ISSN 0378-9721

INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES
BUREAU INTERAFRICAIN DES RESSOURCES ANIMALES
P.O. Box 30786, NAIROBI, KENYA

BULLETIN

March
Mars
2008 Volume 56 No. 1

AFRICAN UNION
UNION AFRICAINE

IBAR PUBLICATION
PUBLICATION DE L'IBAR

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA
BULLETIN DE LA SANTE ET DE LA PRODUCTION ANIMALES EN AFRIQUE

A Quarterly Journal of Original Articles and Abstracts in English and French

Annual subscription: US$ 50.00

ISSN 0378-9721

Revue trimestrielle contenant des articles originaux et des résumés d'études en anglais et en français

Abonnement pour un an: 50 $EU

BULLETIN OF ANIMAL HEALTH AND PRODUCTION IN AFRICA

VOL. 56 NO. 1 CONTENTS MARCH, 2008

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Bull. Anim. Hlth. Prod. Afr., (2007), 56, 5-

PREVENTION AND CONTROL OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN AFRICA

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PREVENTION ET LUTTE CONTRE LA GRIPPE AVIAIRE HAUTEMENT PATHOGENE EN AFRIQUE

Résumé

La Grippe aviaire hautement pathogène (GAHP) est une maladie zoonotique transfrontalière. Son apparition dans un pays représente un obstacle majeur aux activités d'élevage et constitue un grave danger pour la santé publique au niveau régional et mondial.

Depuis février 2006, la GAHP a infecté onze pays africains (le Nigeria, l'Egypte, le Niger, le Cameroun, le Burkina Faso, la Côte d'Ivoire, le Soudan, Djibouti, le Ghana, le Togo et le Bénin). Le reste de l'Afrique est menacé à cause de la persistance du virus en Egypte et au Nigeria.

Le Bureau Interafricain des Ressources Animales de l'Union Africaine (UA/BIRA) qui a pour mandat de lutter contre les maladies animales au niveau continental, en coopération avec d'autres institutions internationales telles que les partenaires de la Plate-forme ALIVE, a coordonné et aidé le continent dans la gestion des risques de GAHP à travers: l'évaluation des lacunes à combler et des besoins du continent ; et l'élaboration de Plans d'urgence nationaux (PUN) et de Plans d'action. Les PUN et les Plans d'action n'ont, toutefois, pas complètement réussi à gérer la maladie et les leçons tirées de la crise de GAHP devraient donc tracer la voie pour ce qui est des besoins immédiats et à long terme.

Summary

Highly Pathogenic Avian Influenza (HPAI) is a zoonotic trans-boundary disease. Its occurrence in a country constitutes a major constraint to profitable livestock operations and poses a high public health risk at regional and global levels.

Since February 2006, HPAI has infected eleven African countries (Nigeria, Egypt, Niger, Cameroon, Burkina Faso, Côte d'Ivoire, Sudan, Djibouti, Ghana, Togo and Benin). The rest of Africa remains at high risk due to the persistence of the virus in Egypt and Nigeria.

African Union/Interafrican Bureau for Animal Resources (AU/IBAR), which has the continental mandate for control of animal diseases, together with other international institutions, including the African Livestock (ALIVE) Platform partners, have coordinated and assisted the continent in managing the risks posed by HPAI, such as assessment of continental gaps and needs, preparation of national Emergency Plans (EP) and subsequent Action Plans. The EP & Action Plans have not been entirely successful in managing the disease and lessons learnt from the HPAI crisis should therefore shape the way forward both in terms of sustainable immediate and longer term needs.

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Introduction

In Africa, the Highly Pathogenic Avian Influenza (HPAI) virus (H5N1 strain) was first confirmed in February 2006 in Nigeria. Ten more countries followed with reports still being made to date. Sixteen human fatalities out of 40 cases have been recorded since (15/38 Egypt, 1/1 Nigeria and 0/1 Djibouti). Due to production systems, socio-cultural and trade practices (legal or illegal, traditional marketing systems, live bird markets, mixing species etc), Africa is especially vulnerable to HPAI, causing income losses directly through mortality and reduced productivity and indirectly through restrictions on regional and international trade in livestock and their products.

