine animale, bien qu’il soit une excellente source de protéine et d’oligo-éléments, est aussi l’unique et la plus importante source de maladie véhiculée par l’aliment. Le présent article passe en revue les méthodes d’approche à base de risque en vue d’une meilleure gestion des maladies véhiculées par l’aliment dans les pays en développement, avec un accent particulier sur les marchés locaux et les maladies émergentes. Les méthodes élaborées par le Codex Alimentarius Commission sont expliquées. Les avantages des méthodes d’approche à base de risque ainsi que les difficultés rencontrées pour les adapter sont discutés en ayant recours aux exemples des études de cas en cours sur les maladies véhiculées par l’aliment en Afrique de l’est et de l’ouest.

Mots-clés : Analyse de risque, maladies émergentes, maladie véhiculée par l’aliment.

Summary

Rapid urbanization, globalization and intensification of livestock production in developing countries are causing dramatic changes in food production, supply and consumption with the potential of generating unprecedented opportunities for poor farmers, processors and traders. But, changing production systems and eco-systems also offer new niches for emerging and re-emerging diseases. Animal source food, though an excellent source of protein and micro-nutrients, is also the single most important source of food-borne disease. This paper reviews risk-based approaches for better management of food-borne diseases in developing countries, with an emphasis on local markets and emerging diseases. Methodologies developed by the Codex Alimentarius Commission (CAC) are explained. The benefits of risk-based approaches as well as the challenges of adapting them are discussed using examples from ongoing case-studies involving emerging food borne diseases in East and West Africa.

Key words: risk analysis, emerging diseases, food-borne disease

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Introduction

The purpose of this paper is to review food-borne diseases of animal origin, with emphasis on those that are new and emerging, and to suggest how these can be better managed in developing countries where informal markets predominate. The paper starts by reviewing evidence concerning the direct and indirect disease burden of unsafe food and goes on to discuss how disease is attributed to different sources, showing the importance of animal source foods in the aetiology of food-borne disease. Next the changing epidemiology of food-borne disease is discussed. Some case studies are then presented which show how emerging food-borne disease can be successfully managed. Based on this, suggestions are made as to the opportunities and constraints of using risk-based approaches to improve the safety of food in informal markets in developing countries.

The direct burden of food-borne disease

More than 200 diseases are spread by food: hazards potentially present include viruses, bacteria, parasites, toxins, metals, allergens and prions. Indeed, food and water borne illness is believed to be the single most common disease of humanity. The most typical manifestation is acute gastrointestinal disease and recent studies in developed countries have found incidences from 0.2 to 1.3 episodes per person per year, of which a third or more may be due to food-borne illness1.

In developing countries, the prevalence is likely to be even higher because of a number of interacting factors including: higher proportions of vulnerable people (e.g. young, immuno-suppressed and malnourished); a greater variety and often higher levels of pathogens; less food preservation infrastructure; environmental conditions that favour pathogen survival and growth; generally inadequate food safety systems; and lack of knowledge about the detection and management of food safety problems. These effects are important. For example, malnutrition, from which nearly a billion people suffer world-wide, can result in a 30-fold increase in the risk for diarrhoea associated death2. As a result, food and water-borne diarrhoeal diseases are leading causes of illness and death in less developed countries, killing an estimated 2.1 million people annually, most of whom are children3.

Although the negative impact of food-borne disease is predicted to be immense, quantitative data on disease burden is only starting to emerge for developed countries and is generally lacking for poor countries. The costs of disease can be assessed in terms of losses at the individual, industry and societal level (Table 1) and extend far beyond the direct costs of treatment; costs of illness need to include transaction costs of obtaining treatment occurred by the victim and care-givers, costs of nursing and rehabilitation, costs of lost productivity and costs of behaviour change such as switching to more expensive food or expenditure on food safety measures. Unlike other diseases, animal-source food-borne disease imposes a ‘double burden’, in so far as it also entails costs on the animal production industry. Moreover, presence of food-borne disease can also have negative impacts on international trade, and, what is often more important for developing countries, tourism.
Table 1: Costs of food-borne illness

<table>
<thead>
<tr>
<th>Individual/household costs</th>
<th>Industry costs</th>
<th>Regulatory and public health costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical</td>
<td>Cost of lost animal production</td>
<td>Disease surveillance</td>
</tr>
<tr>
<td>Income or productivity loss</td>
<td>Cost of pathogen control</td>
<td>Research</td>
</tr>
<tr>
<td>Other illness costs</td>
<td>Outbreak</td>
<td>Outbreaks</td>
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<tr>
<td>Psychological (psychic)</td>
<td></td>
<td></td>
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<tr>
<td>Averting behaviour</td>
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Emerging evidence on chronic sequelae of food-borne disease

For many years food-borne illness was regarded as a common, but not especially serious, disease. This perspective is changing as diseases emerge or re-emerge which are untreatable (e.g. Cryptosporidium), deadly (e.g. tuberculosis) or both untreatable and deadly (e.g. new variant Creutzfeldt Jakob syndrome and avian influenza). As knowledge has grown on the seriousness of infection so has awareness of the importance of chronic sequelae. The USDA Food and Drug Authority estimate that 2 to 3% of people with acute food-borne illness go on to develop secondary long term illness and the long-term consequences to human health and the economy may be more detrimental than the initial acute disease.

Chronic sequelae attributed to food-borne disease include: ankylosing spondylitis; arthropathies; renal disease; cardiac disease; neurological disorders; abortions and developmental abnormalities; and, nutritional and other mal-absorptive disorders (incapacitating diarrhea). For example, Guillain-Barré Syndrome, (GBS), an auto-immune reaction that can cause paralysis, may occur after acute gastrointestinal infection. Campylobacter jejuni, usually acquired from poultry, is the most common preceding infection but the disease may follow other infections. GBS may afflict patients for the remainder of their lives and result in premature death. It is the most common cause of acute flaccid paralysis in the world and has a case fatality of 2-8%. The medical costs of GBS associated with C. jejuni infection have been investigated more fully than other long-term sequelae of food-borne diseases. A recent report estimates that of the 2,600 to 9,500 new cases of GBS which occur annually in the USA, between 525 and 3,800 are triggered by infection with C. jejuni which means that long-term sequelae to C. jejuni are of the same magnitude as the costs of acute illness, despite the fact that the long-term complications are at least 100 times less common.

Toxoplasmosis, another important zoonotic emerging disease is a major cause of congenital abnormalities. Screening programmes from Europe have shown a prevalence that varies from 1 in 1000 to 0.3 in 1000 births; the limited studies suggest prevalence may be higher in developing
countries. Consumption or contact with contaminated meat is thought to be the most important source of infection. Congenital impairments associated with maternal toxoplasmosis infection passed to the foetus include hearing loss, visual impairment such as retinal lesions and strabismus, and slight to severe mental retardation. These impairments are still present in 80% of persons who reach the age of 20 years. Chronic toxoplasmic encephalitis may occur when a person's immune system is impaired. Indeed, toxoplasmic encephalitis marked by dementia and seizures has become the most commonly recognized cause of central nervous system opportunistic infections in AIDS patients, and is probably more important in developing than in developed countries. Additionally, it appears that certain cancer treatments weaken the immune system, and old infections in the muscles can become reactivated, causing severe complications or death. The evidence that microorganisms or their products are associated with these long-term sequelae ranges from convincing, as in the two examples cited, to circumstantial.

Evidence continues to emerge and be re-evaluated and it is likely that other syndromes of currently unknown aetiology, both acute and chronic, may prove to be associated with food-borne disease. In recent years several diseases have been recognized as certainly food-borne (e.g. meningitis caused by *Listeria monocytogenes*) or probably food-borne (e.g. stomach ulcers caused by *Helicobacter pylorus*). Debate continues on the involvement of other pathogens; for example there is some evidence that Crohn's disease may be linked to *Mycobacterium paratuberculosis*.

Attribution of food-borne disease: importance of animal source foods

Development of a rational system of interventions to improve food safety requires an understanding of the source from which humans are acquiring food-borne pathogens. However, food attribution is not straightforward. Approaches to food attribution can be grouped into two categories, namely epidemiological and microbiological. Epidemiologic approaches are based on public health surveillance and include food-borne outbreak data and case-control studies. Microbiologic approaches are based on related sampling from human, animal, and food sources to look for relatedness. Methods include pathogen subtyping, as used in Denmark's *Salmonella* Accounts, and microbial source tracking (MST) methods, as well as risk assessments of specific pathogens in specific foods. Both approaches have unambiguously revealed the importance of animal-source foods in the burden of infection. The importance of animal-source food is both a justification for veterinary involvement in control of food-borne disease, and a fact many stakeholders are unaware of. As such it merits further consideration.

Of the nine most important culture-confirmed pathogens in the USA, seven have an animal reservoir. More than 75% of identified illness is caused by just three zoonotic, food-borne pathogens: *Salmonella spp.*, *Listeria monocytogenes*, and *Toxoplasma gondii*. Between 1998 and 2002, most (69%) food-borne disease outbreaks with an identifiable vehicle were caused by animal-source foods. This analysis excluded complex foods which contain a mixture of different ingredients. Poultry was the food most often implicated (25%) but beef, pork,
shellfish and finfish were also important, each causing over 10% of the total. In the United Kingdom a similar pattern is seen. Food safety authorities have developed a method for estimating the relative risks associated with specific foods, dividing the number of cases due to a specific food (as derived from their outbreak database) by the estimated total servings of that food consumed in a year. In the four years from 1996 to 2000 most illness was attributed to eating poultry (30%), complex foods (27%), and red meat (17%). Consideration of epidemiological features suggested that eggs were a major source of infection for disease related to complex foods. Chicken consumption accounted for more diseases, deaths and healthcare usage than any other food type. Milk also exerted a considerable impact on healthcare provision. The lowest case-fatality rates were associated with plant-based foods. By contrast, foods of bovine origin tended to have the highest case-fatality rates.

Many developed countries use microbial sub-typing to aid with outbreak investigations, but Denmark is so far the only country where this is done with sufficient regularity in humans, animals, and food to attribute sporadic pathogen illnesses to specific food and animal sources. Results for Salmonella have been available since 1998 and changes of subtypes in animal reservoirs are clearly reflected in changes in food and human cases. Campylobacteriosis is now recognized as the most common cause of infectious bacterial enteritis worldwide. In Belgium, poultry was withdrawn from the market for four weeks in 1999 because of concerns that it might be contaminated with dioxin. Rates of campylobacter infection fell by 40% during this ban, and then returned to the previous level after chicken was put back on the shelves. This natural experiment provides strong support for the importance of poultry in disease aetiology.

In addition, infections caused by plant foods can be attributed to animal reservoirs, for example, the recent outbreaks of Escherichia coli O157:H7 linked to contamination of spinach with cattle manure.

Changing epidemiology of food-borne disease

The epidemiology of food-borne disease is changing rapidly: over the last two decades, bacterial infections caused by Campylobacter spp. and enterohaemorrhagic Escherichia coli have emerged, well-recognized pathogens, such as Salmonella enteritidis have increased dramatically in disease incidence and foodborne pathogens have become increasingly resistant to antimicrobial agents. Emerging and re-emerging diseases are a particular source of concern making up more than half of all human infectious diseases known today. Emerging diseases can be defined as those which: 1) have been newly discovered; 2) have recently increased in incidence or prevalence; 3) have recently expanded in geographic or climatologic range; or 4) have jumped from animal populations into humans. Re-emerging infectious diseases, on the other hand, are diseases that have been discovered previously in a species and are often at enzootic levels in that species but, for some reason, have significantly increased in incidence at a given point in time or in a specific geographic region; zoonotic avian influenza, E. coli O157:H7, new variant Creutzfeldt-Jakob syndrome are examples of emerging diseases while brucellosis and...
leptospirosis are, in many areas, re-emerging diseases. More than 1,400 pathogens are known to infect humans and 58% of these are zoonotic, but among the emerging diseases, approximately 73% are zoonotic; and among food-borne emerging diseases the proportion is even higher (Table 2).

Emergence in food-borne diseases is driven by the same forces as emergence in other infectious diseases, namely changes in demography, culture, industry, and technology; the shift towards a global economy; microbial adaptation; and the breakdown in the veterinary and public health infrastructure. In the 21st century human and animal populations are higher than ever before, bringing larger numbers of animals and humans into contact. Population growth has been most rapid in developing countries; unfortunately increase in livestock production has not been accompanied by improvements in hygiene and bio-security, or effective regulation. Moreover, farmers are operating in new and rapidly changing farming systems and lack the experience and technological support to identify the disease problems that these entail while consumers lack information on the dangers to which they are exposed. Intensive farming systems are often associated with a high use of veterinary drugs (sometimes used to mask poor husbandry) which invariably exerts pressure for the development of drug resistance.

Pressure on natural resources and wilderness destruction is creating new opportunities for humans and their domestic animals to come into contact with wild animals thus facilitating disease transmission. Deforestation and settlement of new tropical forest and farm margins have exposed farmers and domestic animals to

| Table 2: Important emerging food-borne pathogens and their animal reservoirs |
|-------------------------------------------------|----------------|----------------|
| **Infection** with animal source food **Main reservoir** |
| Campylobacter jejuni | Yes | Poultry |
| Campylobacter fetus ssp. fetus | Yes | Cattle |
| Cryptosporidium parvum | Yes | Cattle |
| Cyclospora cayetanensis | No | Birds? |
| Escherichia coli O157:H7 and related | Yes | Cattle |
| Listeria monocytogenes | Yes | Ruminants |
| Norwalk-like viruses | No | Human |
| Nitzschia pungens | Yes | Shellfish |
| Salmonella Enteritidis | Yes | Food animals |
| Salmonella Typhimurium DT 104 | Yes | Food animals |
| Vibrio cholerae 01 | No | Human |
| Vibrio vulnificus | Yes | Shellfish |
| Vibrio parahaemolyticus | Yes | Shellfish & fish |
| Yersinia enterocolitica | Yes | Swine |
new vectors and diseases they carry such as the Mayaro and Oropouche virus infections in Brazilian woodcutters who cleared the Amazonian forest. The opening up of isolated ecosystems has contributed to emergent disease episodes. Remote eco-niches, such as islands, with immunologically naive potential reservoir hosts and vectors are often particularly vulnerable to an introduced virus, resulting in so-called "virgin soil" epidemics, such as the small-pox and measles epidemics which resulted in demographic collapse after their introduction to the Americas.

Rapid transport allows pathogens to travel vast distances even before symptoms have become visible and the connectedness of even remote, rural communities means highly pathogenic infections can spread by finding new victims rather than "burning out" as those infected died or become immune. While human populations are becoming more mobile, they are also becoming more vulnerable: with higher numbers than ever before of the young, the poor and the immunosuppressed.

Climate change, whether natural or man-made, may be contributing to the emergence of infectious diseases. For example, in 1993 increased rainfall in the south-western United States led to increased vegetation, larger rodent populations, more rodent–human contact and the first recognized outbreak of hantavirus in North America. The tremendous societal, environmental and technological changes that occurred in the last century are likely to continue or accelerate in the present one: new emerging and re-emerging diseases, many of which are likely to be zoonotic and/or transmitted via animal-source foods can also be anticipated.

Effective control of animal-source-food disease

Given the enormous impacts of food-borne diseases and their rising incidences in both developed and developing countries, improving food safety should be a priority. It is also an issue of great concern to the general public which makes it an attractive issue to address. Substantial progress has been made in preventing food-borne diseases long before the antibiotic era by simple measures such as disinfection of drinking water, sewage treatment, milk sanitation and pasteurization, and better nutrition and housing. More recently, the efficient treatment of diarrhoeal diseases through oral re-hydration has probably led to the prevention of many food related deaths in developing countries.

These approaches need to be continued, but the complex emerging food-borne diseases of the last few decades have been difficult to manage using traditional practices of good general hygiene and technological fixes. A number of factors make these diseases hard to control: many emerging food-borne diseases are asymptomatic in their animal host, so detection is difficult and there are less incentives for farmers to eradicate disease; globalisation of trade means that many more actors are involved in trade, so co-ordination is more difficult while the vastly expanded extent of trade means many people in distant localities can be simultaneously affected; highly integrated, large-scale farming systems can allow pathogens to become endemic throughout the industry; food safety is difficult to guarantee and advertising food safety may have paradoxical effects of increasing consumer anxiety, dampening the
emergence of market mechanisms for food safety; and in the majority of countries public food safety regulation is ineffective.

However, there are some notable cases of large-scale successful control and prevention of food-borne disease, mainly in developed countries. For example, in Iceland, measures at production, retail level and in the household were introduced to prevent *Campylobacter* transmission. Flocks were comprehensively tested and birds from positive flocks could only be sold frozen. This was accompanied by consumer education campaigns and improvements in hygiene and biosecurity on farm. Parallel declines (>70%) were subsequently observed in the carriage of *Campylobacter* in broiler flocks and in human infections.\(^{21}\)

In Denmark, a focused and integrated program, reduced *Salmonella* by up to 95% in eggs, poultry and pork, by monitoring herds and flocks, eliminating infected animals, and diversifying animals (animals and products are processed differently depending on *Salmonella* status) and animal food products according to the determined risk. In 2001, the Danish society saved US $25.5 million by controlling *Salmonella*. The total annual *Salmonella* control costs in 2001 were US $14.1 million, corresponding to US $0.075 for each kg of pork produced and US $0.02 for each kg of broiler or egg. These costs were paid almost exclusively by the industry.\(^{13}\)

In another example from America, Texas American used its expertise in pathogen control to attract new customers and estimates that 25 to 30% of its new sales between 1998 and 2001 occurred because of its superior safety record.\(^{22}\)

These success stories allow some lesson-learning on the important elements of food safety systems for emerging diseases associated with animal-source food which may be applicable to developing countries. In both Europe and the United States, pre-harvest measures have generally been more effective than the post-harvest measures on which regulators have traditionally focused. For example the largest reductions in *Salmonella* and *Campylobacter* levels have come from implementing controls in farm-to-retail processing rather than in instituting them in domestic kitchens.\(^{23}\) But although animal-based interventions have proven to be most effective, their successfully application requires integration, or at least close collaboration, of health and medical systems that is often difficult to operationalise. In several developed countries there has been a move to consolidating food safety responsibilities but in developing countries (and in some developed countries such as the USA) food safety remains fragmented.

Preventative approaches to food safety have been dramatically more effective than the prescriptive and punitive approaches they are replacing. An example is the Hazard Analysis and Critical Control Point (HACCP) approach which has emerged over the last decade as the primary approach to securing the safety of food supply. This system can be described as an operation-specific, internally managed system of preventative control, which identifies, evaluates and controls hazards of significance to food safety. However, HACCP has been difficult
to apply to small businesses and where regulatory agencies lack resources to adequately support it, the uptake and impact has been low. 

Risk-based approaches are now considered the most appropriate way of managing food safety. Risk-based approaches take two factors into account: undesirable effects and the probability of their occurring. This avoids wasting money on problems which present little risk. The risk-based approach consists of assessing the risk of a hazard (where risk is a combination of undesirable effects and likelihood), deciding what is the acceptable level, considering the options for achieving these and implementing, monitoring and evaluating selected options. Like HACCP, which is itself a method of risk management, risk analysis follows a structured and sequential approach, but by encompassing the entire pathway ‘from stable to table’, ‘farm to fork’ or ‘boat to throat’, it extends the approach.

**Codex Alimentarius risk analysis frameworks for food safety**

The Codex Alimentarius Commission is an intergovernmental body, established jointly by the World Health Organisation and the Food and Agriculture Organisation, concerned with protecting the health of consumers and ensuring fair practices in international food trade through the development of food standards. The Codex Alimentarius, or food code, is an internationally agreed set of food standards, recommendations, and guidelines for consumers, food producers and processors, national food control agencies, and the international food trade. The Uruguay Round of Multilateral Trade Negotiations recognised Codex standards as the international reference standards; in order to ensure that decisions are science-based, transparent, fair and systematic the Codex Alimentarius adopted risk analysis as the standard approach for food safety issues.

Quantitative microbial risk analysis is hence a new discipline but it is grounded in the disciplines of chemical and toxicological risk analysis which were developed around the middle of the last century. Following from these it is conceptualized as consisting of three inter-dependent components: risk assessment, risk management and risk communication. Risk assessment is perhaps the most methodologically developed aspect, comprising four steps: hazard identification, exposure assessment, hazard characterization and risk characterisation. Risk management both precedes and succeeds risk assessment.

The decision to undertake a risk assessment is a part of risk management and an early task is the construction of a risk profile which places the food safety issue within a particular food safety context. Based on the findings of the risk profile, the risk managers can decide whether a formal risk assessment is warranted and, if so, what its objectives should be.

The first step of the risk assessment is Hazard identification - a qualitative process intended to identify micro-organisms or microbial toxins of concern in food or water. It can include information on the hazard of concern as well as relevant related data, such as clinical and surveillance data. Exposure assessment should provide an estimate, with associated uncertainty, of the occurrence and level of the pathogen in a specified portion of food at the time of consumption, or in a specified volume of water using a production-to-consumption pathway approach. While a mean value may
be used, more accurate estimates will include an estimate of the distribution of exposures, and this is usually obtained by Monte Carlo simulation\textsuperscript{26}. This will typically include identification of the annual food and water consumption frequencies and weights or volumes for a given population or sub-populations(s), and should combine the information to estimate the population exposure to pathogens through a certain food or water commodity. The next step Hazard characterization provides a description of the adverse health effects that may result from ingestion of a microorganism. When data are available, the hazard characterization should present quantitative information in terms of a dose response relationship and the probability of adverse outcomes. Finally Risk characterization is the integration of the three previous steps to obtain a risk estimate (i.e. an estimate of the likelihood and severity of the adverse health effects that would occur in a given population, with associated uncertainties).

The four steps together constitute risk assessment. According to the Codex Alimentarius framework, the functions of risk assessment and risk management should be functionally separated. The task of the risk manager is to evaluate the different options for managing risk and on the basis of this to decide, implement and monitor the strategies needed to reduce risk to the levels considered appropriate. Decisions as to the appropriate level of risk are the mandate of relevant authorities (ideally made on the basis of stakeholder consultation) and should take into account all stakeholder concerns. This involves trading off different objectives - social, economic environmental and others – and the appropriate level will depend on local circumstances and aspirations.

Risk-based approaches are the best way to improve food safety. They are becoming standard in developed countries and recent years have seen increasing interest in their potential to address food safety issues in developing countries.

Although application is still in its infancy there are already some promising examples from research by the International Livestock Research Institute, University of Nairobi and Makerere University in East Africa. For example, a study of urban agriculture in Kampala, Uganda (which will be reported elsewhere) found that dairy farmers spontaneously used risk mitigation strategies that improved the safety and quality of informally produced milk. An average of 17 different strategies per household was used, varying from zero-grazing, to use of concrete floors, to washing utensils with hot water and soap. Farmers who believed that urban dairying was illegal or who had experienced harassment were less likely to use risk mitigation strategies.

In another example from Kenya, a qualitative risk-assessment for \textit{E. coli} O157: H7 suggested that even though the pathogen was being shed by a small proportion of dairy cattle, the risk to consumers was low. This was partly because the small volume of containers used in the informal milk sector limited the extent of cross-contamination, but mainly because of the almost universal practice of boiling milk. Conventional analysis based on hazards would have over-estimated the likely importance of this disease, as it does not take into account risk mitigating behaviours, in this case, boiling milk\textsuperscript{27}.

Studies on urban agriculture in East and West Africa supported by the International Development Research Centre
used participatory methodologies to estimate the benefits as well as potential harms of urban livestock keeping. This is a first step towards helping stakeholders negotiate and decide appropriate levels of protection that take into consideration the benefits as well as risks of livestock keeping.

Conclusions

Food safety concerns have greatly increased in recent decades, but the extent and impact of the problem is still imperfectly understood, especially in developing countries. Fortunately new approaches and tools are emerging with great potential for the better management of food safety: risk-based approaches are acknowledged to be the most effective ways of providing food safety and are especially relevant to developing countries where many other considerations need to be taken into account when setting food appropriate standards; HACCP has proven to be very effective in preventing food safety problems and is becoming industry-standard in developed countries; previously fragmented food safety systems become more integrated; and the importance of interventions at the farm and along the supply chain are being recognised.

However, these insights and innovations have yet to be applied to informally marketed food in developing countries despite the fact that this food system is likely to pose high risks to consumers, intermediaries and producers. Improving the safety of animal-source foods in developing countries is a major challenge but one which can be overcome, delivering important benefits to consumers and producers alike.

Acknowledgement

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SCROTAL CIRCUMFERENCE AFTER ADJUSTMENT FOR AGE AND BODY CONDITION IN BULLS INDIGENOUS TO EAST AFRICA

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CIRCONFÉRENCE SCROTALE APRES AJUSTEMENT DE L’ÂGE ET DE L’ETAT CORPOREL CHEZ LES TAUREAUX LOCAUX DE L’AFRIQUE DE L’EST

Résumé

Afin d’étudier les effets de l’âge, des variations saisonnières de l’état corporel (EC) et du poids vil (PV) sur la circonférence scrotale (CS) chez les animaux élevés dans un milieu tropical pastoral traditionnel, 303 taureaux zébus à courtes cornes de la Tanzanie ont été examinés. Les taureaux étaient répartis en groupe normal (groupe 1 ; n=169) et groupe anormal (groupe 2 ; n=134) après analyse des données histopathologiques testiculaires. Le PV était déterminé par la mensuration du tour de poitrail (TP). Les équations Y (CS en cm) = 1,3X1 (âge en mois) + 23,1 et Y (CS en cm) = 0,13X2 (TP en cm) + 9,98 évaluèrent la CS. Les valeurs ajoutées de la circonférence scrotale pour PV et EC étaient de (moyenne ± écart type) 25,6 ± 1,5 ; 28,1 ± 1,1 ; 29,3 ± 2,1 et 31,2 ± 1,6 cm pour la tranche d’âge < 2 ; 2-3 ; 3-4 > 4 ans respectivement dans le groupe 1. Les valeurs enregistrées pour le groupe 2 étaient respectivement de 17,7 ± 3,1 ; 22,1 ± 2,5 ; 24 ± 2,1 et 26,1 ± 1,7 cm pour la tranche d’âge < 2 ; 2-3 ; 3-4 et < 4. CS a indiqué une augmentation significative (P<0,001) avec l’avancement de l’âge, mais elle était nettement différente entre les groupes 1 et 2 (P<0,01). Il a été conclu que les changements de CS ne reflétaient pas les changements de PV, de l’EC, de l’âge et de la taille du taureau.