There are no quantified estimates of the costs of the disease. However to date more than 32 million poultry died from the disease or were culled in Africa.

Issues, threats and challenges of responding to HPAI crisis in Africa

Prevention Phase

Following the conclusions of the Joint AU-IBAR/FAO symposium on HPAI prevention and control in Africa (Nairobi, September 14-16, 2005), the 7th Conference of the Ministers responsible for animal resources (Kigali, October 31 - November 2, 2005) asked African countries to strengthen their surveillance capacities in regard to HPAI. From then onwards, the countries undertook the drafting of HPAI emergency preparedness plans.

In disease free countries, the main preventive measures taken are partial or total ban of poultry and poultry product importation from infected countries, the setting-up of national inter-ministerial multidisciplinary technical committees to coordinate HPAI prevention and control programs. The Committee supervised the drafting of HPAI national integrated emergency preparedness and contingency plans.

Most countries have drafted plans, many of which lack sufficient data, coherence and basis for implementation, since they were conceived as emergency action plans. In addition, few countries allocated the necessary financial resources for prevention activities. Finally, with the exception of Algeria, Guinea, Morocco, and Senegal, no HPAI emergency preparedness plans have been subjected to simulation exercise to identify their weaknesses. In this respect, most plans need adjustments and updating.

Containment and Eradication phases

The spread and characteristics of HPAI outbreaks in Africa

The origin of the first confirmed outbreak of HPAI, H5N1 in Nigeria is still unknown. Trade in poultry and poultry products (legal and illegal) and other movements of live birds seem to have played a major role in the introduction and spread of the disease on the continent. In sub-Saharan Africa, subsequent outbreaks followed a similar pathway (from Nigeria to Niger and Burkina Faso; from Burkina Faso to Côte d'Ivoire), with the exception of a case in Northern Cameroon where HPAI virus was isolated from a wild duck. The involvement of wild birds in the transmission of HPAI could not be proved in spite of extensive and still ongoing laboratory investigations.

After a succession of HPAI, H5N1 outbreaks from February until May 2006, an

inter-epizootic silence was noticed before the resurgence of new cases in Côte d'Ivoire (on November 6, 2006 in turkey hens imported from a nearby country), in Nigeria (February, 2007), in Egypt (February 25, 2007) and most recently in Ghana, Togo and Benin. The spread and impact of HPAI, H5N1 were most pronounced in the industrial and semi-industrial sectors of production in Egypt (more than 30 million dead or culled chickens), Sudan (more than 1million chickens dead or culled) and Nigeria (more than 700,000 dead or culled chickens). In other affected countries, outbreaks remained localized and affected chicken as well as other domestic birds like ducks (Niger, Cameroon, Côte d'Ivoire), guinea fowls (Burkina Faso) and turkeys (Côte d'Ivoire). Some cases were also confirmed in vultures in Burkina Faso, Cameroon and Côte d'Ivoire.

HPAI control strategies implemented and their results

Infected countries implemented emergency measures, notably: Intensive community awareness and epidemiological surveillance for early detection and early reporting of new cases; outbreak management through the institution of control measures including quarantine of infected or suspected zones, culling and destruction of infected or suspected poultry and disinfections of premises and materials; controlled movement and ban on trade in poultry; management of the socio-economic impact at poultry owner level (compensation); vaccination; minimizing risks and care of human flu cases of avian origin; advocacy for improved bio-security at farm level and on live bird markets; and updating of zoo sanitary rules and protocols.

Some of the constraints, threats and challenges encountered in implementing the measures include: limited intervention capacities of affected countries (in terms of human, technical, financial and material resources); inexperience in the management of such zoonotic crises, including absence of crisis communication centers; weak epidemio-surveillance networks; organizational problems of coordination between various services; absence of direct chain of command within veterinary services; disorderly use of vaccination as a tool in the control strategy; inability to implement basic bio-security measures at farm level and in live poultry markets; failure by many countries to timely and/or adequately revise rules pertaining to zoo sanitary measures; and poor management of HPAI socio-economic impacts leading to delays in releasing of funds for compensation. Others relate to cultural practices in poultry keeping; poor infrastructure; and inadequate government support.