Mots-clés : Circonférence scrotale, taureaux zébus, âge, état corporel.

Summary

In order to study the effects of age, seasonal variations in body condition (BC) and body weight (BW) on scrotal circumference (SC) in animals experiencing traditional pastoral tropical environment, 303 Tanzania shorthorn (TSH) zebu bulls were examined. The bulls were classified into normal (n = 169; group 1) and abnormal (n = 134; group 2) after testicular histopathological data analysis. BW was determined by heart girth (HG) measurement. Equations Y (SC in centimetres) = 1.3X1 (age in months) + 23.1 and Y (SC in centimetres) = 0.13X2 (HG in centimetres) + 9.98 estimated SC. Scrotal circumference values adjusted for BW and BC were (mean ± standard deviation) 25.6 ± 1.5, 28.1 ± 1.1, 29.3 ± 2.1 and 31.2 ± 1.6 centimetres for <2, 2-3, 3-4 and >4 year age bracket, respectively, in group 1. Values for group 2 were 17.7 ± 3.1, 22.1 ± 2.5, 24.0 ± 2.1 and 26.1 ± 1.7 centimetres for <2, 2-3, 3-4 and <4 year age bracket, respectively. SC showed significant (P < 0.001) increase with advancement of age but was significantly different between group 1 and 2 (P < 0.01). It was concluded that changes in SC reflected changes in BW, BC, age and size of the bull.

Key words: scrotal circumference, zebu bulls, age, body condition

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Introduction

Zebu bulls in the traditional pastoral livestock sector of Tanzania are selected for natural mating on the basis of animal size and body appearance, rather than reproductive and mating performance qualities. However, assessment for reproductive and mating qualities is crucial in these areas because, as observed by Kashoma\textsuperscript{1}, approximately 44\% of mature zebu bulls under a multiple male mating system can be sub-fertile at any one single moment. The multiple male mating system is based on natural mating using several bulls constantly running with cows, therefore improving accuracy of selection of fertile bulls is crucial for successful breeding\textsuperscript{2}. Worldwide, assessment for bull fertility is conducted partly through measurement of SC\textsuperscript{3,4}. Measurement of the scrotal circumference is regarded as a cheap, repeatable and objective way to estimate fertility\textsuperscript{5,6}. Fertile bulls of a certain age are required to obtain a minimum SC for that age.

Scrotal circumference (SC) is a reliable indicator of testicular function in bulls,\textsuperscript{6,7} because it is heritable, positively related to testicular weight, spermatogenesis, seminal characteristics and spermatozoa output\textsuperscript{8,9,10}. Bulls with larger SC tend to produce more normal sperm cells\textsuperscript{11,12}.

Scrotal circumference varies with breed, age, body weight and the season of the year\textsuperscript{13,14}. There are many other factors that can affect SC measurements. Worldwide, seasonal variations in testicular function, semen quality and fertility of bulls have been reported as being common in the dry season\textsuperscript{15}. The dry season is also a critical period for the pastoral cattle of Tanzania largely because of feed scarcity and extreme temperatures\textsuperscript{16}. Therefore, before using guidelines spelling out limits of SC, it is necessary to know the variability that exists within a given breed.

There is little information available on scrotal circumference measurements of zebu bulls of East Africa\textsuperscript{1,16} and to our knowledge no SC recommendations for minimal values have been established on the Tanzania shorthorn zebu. Zebu bulls of East Africa are \textit{Bos indicus} cattle with origins traceable to Asia\textsuperscript{17}. The \textit{Bos indicus}, group is comprised of different breeds and each breed has developed into animals of different strength and character to withstand a particular local physical environment. For example, the Tanzania shorthorn (TSH) zebu is different by having a small body size\textsuperscript{16,17}. Because of the reasons given above it is essential to establish SC guidelines with adjustments made for breed, age, body condition or weight and agro-ecological environmental factors. This work was therefore undertaken with the objective of establishing the minimum and maximum SC limits for different age categories of TSH zebu bulls raised under traditional livestock system.

Materials and methods

Study area

This study was carried out in the Morogoro abattoir on bulls brought for slaughter from different parts of Tanzania. Over a period of 12 months, 303 extensively reared, TSH zebu bulls from the traditional pastoral livestock production sector were examined. In this sector, TSH zebu bulls are raised under free range and multiple male mating system.
Scrotal circumference

Each bull was adequately restrained with a rope and placed in a crush. Measurements of the SC were performed by grasping the scrotum from behind and forcing both testes to the bottom until ventral scrotal skin folds were eliminated. The testes were kept firmly in place by holding the neck of the scrotum with one hand. The area of the scrotum with the greatest circumference was then identified for measurement. Measurements were conducted using a graduated metal tape and recorded only for bulls with two descended testes. In order to standardise the readings and reduce error due to repeated readings using a flexible metal tape, the procedure by Whittaker et al. was used whereby SC was determined using a metal tape pulled to a constant tension of 200 g. The measurement was repeated three times and the average was recorded to the nearest half centimetre.

Body condition score, heart girth and age measurements

The body condition score (BCS), a subjective estimate of the overall nutritional status which took into account the larger variation in body condition found in African zebu cattle, was made after visual assessment using a one to nine-point scoring scale of Nicholson and Butterworth. Score 1 represented emaciated, 5 moderate amount of fat cover and 9 very fat animals.

Heart girth (HG) was measured in centimetres using a metal tape. In order to standardise the readings and reduce error due to repeated readings, the procedure by Whittaker et al. was used whereby HG was determined using a metal tape pulled to a constant tension of 300g. Bulls standing on four legs were restrained to maintain the head in an upright position. The tape was placed around the bull at the point of the smallest body circumference just behind the forelegs and measurements taken. HG measurements were converted into weights in kilogram by an equation \( Y = 4.81 \times HG - 432.73 \) (where \( Y = \) weight in kilogram; \( HG = \) heart girth in centimetres) recommended by Nicholson and Sayer for the African Bos indicus zebu. Age was estimated after visual assessment of wear of dentition using a standard method described by Goetz.

Ultrasonographic scanning

Scrotal contents including testes and epididymis were examined before and after slaughter using a 5 MHz linear array transducer connected to a B-mode ultrasound scanner (SonoAce SA 600V, Kretz Technich AG, Zipf, Austria). Before scanning, obstetric gel was applied to the probe. A procedure described by Kähn was used to align the probe. The probe was aligned at the centre of each testis so as to be oriented vertically (parallel to the long axis of the testis) and reveal the mediastinum as a continuous band across the image. The alignment was used to standardise measurements because the probe was found at the centre of testicles in all samples. Subsequently ultrasonographic measurements for the three dimensions of the testis (testicular length, width and depth) as well as scrotal circumference were recorded. The testicular tissue was then scanned in different planes (transverse and longitudinal) for consistency and abnormalities. After slaughter testicular length, width and depth were measured by a calliper.
Semen collection and analysis

Semen and sperm cells from live animals were collected using a testicular biopsy procedure described by Heath et al.\(^{23}\) and aspiration biopsy procedure of Roberts\(^{24}\). A site on the bottom aspect of a testicle nearest to the caudal epididymis and a site at the middle of the caudal epididymis were shaved and disinfected with methylated spirit. A sterile 18-gauge needle was then threaded one centimetre deep into the testicle and into the epididymis. In each case a sterile 5 ml syringe was used to aspirate semen or sperm cells by suction. The semen was placed on a clean microscopic glass slide, diluted with approximately equal amount of normal saline, thoroughly mixed and finally spread over the slide before air drying. The glass slides were marked to provide the bull’s identity. In the laboratory, air dried slides were fixed with 0.2% glutaraldehyde solution. Staining was according to Boguth.\(^{25}\) In brief, the slides were covered with carbol fuchsin solution for five minutes, washed with acetic alcohol solution for five minutes, placed in methylene blue solution for two minutes and finally washed with absolute alcohol before air drying. The smears were ultimately examined for spermatozoa abnormalities at 100X magnification using a Minitube® HT007 dark phase microscope (Minitube Abfull- und Labortechnik GmbH, Tieferbach, Germany). A random sample of 100 spermatozoa was examined and classified into normal or abnormal according to standard criteria for semen analysis described by Stolla et al.\(^{26}\)

Histopathological analysis

In the laboratory, the scrotum was excised and testes together with epididymis exposed. A testicular tissue sample was taken from the middle region of each testis and fixed in Bouin’s solution. After 48 hours of fixation, samples were washed with running water for about 6 hours. Then tissues were trimmed and preserved in 70% ethyl alcohol until processed. The standard procedure for processing fixed tissue for histological examination was used and the tissue was stained with haematoxylin and eosin. For each tissue 40 areas picked randomly by movements from left to right were examined by using a light microscope at X100 magnification. Abnormal testis were indicated by degenerated or hypoplastic seminiferous tubules with over 25% testicular tissue loss.

Statistical analysis

Data were handled in Microsoft excel whereas all statistical analyses were performed using the Statistical Analysis System\(^{27}\) windows program. Analyses classified bulls into normal (healthy testicular tissue; group 1) or abnormal (unhealthy testicular tissue; group 2).

A general linear model analysis of variance procedure was used to determine differences of SC attributable to age and heart girth, with age and bull as main effects and heart girth as a covariate. The linear relationships of SC with age and heart girth were estimated, using a linear regression adjusted for bull differences. Adjustments for body weight, age and body condition were made according to Quirino and Bergmann\(^{28}\) and Bell et al.\(^{29}\). Significant differences among groups were found with t-test and \(\chi^2\)-correlation. Means and standard deviations were calculated within the age groups.

Results

The groups

Bulls placed into group 1 were 169 and those placed into group 2 were 134. That is, 55.77% of the bulls had sound testicular
tissue with high percent testicular tissue intact. This result was after ultrasonic echotexture scan and histological analysis of tissue.

Heart girth and body weight measurements

Table 1. Relationship of heart girth and body weight in Tanzania shorthorn zebu bulls.

<table>
<thead>
<tr>
<th>Age bracket</th>
<th>Group 1 (n = 169)</th>
<th>Group 2 (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart girth (centimetre)</td>
<td>Body weight (kg)</td>
</tr>
<tr>
<td></td>
<td>minimum and maximum</td>
<td>minimum and maximum</td>
</tr>
<tr>
<td>&lt;2.0</td>
<td>121.8 ± 3.9 (n = 11) and 114 - 128</td>
<td>153.2 ± 18.9 (n = 11)</td>
</tr>
<tr>
<td>&gt;2.0 - 2.5</td>
<td>133.0 ± 4.2 (n = 26) and 126 - 138</td>
<td>206.8 ± 20.2 (n = 26)</td>
</tr>
<tr>
<td>&gt;2.5 - 3.0</td>
<td>139.7 ± 4.8 (n = 37) and 132 - 150</td>
<td>238.7 ± 23.9 (n = 37)</td>
</tr>
<tr>
<td>&gt;3.0 - 4.0</td>
<td>153.0 ± 6.8 (n = 59) and 145 - 167</td>
<td>303.1 ± 32.7 (n = 59)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>163.6 ± 4.2 (n = 32) and 158 -175</td>
<td>354.9 ± 20.4 (n = 32)</td>
</tr>
<tr>
<td>Overall mean</td>
<td>146.9 ± 13.4 (n = 169) and 114 -176</td>
<td>251.4 ± 23.2 (n = 169)</td>
</tr>
</tbody>
</table>

HG measurements for groups 1 and 2 are presented in Table 1. Body weight obtained by the formula \( Y = 4.81HG - 432.73 \) described by Nicholson and Sayer²⁰ correlated highly with age in Group 1 (\( r = 0.4 \)).