With the exception of the prevailing situation in Nigeria and in Egypt, an explosion of HPAI outbreaks throughout the African continent as predicted by many international experts did not materialize in spite of the limited operational capacities of national Veterinary Services. However, the situation still remains worrisome especially with the continuing confirmation of new human cases in Egypt and more recently in Nigeria and Ghana (May 2007). There is a high probability that an infection, starting from these outbreaks, may spin out of control, propagate and spread throughout the entire continent and the Arabian Peninsula. Thus, the H5N1 virus is likely to

cause heavy economic losses at regional and national levels and more importantly, at the level of the rural poor African family.

The proximity of poultry and people in the villages increases the risk of human infection. The diversity of the ecosystems infected leads to the fear that the disease may become endemic, meaning that it may quietly circulate only to re-emerge given favourable climatic conditions or in an encounter with a highly susceptible animal population.

Current involvement and contributions from various institutions and organization

The Interafrican Bureau for Animal Resources (IBAR) is a specialized technical office in the Department of Rural Economy and Agriculture (DREA) of the African Union Commission (AUC). It is responsible for spearheading and coordinating all programmes pertaining to animal resource development on the continent. It does this through six inter-related mandates including the control of major trans-boundary animal diseases (TADs); capacity-building and harmonization of livestock-related policies and legal framework in all 52 member states of the African Union.

IBAR has played the lead role in coordinating all preventive and intervention measures directed at HPAI on the continent in partnership with FAO, OIE and other leading institutions and organisations. IBAR is a member of and currently chairs the executive committee of the ALive Platform, which is responsible for the preparation of a "needs assessment document" and Continental Action Plan.

The following institutions and organizations in partnership with IBAR are currently at the forefront of combating the HPAI crisis in Africa:

1. The African Development Bank (AfDB) - emergency support amounting to US$6.5 million which has been channelled through IBAR to 13 countries, the infected countries and some of those at high risk of infection.
2. Through the ALive partnership, an assessment of financial needs and gaps was carried out and the results of the study presented at the Fourth International Conference on Avian Influenza in Bamako/Mali, December 6-8, 2006. An updated (2007) version is also available and presented at International Ministerial Conference on Avian and Pandemic influenza, New Delhi, 4-6 December 2007.
3. Based on the continental strategy, IBAR formulated a "tailor-made" project "Support Programme to Integrated National Action Plans (SPINAP)" for EC funding, to coordinate the implementation of national action plans and different donor contributions at continental level. This program budgeted at Euro 21.5 million was approved in April 2007 and is currently in its inception phase. Out of the total amount, 18.1 millions Euros will be channelled through IBAR to 47 eligible ACP countries.
4. IBAR and ILRI with financial support from GTZ organized training

courses on rapid detection of avian influenza virus for veterinary and medical laboratory staff from African countries (39 countries). More than 85 laboratory staffs have been trained and have improved capacity for their respective national and regional laboratories.

5. Three FAO regional and several national, Technical Cooperation Projects (TCP) have been undertaken, to support national emergency preparedness to face possible introduction of the disease.
6. The Peoples' Republic of China availed for six months technical assistant teams of five experts for experience sharing on prevention and control of HPAI; two were stationed in Eastern Africa and three in Bamako/Mali, covering West and Central Africa.
7. The Ministry of Agriculture of the Royal Thai Government contributed to the training of African experts (two from each infected country – one each from the fields of veterinary and human health services) with the financial support of French Cooperation and UNDP.

Success stories

1. The epidemio-surveillance network set up by the PACE programme (2000–2007) in 30 sub-Saharan countries has been easily adapted to take on board HPAI. It also has the potential to improve and expand to other sub-Saharan countries.
2. National governments responded positively in developing and implementing emergency preparedness plans. These were mainly prepared in response to emergency outbreaks and therefore need review and updating in order to meet current challenges especially in their implementation.
3. Coordination of donors and international technical Institutions activities through the ALive Partnership.
4. Establishment of Regional Animal Health Centres (RAHC) set up by IBAR,OIE and FAO to better coordinate their activities in Africa, including the constitution of a virtual vaccine Bank to meet the needs from infected countries. The centres for West Africa in Bamako and East/ Central Africa in Nairobi are already operational.