\( N = \) number of bulls; mean ± standard deviation
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>BCS</th>
<th>Heart girth (cm)</th>
<th>Adjusted HG (cm)</th>
</tr>
</thead>
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<td></td>
</tr>
<tr>
<td>4</td>
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<td>115.0 ± 1.4</td>
<td>116.5</td>
</tr>
<tr>
<td>5</td>
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<td>123.3 ± 3.4</td>
<td>120.0</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>125.0 ± 0.0</td>
<td>123.4</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>126.0 ± 0.1</td>
<td>126.9</td>
</tr>
<tr>
<td>&gt;2.0 – 2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>128.5 ± 1.0</td>
<td>128.2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>129.0 ± 0.8</td>
<td>131.0</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>134.0 ± 1.4</td>
<td>133.8</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>137.3 ± 1.8</td>
<td>136.6</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>139.0 ± 0.6</td>
<td>139.5</td>
</tr>
<tr>
<td>&gt;2.5 – 3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>137.0 ± 4.6</td>
<td>135.8</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>139.4 ± 3.3</td>
<td>139.5</td>
</tr>
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<td>142.7 ± 2.6</td>
<td>143.2</td>
</tr>
<tr>
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<td>149.5 ± 0.7</td>
<td>146.7</td>
</tr>
<tr>
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<td>150.0 ± 0.0</td>
<td>150.6</td>
</tr>
<tr>
<td>&gt;3.0 – 4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>142.5 ± 4.0</td>
<td>144.3</td>
</tr>
<tr>
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<td></td>
<td>148.3 ± 1.6</td>
<td>148.5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>153.3 ± 4.1</td>
<td>152.6</td>
</tr>
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<td>7</td>
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<td>155.5 ± 3.8</td>
<td>156.7</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>160.3 ± 3.7</td>
<td>160.9</td>
</tr>
<tr>
<td>&gt;4</td>
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<tr>
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<td>156.5 ± 0.7</td>
<td>156.0</td>
</tr>
<tr>
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<td></td>
<td>158.6 ± 2.0</td>
<td>159.3</td>
</tr>
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<td></td>
<td>163.8 ± 3.1</td>
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<td>8</td>
<td></td>
<td>165.5 ± 3.8</td>
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</table>
Table 3. Variations in SC of TSH zebu according to age and heart girth (HG).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number</th>
<th>HG** ranges</th>
<th>Mean HG</th>
<th>SC**</th>
<th>Mean SC</th>
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<tbody>
<tr>
<td>1.5 - 2.0</td>
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<td>115 - 120</td>
<td>121.9</td>
<td>25.6 ± 0.5</td>
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<tr>
<td></td>
<td>2</td>
<td>121 - 125</td>
<td>±</td>
<td>26.0 ± 1.4</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>126 - 130</td>
<td>7.8</td>
<td>27.0 ± 0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0 - 2.5</td>
<td>8</td>
<td>126 - 130</td>
<td>128.9</td>
<td>27.1 ± 1.33</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>131 - 135</td>
<td>±</td>
<td>27.2 ± 0.9</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>136 - 140</td>
<td>8.1</td>
<td>27.9 ± 0.7</td>
<td>2.6</td>
</tr>
<tr>
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<td>13</td>
<td>131 - 135</td>
<td>137.1</td>
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<td>28.1</td>
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<td></td>
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<td>±</td>
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<tr>
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<td>2</td>
<td>141 - 145</td>
<td>8.0</td>
<td>28.5 ± 0.7</td>
<td>1.1</td>
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<td>6</td>
<td>146 - 150</td>
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<td>28.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>3.0 - 4.0</td>
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<td>141 - 145</td>
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<td>28.8 ± 1.0</td>
<td>29.3</td>
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<tr>
<td></td>
<td>22</td>
<td>146 - 150</td>
<td>±</td>
<td>29.3 ± 1.5</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>151 - 155</td>
<td>9.8</td>
<td>29.9 ± 1.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>156 - 160</td>
<td></td>
<td>29.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>161 - 165</td>
<td></td>
<td>30.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>166 - 170</td>
<td></td>
<td>30.3 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>2</td>
<td>150 - 155</td>
<td>159.6</td>
<td>29.5 ± 0.7</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>156 - 160</td>
<td>±</td>
<td>30.6 ± 1.6</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>161 - 165</td>
<td>7.6</td>
<td>31.5 ± 1.3</td>
<td>1.6</td>
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<tr>
<td></td>
<td>11</td>
<td>166 - 170</td>
<td></td>
<td>32.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>171 - 175</td>
<td></td>
<td>33.5 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

Mean 169 146.9 ± 3.4 28.9 ± 1.8

**SC in centimetres, HG in centimetres, Number = number of bulls

79; P = 0.01; n = 169) and Group 2 (r = 0.79; P = 0.01; n = 134). Mean body weight in Group 1 was 251.4 ± 23.2 kg and 245.7 ± 69.2 kg in group 2. Body weight for the different age brackets within a group differed significantly (P = 0.01). There was however no difference in body weight between the two groups of the same age bracket. In general, body weights reflected the age bracket of the animal rather than the pathology of the reproductive organs.

**Body condition score measurements**

The body condition of a bull contributed greatly to differences in heart girth. That is, BCS increased variations in HG within the
particular age bracket. Variations of HG within the same age bracket were not influenced by the pathology of the reproductive organs. Therefore, an animal with pathology of the reproductive organs (i.e. group 2 bull) may have a similar HG and therefore body weight with an associate carrying normal organ when the age bracket and BCS were similar.

Simple linear regression analysis revealed that within the same age category, HG measurements can be predicted by an equation $HG = 4.8 \text{ BCS} \pm 119.6$ ($r = 0.82$; $P = 0.01$; $n = 169$) in group 1 and an equation $HG = 5.1 \text{ BCS} \pm 108.1$ ($r = 0.82$; $P = 0.01$; $n = 134$) in group 2. Text table 2 shows how BCSs influenced the spreading of body weights within each age bracket in Group 1. A similar pattern of spreading of body weights was observed in Group 2.

**Scrotal circumference**

SC measurements varied between the different age groups and body weights (Table 3). SC increased gradually with advancement in age of the bull up to 4 years of age ($P = 0.01$), and thereafter increased marginally with little change. Linear regression analysis revealed that an equation $Y = 1.3X \pm 23.1$ (where $Y =$ scrotal circumference in centimetres and $X =$ age in years) estimated scrotal circumference with a correlation coefficient ($r$) of 0.67 ($P < 0.01$). Similarly, for heart girth measurements, an equation of $Y = 0.13X \pm 9.98$ (where $Y =$ scrotal circumference in centimetres and $X =$ heart girth in centimetres) was used to estimate scrotal circumference with a coefficient of correlation ($r$) of 0.61 ($P < 0.01$). Testicular pathology of the reproductive organs was a significant factor that caused significant variations in scrotal circumference because there was a difference in scrotal circumference between bulls of Group 1 (healthy testicular tissue) and Group 2 (pathological testicular tissue) when age, body weight and body condition score were similar. Further data is given in Table 4. SC is significantly smaller in bulls of group 2 carrying a pathological testis than those in group 1 with a healthy testis.

**Adjustments**

After using the formula $SC = 0.21HG \pm 0.29BCS$ for adjusting heart girth and body condition scores, where scrotal circumference (SC) and heart girth (HG) are measured in centimetres and body condition scores in numerical values ranging between one and nine, the minimum limits for scrotal circumference are as given in Table 4. Values for Group 1 are used to tabulate the limits, because in Group 2, pathology modified the dimension value of the SC.

**Discussion**

This study determined various body measurements in order to characterize the size and weight of the TSH zebu. HG measurements in this study provided key equations on estimation of weight and size of the TSH zebu. For zebu bulls analyzed in this study HG measurements ranged between 112 centimetres and 175 centimetres. Consequently the corresponding weights of the animals ranged between 108 and 358 kg. This weight of the TSH zebu is similar to that of the zebu reported earlier by Mpiri et al. but smaller than that of most zebras. The weight of zebu of South America ranges between 600 and 1100 kg while that of the Australia zebu ranges between 450kg and 700kg. Similarly the weight of a mature boran zebu of Ethiopia ranges between 400kg and
### Table 4. Minimum scrotal circumference for Tanzania shorthorn

<table>
<thead>
<tr>
<th>Age Categories (years)</th>
<th>Body condition scores**</th>
<th>Heart Girth in centimetres</th>
<th>SC Ranges in centimetres</th>
<th>Adjusted SC in centimetres</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 - 1.5</td>
<td>1 - 3</td>
<td>110 - 115</td>
<td>21.5 - 23.0</td>
<td>21.5 - 22.8</td>
</tr>
<tr>
<td></td>
<td>4 - 6</td>
<td>114 - 118</td>
<td>22.5 - 24.5</td>
<td>22.6 - 24.7</td>
</tr>
<tr>
<td></td>
<td>7 - 9</td>
<td>116 - 121</td>
<td>24.0 - 26.5</td>
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</tr>
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<td>1 - 3</td>
<td>113 - 120</td>
<td>24.0 - 26.0</td>
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<td></td>
<td>7 - 9</td>
<td>123 - 128</td>
<td>27.5 - 29.0</td>
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<td>2.0 - 2.5</td>
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<td>126 - 130</td>
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<td>26.8 - 27.8</td>
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<td>2.5 - 3.0</td>
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<td>136 - 144</td>
<td>27.0 - 28.5</td>
<td>27.2 - 28.4</td>
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<td></td>
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<td>27.8 - 29.7</td>
</tr>
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<td>3.0 - 4.0</td>
<td>1 - 3</td>
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<td>28.5 - 29.5</td>
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</tr>
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<tr>
<td></td>
<td>7 - 9</td>
<td>160 - 167</td>
<td>29.5 - 31.0</td>
<td>29.8 - 30.9</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>1 - 3</td>
<td>158 - 155</td>
<td>29.0 - 30.5</td>
<td>28.8 - 30.2</td>
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<td></td>
<td>7 - 9</td>
<td>168 - 175</td>
<td>31.0 - 33.0</td>
<td>31.4 - 35.6</td>
</tr>
</tbody>
</table>

** Body condition score according to Nicholson and Butterworth, 19 SC = scrotal circumference

700kg\(^{20}\).

Age of bulls in this study ranged from 2 years to just over 4 years. According to data on the TSH zebu, this is the age range for young bulls recruited for breeding in the traditional livestock management system\(^{16}\). Accordingly, this study covered an age group which is repeatedly used for breeding and therefore represents a large population of breeding TSH zebu bulls. The data obtained in this study may be used therefore in almost all TSH adult cattle.

Bull’s scrotal circumference in this study ranged between 21.5 and 33 centimetres. The SC measurements were positively correlated with age (r=0.69; P<0.01; n=169). The increase in SC with age was gradual until bulls were 4 years.
The increase was greater in the young than in the old. This means, there was a large variation in SC in young bulls and thereafter there was a slow increase causing an insignificant variation in SC. Mature bulls had a fixed SC. This finding is consistent with many other publications that report SC increases with age and that young bulls have a great potential of changing SC size\(^{30,31,32,33}\). This means, growth rates of individual bulls are reflected by changes in SC. Once animals become mature and stop growing, scrotal circumference stops increasing. Therefore, SC recommendations should have ranges for young bulls and fixed values for mature bulls.

The most interesting finding of the present study is that SC measurements in TSH zebu bulls are positively related with heart girth (r=0.77; P<0.01; n=169) and body condition score (r=0.33; P<0.01; n=169). Heart girth is an indicator of body weight of the animal. Body condition score is an indicator of the nutritional status of the animal. This means variations in body weight caused by variations in growth rates or nutritional status are reflected by changes in SC. In addition, bulls with small body size have relatively small scrotal circumference. This effect was reflected by TSH zebu bulls in which SC of 26.3 – 29.7 centimetres for 2 – 3 year old bulls is obviously smaller than that of 31.7 – 34.7 centimetres reported for other bulls of the same age bracket\(^{34,35}\). Many researchers have reported similar correlations (coefficient correlations ranged from 0.45 to 0.78; P 001)\(^{36,37,38,39}\). Since changes in heart girth are reflected in body condition score of the animal, one may use either body condition score and age or heart girth and age to adjust SC. Variations in body weight that follow nutritional deficiencies common during the dry season are also reflected by changes in SC; with healthy testes maintaining the maximum SC value for that age bracket. For the same reason, threshold for SC should be given in ranges to take care of variations of body condition.

Diagnostic ultrasound is an accurate tool for determining testicular size and assessing scrotal and testicular pathology in bulls.\(^{40}\) In this study, scanning was used to obtain accurate measurements of testicles, which cannot be obtained through use of calipers. Calipers are normally regarded as basic tools which can be used in field conditions of Africa. Therefore, threshold for calipers should be different from those generated by use of ultrasonography.

In conclusion, heart girth values from this study confirm that TSH zebu is smaller than other Bos indicus breeds of cattle. The results also confirm that the minimum and maximum standards for SC in TSH zebu bulls are smaller than those for other zebu breeds. This is a reflection of the small size of the TSH zebu. We recommend SC given in Table 4 for use in the Tanzania shorthorn zebu bull.

Acknowledgements

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ETAT DE LA COMPLEMENTATION MINERALE DES BOVINS DE LA VINA (CAMEROUN)

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STATE OF MINERAL SUPPLEMENTATION IN CATTLE IN VINA (CAMEROON)

Summary

Different methods of mineral supplementations in cattle in Vina division of Adamawa Cameroon were evaluated to find out if they are capable of preventing symptoms linked to mineral deficiency. A survey was carried out using a questionnaire administered to cattle rearers. Mineral disorder symptoms have been found in 70% of herds. Pica, general emaciation and rough hair coat diseases was most found. Except of pica and general emaciation, the observation of mineral disorder was high in rainy season. All cattle rearers give mineral supplements to their herds. In terms of type of supplements, 60% use NaCl salt, 14% bicarbonate salt and only 1% used industrial mineral premix. NaCl salt is more frequently used; 24% of cattle rearers use NaCl everyday while 5% use veterinary products for mineral supplementation. Generally, there was a negative and significant correlation between the use of common salt and the occurrence of pica diseases.

Key words: Mineral nutrition, survey, Vina (Cameroun)

Résumé

Les méthodes de complémentation minérale bovine pratiquées dans la région de la Vina (Adamawa, Cameroun) ont été évaluées dans le but de savoir si elles permettent d'éviter des manifestations de symptômes liés aux carences minérales. L'étude s'est faite sur la base d'une enquête qui a permis de recueillir des informations sur les symptômes de carence minérale et le mode de complémentation pratiquée par les éleveurs de cette région. Au moins un symptôme relevant d'une possibilité de trouble minéral a été observé dans 70% de troupeaux enquêtés. Les symptômes les plus observés ont été la manifestation de pica, la cachexie et les poils piqués. À l'exception des symptômes de pica et de cachexie qui ont été plus observés pendant la saison sèche, tous les autres symptômes ont été plus fréquents en saison pluvieuse. Comme complémentation minérale, tous les éleveurs donnent à leurs animaux le sel de cuisine et le natron. 60% des éleveurs préfèrent le sel de cuisine contre 14% pour le natron et seulement 1% préfère les compléments minéraux industriels. Le sel de cuisine est fréquemment utilisé; 24% des troupeaux utilisent le sel de cuisine tous les jours contre 5% pour les produits d'officine vétérinaire. Une corrélation négative et significative a été observée entre la fréquence d'utilisation du sel de cuisine et du natron avec la manifestation de Pica chez les bovins.

Mots clés : Nutrition minérale, enquête, Vina, Adamawa, Cameroun

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Introduction

L'élevage bovin en zone tropicale est caractérisé par une faible productivité et reproduction\(^1\). Les contraintes liées à ce faible niveau de production et reproduction sont multiples : génétique, environnementale (climat, infections parasitaires), technologique (hygiène, technique de mise bas, allaitement) et nutritionnelle\(^2\). Selon Smith & Akinbamijo\(^2\), 20% des cas de baisse de reproduction sont liés à l'espèce bovine alors que la nutrition contribue à 50% de cette baisse si les pathologies parasitaires sont maîtrisées.

Dans l'Adamaoua (région dont fait partie la Vina), le système d'élevage majoritairement utilisé est de type traditionnel et le pâturage est essentiellement et quelquefois même l'unique ressource alimentaire des bovins\(^3\). Cependant, la strate herbacée de ce pâturage est qualitativement pauvre et quantitativement insuffisante par rapport à l'effectif bovin localement exploité. En effet, depuis 1968, Letouze\(^6\) estimait une surcharge bovine sur près de 30% des pâturages de l'Adamaoua. Rippstein\(^5\) quant à lui a estimé la charge des pâturages de l'Adamaoua entre 0,7 et 2,2 têtes par hectare alors que pour une exploitation, il est recommandé une charge de 0,5 à 1 tête par hectare. Cette situation s'empire de nos jours avec la dégradation des pâturages suite à l'extension des espaces cultivés, à la création des réserves de chasse ou à l'urbanisation\(^6\). Pour faire face à tout cela, il est important d'apporter à l'animal des aliments de complémentation pour combler les déficits des apports des fourrages.

Dans la Vina (Adamaoua – Cameroun), les études en nutrition bovine se sont généralement limitées à des apports énergétique et protéique pour réduire les pertes de poids observées en période de soudure (saison sèche) chez les animaux de pâturage\(^6\), 7, 8. Malgré ces efforts de complémentations protéino-énergétiques des rations des bovins de l'Adamaoua, on a toujours observé de faibles productivités et reproduction chez ces bovins; ce qui concorde avec les travaux de McDowell\(^9\) menés dans de nombreux pays tropicaux. Selon McDowell\(^10\), les faibles performances des bovins seraient liées aux déséquilibres minéraux. En effet, ses travaux ont montré qu'une complémentation en sélénium a permis de réduire le taux de mortalité chez les bovins dans la région d'Amérique Latine où cet élément était en carence. L'effet d'apport minéral sur les performances des bovins avait déjà été prouvé dans une étude comparative où il a été montré que des pâturages fortement carencés en cobalt et cuivre entraînaient une productivité bovine plus faible par rapport aux pâturages présentant une déficience protéino-énergétique élevée\(^11\).

D'autre part, ces auteurs ont montré à travers un apport en un mélange d'éléments minéraux, une réduction de plus de 50% du taux d'avortement et de mortalité de jeunes veaux.

Le problème du statut minéral des bovins de l'Adamaoua a été abordé dans quelques travaux. Lou Issa\(^12\) et Njwe & Lou Issa\(^13\) ont rapporté des cas de carence en Ca, P, Cu et Zn dans les fourrages et de carence en Ca, Mg, Na, K et Zn chez les bovins. Moffor & Mbah\(^14\) et Moffor et al.\(^15\) ont montré que les teneurs en minéraux des fourrages varient avec le type de sol ; les fourrages des sols sur basalte et les animaux qui y pâturent ayant les teneurs les plus élevées, comparé à ceux des sols sur granite. Il ressort de ces travaux que le problème minéral dans
l'Adamaoua, quoiqu'il soit réel, n'est pas bien élucidé alors qu'un mauvais apport en éléments minéraux a des conséquences néfastes sur le rendement des bovins. En cas de carences ou toxicités minérales, il est possible d'observer des signes cliniques de maladie chez les bovins. On attribue les manifestations de pica (géophagie) à une carence en sodium chez l'animal, la décoloration de poils à une carence en cuivre et les dermites à une carence en zinc. Bien que ces manifestations surviennent lorsque les carences sont déjà très prononcées, elles permettent tout de même de constater les effets sur un élevage d'un faible apport en éléments minéraux dans une région donnée. En région tropicale, la complémentation minérale est généralement assurée par un apport unique en sel de cuisine (NaCl) aux bovins ; bien que cette pratique donne un plus à l'animal, elle ne comble que les besoins en sodium et chlorure. Il a été ainsi montré que les animaux recevant uniquement du sel de cuisine ont un taux de vêlage moyen de 52,6% par rapport à un taux de 75,5% pour ceux recevant en plus du sel de cuisine, une complémentation en d'autres sels minéraux. De plus, une étude a montré que les veaux des vaches recevant un apport complet de sels minéraux avaient un poids moyen de 88,7 kg à la mise bas, contre 44,8 kg pour les veaux des vaches qui ne recevaient que du sel de cuisine.

Mener une complémentation minérale efficace, susceptible d'apporter aux bovins les éléments minéraux en carence dans leur alimentation de base est nécessaire pour avoir au niveau de la zone d'élevage de la Vina un bon rendement de production et de reproduction. Toutefois, avant de rechercher les éléments minéraux nécessaires pour cette complémentation, il est important de connaître si le mode de complémentation minérale appliqué localement permet d'éviter les risques de carence minérale.

L'objectif de la présente étude était de voir s'il existe chez les bovins de cette région des symptômes liés à une carence ou toxicité minérale, de ressortir la période de l'année où ils sont plus observés et de ressortir le mode de complémentation minérale adapté aux conditions locales.

Matériel et méthodes

L'étude a été menée dans les zones fourragères de la Vina et plus précisément dans les localités environnantes de la ville de Ngaoundéré (Cameroun). Le climat de la zone est de type soudano guinéen avec une saison sèche qui va de novembre à mars et une saison pluvieuse qui va d'avril à octobre. Après un test d'enquête préalablement mené, les bergers de 110 troupeaux, qui représentaient dans l'ensemble environ 9000 bovins, ont été interrogés. Le questionnaire se présentait en trois volets.

Pour le premier volet de questions, les bergers devaient nous informer sur la zone de pâturage de leur troupeau. Ils avaient à choisir, en fonction des saisons, entre les pâturages des sols sur granite (sableux), des sols sur basalte ancien (rouge) ou des sols sur basalte récent (rouge foncé).

Le second volet de questions se rapportait aux manifestations de carence ou toxicité minérale chez les bovins. Une série de symptômes de carence minérale était présentée aux bergers qui devaient nous informer du nombre d'animaux dans leur troupeau qui les manifestaient. Le nombre d'animaux présentant un symptôme était ramené en pourcentage par rapport au nombre total des bovins du troupeau. Le sexe, l'âge et la période où ces phénomènes ont
été les plus observés ont également été ressortis.

Le troisième volet quant à lui se rapportait à la pratique de la complémentation minérale. Trois modes de complémentation minérale étaient présentés aux éleveurs : complémentation par le sel de cuisine, par le natron ou par les produits des officines vétérinaires. L’information était de savoir au niveau de chaque troupeau la préférence et la fréquence d’utilisation de ces trois sels de complémentation par les bergers.

A l’aide du logiciel StatBox, une analyse descriptive des données (Analyse en composante principale) a été ensuite réalisée de manière à étudier les corrélations entre l’utilisation des compléments minéraux et l’apparition des symptômes liés à la carence ou toxicité minérale et à les visualiser sur une figure. Les fréquences d’utilisation des minéraux de complémentation par an ont été utilisées pour évaluer leur effet sur les troupeaux, et les symptômes observés par troupeau ont également été ramenés en pourcentage pour ces analyses.

**Résultats et discussion**

**Zones de pâturage**

Près de 95% des bovins pâturent sur des sols sur basalte en saison sèche comme en saison pluvieuse (Tableau 1). En effet, ces sols sont les plus représentatifs. Rippstein a également observé la dominance de ce type de sol au niveau de Wakwa (Adamaoua). En saison pluvieuse, les fourrages y sont plus disponibles pour les bovins et plus de 58% d’éleveurs gardent leur troupeau sur des sols sur basalte ancien. Ceci n’est pas le cas en saison sèche où, avec la baisse de la disponibilité des fourrages sur les sols, les bergers emmènent généralement leurs troupeaux dans les bas-fonds à sols hydromorphes. Ces sols sont humides en saison sèche, ce qui favorise la présence des plantes. En saison sèche, près de 35% des troupeaux y pâturent contre 12% en saison pluvieuse.

**Symptômes observés**

Au moins un symptôme de trouble minéral est observé sur chaque troupeau inspecté (Tableau 2). La manifestation de pica, les poils piqués, la cachexie, la boiterie, l’allongement des sabots, les dermites et la diarrhée ont été observés chez plus de 50% de troupeaux enquêtés, la rigidité des muscles étant le symptôme le moins observé (19%).

La présence de manifestation de pica est le symptôme le plus fréquent. Elle s’est exprimée dans 81% de troupeaux. Cette manifestation est un symptôme de carence grave en sodium, potassium ou phosphore qui se manifeste par une dépravation de goût marqué par le léchage du sol, des écorces d’arbre, des urines ou de la sueur d’autres animaux. Cette manifestation fait aussi suite
Tableau 2: Symptômes observés sur des troupeaux de la Vina.

<table>
<thead>
<tr>
<th>Troupeaux atteints (%)</th>
<th>Périodes majoritaires</th>
<th>Essai de traitement par les éleveurs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Totaux$^1$</td>
<td>Sexes majoritaires$^2$</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Pica</td>
<td>81</td>
<td>25,5</td>
</tr>
<tr>
<td>Poils piqués</td>
<td>73</td>
<td>58,7</td>
</tr>
<tr>
<td>Pelade</td>
<td>39,7</td>
<td>28</td>
</tr>
<tr>
<td>Décoloration des poils</td>
<td>44,4</td>
<td>42,9</td>
</tr>
<tr>
<td>Cachexie</td>
<td>71,4</td>
<td>64,4</td>
</tr>
<tr>
<td>Boiterie</td>
<td>60,3</td>
<td>44,7</td>
</tr>
<tr>
<td>Démolaison des sabots</td>
<td>41,3</td>
<td>57,7</td>
</tr>
<tr>
<td>Allongement des sabots</td>
<td>50,8</td>
<td>53,1</td>
</tr>
<tr>
<td>Dégénérescence musculaire</td>
<td>38,1</td>
<td>37,5</td>
</tr>
<tr>
<td>Rigidité</td>
<td>19,1</td>
<td>50</td>
</tr>
<tr>
<td>Dyspnée</td>
<td>49,2</td>
<td>64,5</td>
</tr>
<tr>
<td>Dermite</td>
<td>52,4</td>
<td>48,5</td>
</tr>
<tr>
<td>Chute</td>
<td>38,1</td>
<td>95,8</td>
</tr>
<tr>
<td>Fracture</td>
<td>41,3</td>
<td>46,2</td>
</tr>
<tr>
<td>Infécordination</td>
<td>46</td>
<td>82,8</td>
</tr>
<tr>
<td>Diarrhée</td>
<td>66,7</td>
<td>47,6</td>
</tr>
</tbody>
</table>

$^1$Pourcentages par rapport à la totalité des troupeaux inspectés (les combinaisons de signes ne sont pas prises en compte)

$^2$Pourcentages par rapport aux troupeaux où les symptômes ont été observés

SS : saison sèche  SP : saison pluvieuse  A : Année  F : Femelle  M : Mâle  J : Jeune

à des carences en oligoéléments tels que le cuivre et le cobalt. La forte présence de cette manifestation dans la Vina n’est pas surprenante car le pica est une manifestation communément rencontrée dans les pâturages tropicaux$^{19}$. Cependant, bien qu’on attribue généralement la consommation du sol (géophagie) à une carence minérale chez les animaux, il convient également de relever que certains sols riches en sodium attirent les animaux à les consommer$^{20}$. Après la manifestation de pica, la cachexie et les poils piqués ont été plus observés avec une présence de 71 et 73% respectivement. Ce sont des symptômes les plus spécifiques de carences en cobalt et également du cuivre et du zinc$^{16}$. Les boiteries dans un troupeau sont également attribuées à des carences en cuivre, zinc mais aussi en manganèse. Ces symptômes ont été observés sur plus de 60% des troupeaux. On peut aussi citer la décoloration des poils (44%), la dermite (52%), la dyspnée (49%) et la pelade (38%). La décoloration des poils et la dyspnée sont généralement attribués à une carence en cuivre ; la dermite et la pelade à une carence en zinc. Les symptômes de carences en cuivre et zinc
ont été très présents au cours de cette enquête.