The Way forward

1 There is an urgent need to update and integrate the various National Emergency Preparedness Plan (EPP) and Action Plans (AP) into sub-regional plans for harmonized implementation within and between regions of Africa; with prioritization of affected countries to contain and eradicate the disease and thereby preventing further spread.
2. The respective AU/IBAR-FAO-OIE Regional Animal Health Centres (RAHCs) should coordinate combined regional strategies in partnership with the relevant REC, including the reinforcement of the

sub-regional epidemio-surveillance networks and the set-up of regional reference laboratories.

3. To address emerging and re-emerging zoonotic diseases on the continent in a sustainable manner, new mechanisms should now be set up and formalized/ institutionalized, based on the achievements and experience of the ad -hoc integrated groups (inter-ministerial committees to plan, coordinate and supervise at national level the activities of integrated surveillance teams and emergency response groups) put in place during the initial emergency phase of the HPAI campaign.
4. Existing national and sub-regional institutions need to be strengthened without necessarily creating new ad-hoc ones to address the new challenges.
5. To improve the operational mechanisms of the RAHCs in such a way that there will be harmonization of jointly prepared programmes (between the respective three institutions) coordinated with a common schedule of implementation. This should be with close involvement of the Regional Economic Communities (RECs) in the planning and supervision of the RAHCs, and will help to avoid gaps and duplications at the same time, ensure the same speed of implementation for complementary components of the same program.
6. To set up in each RAHC a sustainable regional epidemiological network (that will be sustainable at the end of external funding) based on a network of national experts to be trained and upgraded periodically. This should be backstopped by establishing a regional reference laboratory in each REC.
7. IBAR in collaboration with PANVAC and OIE will continue to pursue its objective of establishing a continental virtual vaccine bank

Conclusions

The outbreaks of HPAI in Africa have provided an opportunity to assess the response capacities and major sanitary crisis management systems of African veterinary services. Constraints and weaknesses were evident in national EPP and action plans. Nevertheless, the incursion of the disease was not followed by rapid spread within infected countries where poultry densities were low, but the risk for spread to the entire African continent (the potential threat) is higher than ever before. The ad-hoc measures taken at the time proved to be relatively sufficient and the more useful aspects of these measures such as the consultative mechanisms created in the initial emergency phase need to be adopted and maintained in the future.

There are still several grey areas in the epidemiology of HPAI/ H5N1 in Africa (e.g. clinical disease among domestic ducks rather than chickens in Niger and in Cameroon, the role of vultures in Cameroon and Burkina Faso, and the sparrow hawk in Côte d'Ivoire), and on the other hand, the absence of an epizootic as previously predicted, including the immediate die-out

of the disease in Southern Sudan. Through enhancement of RAHC capacity and the harmonisation of activities between the three institutions some of these areas will be better addressed in the future.

For the moment, there appears to be success in the containment of HPAI in Africa as it is still confined to the affected 11 countries. The lessons learnt so far and how to harness the lessons into regional integrated emergency preparedness and action plans will determine the progress of Africa's future control strategies against HPAI.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 13-18

MODULAR PROCESS RISK MODELS FOR BETTER MANAGEMENT OF *CRYPTOSPORIDIUM PARVUM* – AN EMERGING ZOONOTIC PATHOGEN

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MODELES DE RISQUE DE PROCESSUS MODULAIRE EN VUE D'UNE MEILLEURE GESTION DE *CRYPTOSPORIDIUM PARVUM* – UN PATHOGENE ZOONOTIQUE EMERGENT

Résumé

Cryptosporidium parvum est une maladie émergente particulièrement préoccupante à cause de sa capacité à contaminer le lait, les cultures vivrières et les sources d'eau. Il peut aussi persister dans l'environnement et survivre même après l'épuration de l'eau, dont la javéllisation. *C. parvum* peut provoquer une grave maladie, parfois mortelle chez les enfants et les personnes immunodéficitaires. La compréhension de l'épidémiologie de la maladie avance vite ; cependant, des incertitudes demeurent surtout en ce qui concerne l'importance relative de la transmission zoonotique et anthroponotique. Une étude est conduite à Dagoretti, Nairobi, pour déterminer la contribution des produits laitiers urbains au problème de la maladie ; et quelques résultats préliminaires relatifs à l'analyse des risques sont présentés dans cet article. L'enquête est menée auprès d'une population d'environ 1000 éleveurs et de leurs voisins, à l'aide de données recueillies sur les vaches, les fermes, les ménages et le milieu. Le présent article évoque en détail la conception, l'analyse et l'interprétation des diagrammes de processus, afin d'indiquer les liens spatiaux et temporels entre le pathogène, l'hôte-réservoir, le milieu et la population. Nous montrons comment par l'incorporation et la modélisation des informations sur la survie du pathogène, les diagrammes de processus permettent d'avoir une meilleure compréhension du risque de la maladie et un meilleur choix des interventions pour gérer avec succès le *Cryptosporidium parvum* en milieu urbain au Kenya.

Summary

Cryptosporidium parvum is an emerging disease which is of particular concern because of its ability to contaminate milk, food-crops and water sources; to persist in the environment; to survive water treatment including chlorination; and to cause serious and sometimes fatal disease in children and the immuno-compromised. Understanding of disease epidemiology is rapidly advancing but uncertainties remain especially concerning the relative importance of zoonotic and anthroponotic transmission. A study is being carried out in Dagoretti in Nairobi to evaluate the contribution of urban dairying to disease burden and some preliminary results related to the risk analysis component are reported in this paper. The study is being carried in a population of around 1,000 urban cattle-keepers and their neighbours, with data collected at cow, farm, household and environmental levels. This paper details the construction, analysis and interpretation of process diagrams in order to show spatial and temporal linkages between pathogen, reservoir host, environment and populations. We show how by incorporating and modelling pathogen survival information, process diagrams allow greater understanding of the risk of disease and better targeting of interventions to successfully manage *Cryptosporidium parvum* in urban Kenya.

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Introduction

Cryptosporidium parvum infection is an important disease with serious consequences in the young, the old and the immuno-compromised. The epidemiology is complex; it was previously believed that human disease was zoonotic, transmitted from cattle, water-borne, and affecting mainly those with HIV/AIDS[1]. However, more recently, molecular genetics is has revealed a more complex picture of different strains and sub-types of *C. parvum* with different host preferences, some anthroponotic[2], as well as the role of atypical *Cryptosporidium* in human disease and the importance of the disease in young children[3]. On-going studies by the University of Nairobi and other partners have confirmed the presence of *Cryptosporidium spp.* in cattle in the Nairobi milk-shed. This paper outlines a risk-based approach to the disease and describes in detail the use of modelling to assess exposure to the hazard of *C. parvum*. We start by describing risk assessment as applied to *C. parvum* and situating exposure assessment as one of the four components of risk assessment and also contributing to risk management and risk communication. We then show how modelling can be used in exposure assessment. Finally we discuss the potential of this approach and draw conclusions.

Materials and Methods

There are many different approaches to assessing risk and this paper follows the framework recommended by the *Codex Alimentarius,* the international standards setting organisation for food safety[4]. This defines quantitative microbial risk analysis as a tool to manage food and water-borne pathogens and to elaborate standards of international trade. It comprises risk assessment, risk communication and risk management. Risk assessment, the first stage of risk analysis, comprises four steps each of which answers important questions concerning the hazard:

1. Hazard identification: what agents causing ill effects are present in food or water?
2. Hazard characterisation: what are the adverse health effects, in terms of a dose-response relationship?
3. Exposure assessment: what is the likely intake of the hazard?
4. Risk characterisation: what are the adverse effects of the hazard and their likelihood of occurrence in a given population as well as the attendant uncertainties?