**Sexes où les symptômes sont majoritairement observés**

Pratiquement tous les symptômes sont plus observés chez les femelles. La présence de cachexie s’est par exemple plus exprimée chez les femelles de 64% de troupeaux enquêtés contre 7% pour les mâles (Tableau 2). En effet, les femelles sont plus vulnérables aux carences minérales car durant la gestation ou l’allaitement, leurs besoins en minéraux augmentent\(^2,10,21\). Selon Underwood & Suttle\(^22\), les vaches perdent des éléments minéraux pendant l’allaitement et leurs besoins sont plus élevés. En plus, les sels excrétés dans le lait restent élevés même en cas de carence extrême de Na et Cl chez la vache\(^23\). Des manifestations de carence minérale se sont également exprimées chez les jeunes animaux et le pica vient en tête avec 19% de troupeaux. Ceci concorde avec les observations de Gueguen et al.\(^16\) qui ont exprimé le risque de carence minérale en élevage bovin aussi bien chez les animaux adultes que les jeunes animaux.

**Saison où les symptômes sont majoritairement observés**

Les manifestations de pica et de cachexie ont été plus observées en saison sèche avec respectivement 39 et 59% de troupeaux plus affectés contre 29 et 22% en saison pluvieuse. En effet, avec la baisse de la biomasse des plantes fourragères dans les parcours de la Vina en saison sèche\(^3,4\), l’apport alimentaire est faible et les animaux perdent rapidement de poids. Durant cette même saison, les températures sont plus élevées et les animaux perdent beaucoup plus de sel (sodium) par la sueur et les urines ; ceci pourrait expliquer une manifestation plus importante de pica dans le but de couvrir ces pertes en sodium.

Toutefois, à l’exception du phénomène de pica et des signes de cachexie qui ont été plus observés en saison sèche, tous les autres symptômes l’ont été le plus souvent en saison pluvieuse. La carence minérale en saison pluvieuse pourrait se justifier par le fait que durant cette période, les animaux croissent rapidement suite à un apport protéino-énergétique adéquat qui augmente leurs besoins en minéraux. En saison sèche par contre, le faible apport protéino-énergétique entraîne des pertes importantes de poids vifs réduisant les exigences minérales des animaux\(^1,24\).

**Essais de traitement par les éleveurs**

A l’observation des symptômes dans leur troupeau, les éleveurs essaient eux-mêmes d’administrer quelques traitements avant le passage d’un spécialiste vétérinaire dans leur pâturage (tableau 2). Les symptômes traités par les éleveurs sont ceux qui sont les plus fréquents dans la zone d’étude et observés dans au moins 40% de troupeaux (pica, les poils piqués, pelade, cachexie, boiterie, allongement des sabots, dyspnée, dermite, fracture, infécondité, diarrhée). Pour les traiter, à l’exception de l’allongement des sabots, de la cachexie et de la diarrhée, les éleveurs utilisent les antibiotiques. Les vermifuges sont utilisés en cas de pica et de diarrhée et les compléments alimentaires en cas de pica (sel de cuisine) et cachexie (touretou, sel de cuisine). Les éleveurs utilisent ainsi peu de complément minéral lors de la présence des symptômes dans leurs troupeaux. En fait, ils attribuent ces symptômes beaucoup plus à des affections parasitaires et microbiologiques et ne
pensent à une déficience alimentaire que lorsqu’il y a perte excessive de poids chez l’animal. Cependant, il est important de souligner que les éleveurs utilisent généralement une association antibiotique et sel en cas de présence de pica et une association tourteau, sel en cas de cachexie.

La cachexie est une perte excessive de poids de l’animal et le premier réflexe des éleveurs est un apport d’aliment de complément tel les fourrages ou tourteaux auxquels ils ajoutent le sel de cuisine. Toutefois, le pica qui est une manifestation de géophagie et de mâché anormal d’os d’animaux morts peut avoir pour conséquence une contamination bovine par les microorganismes. En effet, il a été rapporté à Piiaui (Brésil) qu’environ 2 à 3% d’un total de 100000 bovins meurent annuellement du botulinisme lié à l’infection à Clostridium botulinum10. Ces carences minérales ont ainsi des conséquences assez néfastes dans l’évolution du cheptel d’une région. Les éleveurs de la Vina en sont conscients et lorsqu’ils observent les manifestations de pica, ils donnent aux animaux non seulement le sel de cuisine mais également des antibiotiques et des vermifuges. Toutefois, ce traitement par l’apport de sel de cuisine permet de combler les besoins en sodium de l’animal mais pas d’autres elements tels le phosphore et le potassium, donc les carences peuvent également conduire à des manifestations de pica. Ceci a été bien révélé durant l’enquête car malgré l’utilisation du sel de cuisine par pratiquement tous les éleveurs, les phénomènes de pica ont toujours été observés. La géophagie peut également conduire à une forte ingestion d’éléments susceptibles d’empêcher l’absorption des minéraux et causer des carences secondaires. C’est le cas du fer dont la teneur est très élevée dans les sols tropicaux25 et dont la forte ingestion peut causer une déficience en cuivre ou en zinc26. Il est ainsi important de vérifier si le mode de complémentation minérale pratiqué par les éleveurs de cette région peut être efficace pour éviter les troubles minéraux chez l’animal.

**Complémentation minérale**

**Utilisation et préférence des minéraux de complément**

La complémentation minérale par les éleveurs de la Vina est systématique et ils n’attendent généralement pas une manifestation de symptôme de carence pour les appliquer. Du tableau 3 il ressort que pratiquement tous les éleveurs utilisent le sel de cuisine alors que 74% d’éleveurs utilisent le natron et 80% d’éleveurs utilisent les produits d’officines vétérinaires. Toutefois,

| Tableau 3: Troupeaux utilisant une complémentation minérale. |
|-----------------|-----------------|-----------------|
| Nombre de troupeaux (%) | Sel | Natron | Produits officines |
| Toute l’année | 100 | 74,3 | 79,7 |
| Période d’utilisation (%)* | 87,7 | 35,9 | 19,6 |
| Saison pluvieuse | 6,9 | 34,0 | 35,7 |
| Saison sèche | 5,5 | 30,2 | 44,6 |

* Pourcentage par rapport au nombre de troupeaux utilisant l’un des produits de complémentation.
de la figure 1 il ressort qu'en terme de préférence, peu d'éleveurs (1%) préfèrent les produits d'officines alors que 14% préfèrent le natron et la grande majorité (59%) le sel de cuisine. Les observations faites au niveau des préférences ne reflètent pas toujours des observations faites au niveau des minéraux de complémentation utilisés. C'est ainsi que le natron qui est nettement préféré par les éleveurs par rapport aux produits des officines vétérinaires est utilisé par moins d'éleveurs par rapport à ceux qui utilisent ces produits d'officine vétérinaire. Toutefois, dans la majorité des cas, les éleveurs utilisent ou préfèrent plusieurs sels ; la différence de complémentation réside davantage dans la fréquence d'utilisation que dans la nature des compléments.

Fréquence d'utilisation des minéraux de complément

Le tableau 4 présente la fréquence d'utilisation des minéraux de complément par les éleveurs. Cette fréquence représente le nombre de fois (nombre de jour dans l'année) que les bergers utilisent un complément minéral. Il ressort de ce tableau que 24% des bergers utilisent le sel de cuisine chaque jour alors que seulement 7% et 5% utilisent respectivement le natron et les produits d'officines vétérinaires chaque jour. La majorité des bergers utilisent moins de 12 fois par an le natron (28%) et les produits d'officines vétérinaires (49%) alors que seulement 5% des bergers utilisent moins de 12 fois par an le sel de cuisine. Les produits d'officine vétérinaire se présentent ainsi comme ceux où la fréquence d'utilisation est faible alors que le sel de cuisine est fréquemment utilisé. Probablement à cause de leur coût relativement plus élevé, les produits d'officine vétérinaire sont moins accessibles aux bergers que le sel de cuisine ou le natron. Toutefois, si ces résultats présentent la fréquence d'utilisation d'un complément minéral globalement pour une année, les bergers accordent également une grande importance à la saison lorsqu'ils donnent ces compléments à leurs bovins.

Utilisation des minéraux de complément en fonction des saisons

Du tableau 3, il ressort que 87% d'éleveurs utilisent le sel de cuisine en saison sèche et en saison pluvieuse alors que 7% d'éleveurs l'utilisent uniquement en saison pluvieuse et 5% uniquement en saison sèche. Le Natron a été utilisé par 36% d'éleveurs pendant les deux saisons, 34% d'éleveurs pendant uniquement la saison pluvieuse et 30% uniquement pendant la saison sèche. Pour les produits d'officine vétérinaire, 20%, 36% et 45% d'éleveurs l'utilisent respectivement pendant les deux saisons, uniquement pendant la saison pluvieuse et uniquement pendant la saison sèche. De plus, les bergers qui ont

<table>
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<tr>
<th></th>
<th>1 à 12 fois</th>
<th>24 à 36 fois</th>
<th>48 à 58 fois</th>
<th>100 à 160 fois</th>
<th>180 à 200 fois</th>
<th>chaque jour (365 fois)</th>
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</thead>
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<td>5,4</td>
<td>51,4</td>
<td>4,1</td>
<td>2,7</td>
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<td>Natron (%)</td>
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<td>1,4</td>
<td>10,8</td>
<td>2,7</td>
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<tr>
<td>Produits officines (%)</td>
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<td>1,4</td>
<td>0</td>
<td>2,7</td>
<td>12,2</td>
<td>5,4</td>
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</table>
utilisé le natron et les produits d’officine vétérinaire durant les deux saisons ont également utilisé le sel de cuisine. Le sel de cuisine est ainsi le complément minéral de base utilisé par les éleveurs des troupeaux visités, à l’instar des autres régions tropicales.
Tableau 5: Corrélations entre différents symptômes observés et l'utilisation des sels de complémentation

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<tr>
<th></th>
<th>Pi 1</th>
<th>Pp 2</th>
<th>Pe 3</th>
<th>Dé 4</th>
<th>Ca 5</th>
<th>Bo 6</th>
<th>Dé 7</th>
<th>Al 8</th>
<th>Dé 9</th>
<th>Ri 10</th>
<th>Dy 11</th>
<th>De 12</th>
<th>Ch 13</th>
<th>Fr 14</th>
<th>In 15</th>
<th>Di 16</th>
<th>Sel/an</th>
<th>Natron/an</th>
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</table>

En gras, valeurs significatives au seuil $\alpha = 0,05$

Pi 1 Animaux qui mangent les sols (Pica) ; Pp 2 Poil piqué ; Pe 3 Perte de poils (Pelade) ; Dé 4 Décoloration des poils ; Ca 5 Amaigrissement excessif (Cachexie) ; Bo 6 Boiterie ; Dé 7 Déformation des sabots ; Al 8 Allongement des sabots ; Dé 9 Dégénérescence musculaire ; Ri 10 Rigidité des membres ; Dy 11 Difficulté à respirer (Dyspnée) ; De 12 Inflammation des dermes (Dermite) ; Ch 13 Chute de la femelle après mise bas ; Fr 14 Fracture facile ; In 15 Infécondité ; Di 16 Diarrhée ; Sel N Fréquence d'utilisation du sel ; Natron N Fréquence d'utilisation du natron ; Produt Of. Fréquence d'utilisation des produits d'officines vétérinaires
symptômes. Toutefois, leur utilisation n'a pas d'effet significatif (P > 0,05) sur les symptômes. Il n'y a pas de corrélation significative entre l'utilisation des produits des officines vétérinaires et des manifestations des symptômes de cette région. Pourtant les produits d'officine utilisés sont généralement constitués des sels de sodium (NaCl), potassium (KCl) et surtout des sels de sulfate de plusieurs éléments minéraux (SO₄Ca, SO₄Fe, SO₄Mn, SO₄Co, SO₄Cu, SO₄Zn), du Se sous forme de sélénate de Na ou d'iode sous forme d'IK. Ces produits sont donc à priori plus complets que le sel de cuisine ou le natron. Les faibles corrélations de l'utilisation des produits d'officine vétérinaire avec les symptômes pourraient être dû à la faible fréquence d'utilisation de ces produits. On observe également des rapprochements entre les symptômes. La présence du pica dans un troupeau peut induire la présence d'autres symptômes. En effet, comme il a été souligné précédemment, la manifestation de pica exprime en plus de la carence en sodium, potassium ou phosphore, la carence en d'autres éléments minéraux (cuivre, cobalt...) et surtout que l'ingestion des sols de la Vina riche en fer et en aluminium³ peuvent conduire à de faibles absorptions du cuivre, zinc ou phosphore par les animaux¹. Il existe ainsi une corrélation positive entre le pica et les symptômes de maladies qui expriment les carences en ces oligo-éléments telles que la pelade (r = 0,28), la déformation du sabot (r = 0,34), la rigidité des membres (r = 0,30), la dyspnée (r = 0,42), la chute de la femelle après mise bas (r = 0,26), la fracturation facile des membres (r = 0,28) et l'infécondité (r = 0,41). Toutefois, bien que la manifestation de pica soit généralement accompagnée

Figure 2 : Analyse factorielle des données axes F1 et F2 des maladies observées et des sels de complémentation utilisés
d'une perte d'appétit, il n'a pas été observé de corrélation significative entre la cachexie et la manifestation de pica. La corrélation est plutôt positive et significative ($P<0.05$) entre la cachexie et les manifestations de diarrhée ($r=0.28$). Ceci reviendrait donc à penser que la perte du poids observée dans les troupeaux de la Vina serait plus liée à la manifestation de diarrhée chez les animaux. Il existe également des corrélations positives et significatives ($P<0.05$) entre les symptômes généralement attribués à des carences en cuivre et zinc. C'est le cas des poils piqués qui est corrélé positivement à la présence de pelade ($r=0.34$), boiterie ($r=0.30$), dermite ($r=0.58$) ou de la pelade qui est corrélée avec la présence de boiterie ($r=0.35$) et dermite ($r=0.26$). Ceci montre que dans cette région, tout comme ce qui a été observé par plusieurs auteurs dans les régions tropicales, il y a une carence en Cu et Zn chez les bovins qu'il est nécessaire de les apporter par des aliments de complément.

**Conclusion**

Il ressort de cette étude que les bovins de la zone de la Vina pâturent le plus souvent sur des sols sur basaltes (récents ou anciens). Il existe plusieurs symptômes généralement attribués à des troubles de nutrition minérale. Plus de 70% des troupeaux inspectés ont présenté des animaux ayant des manifestations de pica, poils piqués, cachexie. A l'exception des manifestations de pica et de cachexie qui sont plus fréquentes en saison sèche, tous les autres symptômes sont plus observés en saison pluvieuse. Cette saison pourrait être celle où les risques de carence minérale sont les plus élevés.

Pour lutter contre ces carences minérales, pratiquement tous les bergers utilisent le sel de cuisine et le natron pendant toute l'année. Bien que leur utilisation unique ne permette pas d'atteindre une production ou reproduction optimale, il a été observé des corrélations négatives et significatives de l'utilisation du sel de cuisine et de la présence de signes de carence minérale. Toutefois, il est important d'apporter à ces animaux des compléments en d'autres éléments minéraux tels que le cuivre et le zinc dont les symptômes de carence ont été les plus observés ; surtout que l'utilisation des éléments minéraux de complémentation issus des officines vétérinaires reste encore très faible dans la région de la Vina. Pour mener une complémentation minérale efficace dans cette région, il serait aussi important de connaître au préalable les minéraux en carence ou en excès dans la ration alimentaire des bovins. Ceci ne saurait être mené sans déterminer le statut minéral des sols, des fourrages, des eaux d'abreuvement ou de l'animal.

**Remerciement**

Ce travail a bénéficié de la contribution de l'Agence Universitaire de la Francophonie (AUF) à travers la bourse de la formation à la recherche. Les auteurs remercient les éleveurs, les bergers et tous ceux qui ont contribué à la réalisation de cette enquête.

**Références bibliographiques**


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SHORT COMMUNICATION

A CASE OF NEWCASTLE DISEASE IN PARROTS IN NAIROBI, KENYA

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Newcastle disease is an infectious, highly contagious and devastating disease, mostly affecting chickens, turkeys, wild and ornamental birds1. It is reported as the most important viral disease of birds in the World2. The disease is endemic in Kenya3,4,5 causing, most of the times, 100% mortality to unvaccinated birds. Under natural conditions, the virus enters the host through respiratory and digestive tracts2.

Factors associated with the respective viral persistence in poultry are not well established. The extensively-managed, free-roaming village chickens are believed to keep the virus in circulation and act as reservoirs and carriers to other village and exotic poultry breeds6,7. This is especially so when it is appreciated that, although clinically diseased chickens are the most important hosts of Newcastle disease virus, latently infected birds and survivors of natural infection, which still harbor the agent, may act as reservoirs8,9. The viral excretion, mainly via respiratory tract and faeces2, commences before clinical signs of Newcastle disease occur10. Non-chicken poultry are also suspected to play a role in the sustainance and dissemination of the virus. Ducks and geese often reared with chickens under backyard village conditions may be infected and show few or no clinical signs even with strains lethal to the chickens9,11. Wild birds have been shown to harbor Newcastle disease virus and thus partially maintaining it in nature4,12. Virulent Newcastle disease virus has also been frequently isolated from captive caged birds which, in some cases, develop clinical disease13,14,15. Thus, in order to be able to control the disease, the role of non-chicken poultry in sustainance of Newcastle disease virus needs to be established and control measures put in place. This is important in villages since the respective birds are normally not vaccinated.

This paper reports a case of Newcastle disease in pet parrots located close to a retail outlet, selling live indigenous chickens, within an area, in the outskirts of Nairobi, hosting a number of wild birds, mainly pigeons.

Materials, methods

Specimens brought to the clinic and respective case history

Two African grey parrots, one dead and one live (sick), were brought to the poultry clinic for examination and advice on possible treatment. The parrots were kept together with seven others, as pets, in the owner’s sitting room (the owner had kept parrots, as a hobby, for a period of 2 years). All the birds showed signs of sickness (discomfort, sneezing), but only one died, while the rest eventually recovered. There were no new
birds brought in and no record of vaccination against Newcastle disease. Next to the compound where the parrots were raised, there was a retail facility (kiosk) selling live local chickens and eggs and, within the area, there were a number of wild birds, mainly pigeons. Both the local and wild birds appeared healthy. The parrots had been sick for about a week. Treatment with an antibiotic (tetracycline) and an antifungal (griseofulvin) did not cure them.

**Observed clinical signs, gross lesions and samples collected**

The sick bird showed signs of depression, blocked nostrils, sneezing and some diarrhoea. It was sacrificed through cervical dislocation. On postmortem examination, the major lesions were: presence of thickened pericardium, frank hemorhages on the intestinal tract, and laryngitis. The dead bird had pharyngitis with copious mucous that spilled into the crop and the oesophagus. Other lesions were: severe airsacculitis, hemorrhagic enteritis, petechial hemorrhages on the proventriculus; and liver and myocardium congestion. The lungs appeared normal.

Oropharyngeal, cloacal and airsac swabs, liver and spleen samples were submitted for bacteriology, while liver, spleen and tracheal samples were sent for virology.

**Bacteriological and virological results**

Hemolytic *Staphylococcus aureus* was the only significant bacterial isolate from the pharyngeal and other swabs/specimens. A hemagglutinating agent was isolated from the liver, spleen and trachea, and was serologically confirmed as Newcastle disease virus.

**Discussion**

Parrots are ornamental birds that are domesticated and kept by some families in Kenya and other countries. Kenya is also blessed with the presence of various types of wild birds that hover freely over households. Occasionally, Newcastle disease virus (NDV) has been isolated from pigeons and other wild birds that have died, either singly or in large numbers. Isolation of the virus from parrots has been documented in Nigeria, while Newcastle disease has been diagnosed in imported caged birds, especially psittacines, in countries like Canada and United States.

The current NDV isolation in parrots is of interest as the affected birds were reared in a home that was close to a retail outlet of healthy-appearing chickens and wild birds, composed mainly of pigeons. There is a possibility that the local (indigenous) chickens and/or pigeons were the source of the disease (serving as carriers) for the parrots. This is based on the assumption that the parrot caretakers, or other members of the family, could have visited the shopping centre, where the retail live-bird outlet was located, and carried the virus on their shoes, clothes or otherwise to the parrots. Live-bird markets are considered to be major means of spread for the disease, particularly since many village flock owners take their birds to the market as soon as they see signs of sickness, in an attempt to salvage some value from them. Other sources of the virus for a particular area include: wild birds (including pigeons), live infected chickens being transported through the area, infected carcasses, and movement of contaminated objects from an infected site. Infected birds shed virus in their faeces where it can survive
for up to 3 months at temperatures of 20-30°C and longer at cooler temperatures. Once dried, the virus, mixed with dust, can survive on many materials for periods of weeks to months at tropical temperatures and even longer in cooler climates.

There is also the possibility that the parrots were harbouring the virus, as carriers, only to come down with the disease as a result of confinement stress; thus being a potential source of the virus for the chickens, reversing the movement of the virus. If this were true, the parrots would be taking part in the maintenance and dissemination of the virus, considering the fact that the indigenous chickens would be bought and taken to various parts of Nairobi or elsewhere, perpetuating the dissemination of the disease further, as carriers. The indigenous (village) chickens, which form over 70% of the 30 million total population of chickens in Kenya and found in almost every homestead in villages, are normally not vaccinated against any disease. The wild birds, frequenting the homestead, could also pick the virus and spread it widely. It is reported that some species of wild birds, particularly psittacines, may harbor velogenic Newcastle disease virus for extended periods of time.

The causative agent of Newcastle disease is a paramyxovirus strain 1 (APMV1), which belongs to the family Paramyxoviridae, subfamily Paramyxovirinae and genus Rubulavirus. Most pigeon paramyxovirus strain 1 (PPMV1) isolates differ from other avian paramyxovirus strain 1 (APMV1) isolates by having unique monoclonal antibody binding profiles. This characteristic, coupled by difficulty in classifying the PPMV1 isolates by standard Newcastle disease virus (NDV) pathotyping scheme, has led to their being grouped as NDV variants. However, dissemination of these variants from pigeon populations into commercial poultry has been documented. When passaged through chickens by intramuscular inoculations, the viruses were shown to increase in virulence. There is, therefore, a possibility that a PPMV1 isolate may infect chickens or parrots and circulate subclinically until it evolves to a more virulent form capable to cause disease.

This paper, therefore, documents presence of Newcastle disease virus in parrots and cautions on the possibility of these birds playing a role in the maintenance and dissemination of the virus.

Acknowledgement

The authors would like to thank Robert Rono, Zacchary Munene, Julius Kibe, Mary Mutune and Rose Nyawira, of the University of Nairobi, for their input in laboratory diagnosis.

References


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SHORT COMMUNICATION

BACTERIA ISOLATES FROM THE UTERI OF DOES THAT HAD DYSTOCIA

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University of Nigeria, Nsukka, Enugu state, Nigeria

Infection or other disease of the gravid uterus may cause abortions, fetal death, septic metritis or uterine inertia¹. This may occur due to invasion of the uterus by bacteria, fungi and viruses.

The presence of bacteria in the pregnant uterus does not indicate danger to pregnancy¹. However, in severe bacterial infection or when the maternal resistance is depressed, these bacteria cause severe damage to the placenta and endometrium¹. This may lead to fetal maceration, abortions, premature births, dystocia and retained placenta. Fasanya et al² isolated *E. coli*, *Micrococcus* species and *Staphylococcus aureus* from the genitalia of postpartum Savana Brown goats in Nigeria. Recently Ababneh and Degefa³ recovered *E. coli* and *Staphylococcus* in pure or mixed culture from the uterus of postpartum Balady goats in South Africa. As pointed out by Palmer⁴ the postpartum uterus serves as a good environment for bacterial growth because it is warm, fluid filled and contains a variable amount of necrotic debris.

Caesarean surgery usually exacerbates the stress of prolonged labour due to dystocia. This often results in the lowering of immune status of affected animals. Presence of bacteria in the uterus of the immunocompromised animals can cause serious infections. Prevention and control of such infections require a careful selection of appropriate chemotherapeutic agent which is often based on knowledge of likely infecting agents and their antibacterial sensitivity patterns⁶.⁷

The present study was thus carried out to determine the aerobic bacteria commonly present in the uteri of does undergoing caesarean surgery due to dystocia and to determine the antibiotic susceptibility pattern of the isolates.

Animals

Fifty West African Dwarf (WAD) does presented to the University of Nigeria Veterinary Teaching Hospital (UNVTH) due to prolonged labour were used in the study.

Isolation and Identification of Bacteria

Uterine swabs were taken from the uterus of the does during hysterotomy. Each swab sample was plated out on both blood and MacConkey agar under aseptic conditions and incubated at 37°C for 24-48 hours. After incubation, the plates were examined for bacterial growth. Discrete colonies on the culture media were examined for colonial characteristics. A colony of each morphologic type was purified on nutrient agar. Purified cultures were gram stained and examined for microscopic characteristics. Bacteria that could not be identified by

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colonial and microscopic features were subjected to biochemical tests: sugar fermentation, urease production, simmon citrate utilization and reaction on triple sugar iron (TSI) media following the procedures described by Cheesbrough.8

**Sensitivity tests**

The sensitivity tests were done from the pure cultures using the disc diffusion method. The *E. coli* isolates were tested for their susceptibility and resistance to the following antibiotics: Ciprofloxacin (10μg), Norfloxacin (10μg), Pefloxacin (10μg), Ceftazidime (30μg), Amoxicillin (30μg), Gentamycin (10μg), Chloramphenicol (30μg), Cotrimoxazole (30μg), Tetracycline (50μg), Penicillin (20μg) and Streptomycin (30μg). Mueller Hilton agar plates were inoculated by flooding with a 4 hour nutrient broth culture of the test isolates. Standard commercially available antibiotic discs containing the test antibiotics were then placed onto the agar surface. The plates were incubated at 37°C overnight. Susceptibility to an antibiotic was observed as a clear zone on inhibition of bacterial growth around the antibiotic disc while observation of bacterial growth around a disc suggested antibiotic resistance.

Bacteria were isolated from 47 of the 50 uteri samples cultured. A total of 49 bacterial isolates were obtained from the 47 uteri. *Escherichia coli* were isolated in pure cultures from 40 uteri while samples from 5 uteri yielded pure cultures of *Staphylococcus* species. Mixed cultures of *E. coli* and *Staphylococcus* species were obtained from 2 samples.

In summary, of the 49 isolates, 42 (85.7%) and 7(14.3%) were *Escherichia coli* and *Staphylococcus* species respectively. The susceptibility profile of *Escherichia coli*, which is the predominant bacterial agent isolated is presented in Table 1. As shown in the table, the test isolates were highly susceptible to the fluoroquinolines while none of the isolates was sensitive to the beta-lactames used and cotrimoxazole. The resistance patterns exhibited by the *Escherichia* coli isolates are shown in Table 2. The number of antibiotics which the

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Number susceptible</th>
<th>Percentage susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin (Cp)</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Norfloxacin (No)</td>
<td>29</td>
<td>69.0</td>
</tr>
<tr>
<td>Pefloxacin (Pf)</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime (Cf)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin (Am)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin (Gn)</td>
<td>25</td>
<td>59.5</td>
</tr>
<tr>
<td>Chloramphenicol (Ch)</td>
<td>15</td>
<td>35.7</td>
</tr>
<tr>
<td>Cotrimoxazole (Co)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline (Te)</td>
<td>8</td>
<td>19.0</td>
</tr>
<tr>
<td>Penicillin (P)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2: Antibiotic resistance patterns of *E. coli* strains isolated from uteri of dystocia cases (n=42)

<table>
<thead>
<tr>
<th>Antibiotic resistance pattern</th>
<th>Number (%) with pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Am,P,S,Co,Cf</td>
<td>11 (26.2)</td>
</tr>
<tr>
<td>2 Ch,Am,P,S,Co,Cf</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>3 No,Am,P,S,CO,Cf</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>4 Am,P,S,Te,Gn,Co,Cf</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>5 Ch,Am,P,S,Te,Gn,Co,Cf</td>
<td>7 (16.7)</td>
</tr>
<tr>
<td>6 No,Am,P,S,Te,Co,Cf</td>
<td>4 (9.5)</td>
</tr>
<tr>
<td>7 Ch,Am,P,S,Te,Gn,Co,Cf</td>
<td>12 (28.6)</td>
</tr>
<tr>
<td>8 No,Ch,Am,P,S,Te,Gn,Co,Cf</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td>9 No,Ch,Am,P,S,Te,Gn,Co,Cf</td>
<td>1 (2.4)</td>
</tr>
</tbody>
</table>

No = Norfloxacin, Cf = Cefazidine, Am = Amoxicillin, Gn = Gentamycin, Ch = Chloramphenicol, Co = Cotrimoxazole, Te = Tetracycline, P = Penicillin, S = Streptomycin.

isolates were resistant to ranged from four to seven. A total of nine resistance patterns were recorded with Ch,Am,P,S,Te,Gn,Co,Cf (28.6%), Am,P,S,Co,Cf (26.2%) and Ch,Am,P,S,Te,Gn,Co,Cf (16.7%) being the most prevalent patterns.

In this study *Escherichia coli* and *Staphylococcus* species were the aerobic bacteria isolated from the uteri of does that had dystocia. These bacteria were among the organisms frequently isolated from the uterus of postpartum does and cows. These organisms are said to gain access to the uterine cavity and placenta via the cervix at the time of service. It is also suggested that following stress, these organisms reach the fetus and placenta via haematogenous spread or by ascending the genital tract. It is of importance to note that the 47 uteri from which bacteria were isolated contained dead emphysematous fetuses. In intrauterine fetal death, free gas in the peritoneal cavity is said to be due to bacterial infection. This then suggests that these bacteria may either be responsible for the in-utero death or may be opportunistic bacteria which ascended to the uterus following the stress of dystocia. The source of the uterine bacteria not withstanding, the clinical importance of this finding is however significant. During dystocia, uterine rupture may occur leading to bacterial contamination of the peritoneal cavity with subsequent peritonitis and septicaemia. This finding also highlights the importance of choosing appropriate antibiotic for post-operative therapy. During the post-operative period, antibiotics are usually administered to prevent post operative sepsis. These antibacterials are often used without prior knowledge of possible bacterial pathogens or their susceptibility to antibacterial agents. The choice of an antibiotic is usually based on individual preference, availability and convenience.

It is therefore essential for the surgeon to take uterine swabs during caesarean section for culture and sensitivity profile.
Acknowledgement

The assistance of Dr. K. F. Chah of Department of Veterinary Microbiology and Pathology, University of Nigeria, Nsukka in isolation and identification of the bacteria seen in this work is greatly acknowledged.

References


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SHORT COMMUNICATION

HAEMATOLOGY OF THE NIGERIAN LOCAL TURKEY

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University of Ibadan, Ibadan, Nigeria.

2Department of Veterinary Microbiology and Parasitology,
University of Agriculture, Abeokuta, Ogun State, Nigeria

The haematology of the White England turkeys in the tropical environment has been studied extensively1,2,3. However, to the best of our knowledge there has not been any report on the Nigerian local turkey. There are about 200,000 Nigerian local turkeys in Nigeria4 and they are reared under the semi-intensive management system. They are mostly black in colour and weigh between 7 and 10 kg. The Nigerian local turkeys are now being raised intensively by turkey farmers and it is hoped that this effort would assist in alleviating the acute protein deficiency been experienced by vast majority of low-income earners. Also the intensive production of this local breed of turkeys would help in conserving the much-needed foreign exchange that is currently being expended on the procurement of the White England turkeys. The successful development of the Nigerian local breed of turkey would depend heavily on research information on the physiology of the bird, unfortunately such information are currently unavailable. That is why in this paper we present the influence of sex on the haematology of the Nigerian local turkey.

Nineteen apparently healthy Nigerian local turkeys (10 males and 9 females) were used for the present study. They were reared in deep liter pen and fed with feed whose protein concentration was 28%. The feed and water were given ad libitum. The turkeys were treated against nematodes using Piperazine hydrochloride (Wormazine®) (Alfasan International BV3440AB Woerden, Holland) at 1g/litre of water, three weeks before the commencement of the study. Blood was obtained from the jugular vein of each of the Nigerian local turkey into a bottle containing ethylene diamine tetraacetic acid (EDTA) (2mg/ml of blood). Red blood cells (RBC) were counted with haemocytometers. Packed cell volume (PCV) was determined using the microhaematocrit method. Haemoglobin (Hb) concentration was measured by the cyanmethaemoglobin method. From the values obtained the haematimetric indicies (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)) were calculated5. Total WBC count was determined with haemocytometer using the WBC diluting fluid6. Bloods smears, made in duplicate, were fixed in alcohol and stained with Giemsa stain for differential WBC counts. The results were statistically

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evaluated using Student’s t test.

Table 1 shows the erythrocyte values of the male and female Nigerian local turkeys. The values of RBC, PCV, Hb, MCHC, MCV and MCH values were similar (P>0.05) in both sexes.

Table 2 reveals that the effect of sex on the leucocyte values of the Nigerian local turkeys. The total WBC, lymphocyte, heterophils, eosinophil, monocyte and basophil counts were similar (P>0.05) in the male and female but the percentage value of monocyte was higher (P<0.05) in the female than in the male.

The RBC, PCV, Hb, MCH, MCV and MCHC values were similar in the male and female Nigerian local turkey in the present study. Similar observations were made in the Nigerian local duck⁷, Pigeon and Peafowl⁸, captive waterfowl⁹, the black

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>MALE (n=10)</th>
<th>FEMALE (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X 10⁹/µl)</td>
<td>2.72 ± 0.56</td>
<td>3.06 ± 0.40</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>48.20 ± 6.43</td>
<td>44.66 ± 6.70</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.25 ± 1.07</td>
<td>11.58 ± 1.72</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>47.35 ± 12.93</td>
<td>38.40 ± 7.70</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>25.71 ± 2.83</td>
<td>26.39 ± 4.97</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>187.06 ± 58.02</td>
<td>148.28 ± 31.96</td>
</tr>
</tbody>
</table>

All parameters are not significantly different (P>0.05).

<table>
<thead>
<tr>
<th>TOTAL WBC (X10⁹/L)</th>
<th>3.28±1.88</th>
<th>2.73±1.46</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYMPHOCYTE (X10⁹/L)</td>
<td>1.48±0.79</td>
<td>1.19±0.73</td>
</tr>
<tr>
<td>(46.30±13.58)*</td>
<td>(44.33±9.58)*</td>
<td></td>
</tr>
<tr>
<td>HETEROFL (X10⁹/L)</td>
<td>1.79±1.25</td>
<td>1.52±0.81</td>
</tr>
<tr>
<td>(53.30±13.78)*</td>
<td>(56.00±10.63)*</td>
<td></td>
</tr>
<tr>
<td>EOSINOPHIL (X10⁹/L)</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>(0.30±0.48)*</td>
<td>(0.22±0.44)*</td>
<td></td>
</tr>
<tr>
<td>MONOCYTE (X10⁹/L)</td>
<td>0.00±0.00</td>
<td>0.02±0.22</td>
</tr>
<tr>
<td>(0.00±0.00)*</td>
<td>(0.44±0.53)*</td>
<td></td>
</tr>
<tr>
<td>BASOPHIL (X10⁹/L)</td>
<td>0.01±0.02</td>
<td>0.002±0.01</td>
</tr>
<tr>
<td>(0.10±0.32)*</td>
<td>(0.11±0.33)*</td>
<td></td>
</tr>
</tbody>
</table>

a Value expressed as a percentage of Total WBC count
Value significantly different from male turkey at *P<0.05
duck\(^{10}\) and the wood duck\(^{11}\) in which no sex differences were observed in their erythrocyte values. However, the finding in the present study disagrees with the observations in the White England turkey\(^1\), domestic fowl\(^{12}\), Japanese quail\(^{13}\); geese\(^{14}\), guinea fowl\(^{15}\) and the white Pekin duck\(^{16}\) in which higher erythrocyte values were reported in the male than in the female. The male sex hormone, testosterone, was implicated to be responsible for the higher erythrocyte values in the male\(^{17}\). It seems testosterone plays an insignificant role in the erythropoiesis of the Nigerian local turkey.

The mean value of RBC, PCV of the Nigerian local turkey of the present study were higher than that of the White England turkey\(^2\), Nigerian local duck\(^{18}\) and local chicken and ostrich\(^{19}\). The MCV was similarly higher in the Nigerian local turkey of the present study than that of the White England turkey\(^2\), local chicken and ostrich\(^{19}\). It seems the higher RBC, PCV and MCV values of the Nigerian local turkey in the present study than that of the White England turkey\(^2\) may be due to the higher plane of diet given to the Nigerian turkey. However, the higher RBC, PCV and MCV values of the Nigerian local turkey in the present study than the Nigerian duck, local chicken and ostrich may be due to species differences, which could be due to variations in the genetic make-up.

Although the Hb value of the Nigerian local turkey was similar to that of the White England turkey\(^2\), it was however lower than those of the Nigerian local duck\(^{18}\), local chicken and ostrich\(^{19}\). The Hb values obtained previously for the White England turkey were also lower than those reported for the Nigerian local duck, local chicken and ostrich. Olayemi\(^3\) reported a value of 10.27g/dl for White England turkey while Makinde and Fatunmbi\(^1\) obtained values of 12.95g/dl in males and 11.65g/dl in females of the same bird. The lower Hb value of the Nigerian local turkey in the present study may therefore be due to species variation because the Hb value of the White England turkey was also lower.

The total WBC, lymphocyte, heterophil, eosinophil, monocyte and basophil counts were similar in the male and female Nigerian local turkey of the present study. There were also no sexual differences in the total and differential leucocyte counts of the Nigerian duck\(^7\) and Japanese quail\(^{13}\). The total WBC count of the Nigerian local turkey in the present study was significantly lower than those reported for the Nigerian duck\(^{20}\), local chicken and ostrich\(^{19}\).

References

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COMBATING AVIAN INFLUENZA IN DEVELOPING COUNTRIES: THE ROLE OF APPLIED RESEARCH IN IMPROVE RURAL LIVELIHOODS

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International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya.

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

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- des brèves communications.
- analyse des articles proposés par le Rédacteur.
- des éditoriaux.
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Les manuscrits doivent respecter les conditions suivantes: Chaque ligne du texte doit être numérotée.
Le titre doit être concis et ne pas dépasser plus de 15 mots, il est suivi du (des) nom(s) de l'auteur (ou des auteurs) et des établissements où le travail a été effectué, ainsi que de l'adresse pour les correspondances si elle n'est pas la même.
Le résumé ne doit pas dépasser 200 mots. Son texte bref et concis comprendra les principaux résultats et la (les) conclusion(s) de l'étude.
L'introduction expose le but de la recherche.
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Le nom du pays, l'année faisant l'objet du rapport, puis le nom du service ou de l'organisation, le numéro de la première page.
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