In exposure assessment the transmission of the hazard involved is modelled through the food pathway, a chain of processes from a source (e.g. the farm) to the moment of consumption. This transmission model gives insight into the prevalence and the concentration of the hazard along the consecutive processes of the food pathway, taking into account the variability and uncertainty attending this transmission; information that is essential in managing risk. The food pathway is split up into constituent steps and the input-output relation is described for each step; this may be done by observation (direct surveillance of the production process), laboratory experimentation (simulation in the laboratory of the practical situation concerning certain specific steps) or mathematical modelling. A mathematical model takes the description of a real system (for instance, the processing of milk), including the conceptual understanding of

how the process works and any associated data, and translates this into a system of mathematical relations. The mathematical model generated in this way allows the process being described to be clearly and transparently illustrated and more importantly, to be investigated and changed at the mathematical level to see what effects might occur at the large-scale level.

The Modular Process Risk Model (MPRM) is a new general framework for carrying out Quantitative Microbial Risk Assessment[5]. Based on the Process Risk Model[6], it can provide estimates of the amount of pathogen in the food product at each step of the farm-to-fork chain and in the final product. Essentially, each of the steps or key activities at the various intermediary stages is assigned at least one of six basic processes. These are fundamental events that may affect the transmission of any microbial hazard in any food. There are two *'microbial'* basic processes, growth and inactivation, and four 'food handling' processes, mixing and partitioning of the food matrix, removal of a part of the units, and cross contamination. The *'microbial'* processes strongly depend on the characteristics of the microbial hazard, as the effects of environmental conditions on growth and inactivation differ between species (and even between strains). Essentially, the effects of the 'food handling processes' are determined by the food handling process characteristics only, assuming a uniform distribution of micro-organisms over the food matrix.

We used data collected from ongoing studies on small-holder milk production in Kenya to construct a MPRM that can be used for modelling human exposure to *C. parvum*. The material and methods used for data collection are reported elsewhere[7,8]. In brief, questionnaire surveys were carried out at market (n=532) and household level (n=420). In addition samples of raw milk from 261 households were cultured in special media. Suspect *E. coli* columns were confirmed by serology and then assessed for verocytotoxin production.

Table 1: Basic processes associated with different steps in the pathway from milk production to consumption

Step	Description	Unit	Size	Basic process
Production	Cows are milked by hand into open bucket	Bucket	5 litres	Contamination
Production	Milk is transferred from bucket into larger container	Jerry can	10 litres	Mixing; some partitioning
Transport	Milk is transferred from farmers containers to transporters containers	Large jerry can	25 litres	Mixing Partitioning Removal
Distribution	Milk is sold to hawkers	Milk cans	5 litres	Partitioning Removal
Processing	Milk is boiled with tea and sugar	Domestic utensils	0.5 litres	Inactivation Cross contamination
Consumption	Tea is drunk	Domestic utensils	0.1 litres	Exposure
Consumption	Contaminated objects transfer infection	Domestic surfaces and utensils	6 metre square	Exposure

Results

Data from a series of questionnaires at producer trader and household level enabled us to construct a generic pathway to describe the typical movement of milk from 'farm to fork' or 'stable to table' in Nairobi. To each step on the pathway we assigned one or more of the 'basic processes' posited by the MPRM (Table 1). The pathway constructed reflects our finding that most milk consumed in Kenya is purchased in the informal sector. It is un-pasteurised and transported and sold in unsealed containers. Most milk is produced by small-holder farmers and the majority of intermediaries in the milk value chain also operate at a small-scale and in the informal sector.

Because most milk in Kenya is produced by small-scale farmers, mechanised milking is rarely used and farmers typically milk by hand into a metal bucket. This milk is then transferred into larger containers on farm or belonging to the transporters who carry milk from its peri-urban production sites to urban areas where it is distributed and sold. Most transporters are owner-operated or employees of small businesses and use general-purpose vehicles (bicycles, pick-up trucks) without special tanks for transporting liquids. Milk is typically transported in plastic containers over relatively short distances (10-60 km) and refrigeration is not used. In Kenya, like most developing countries, most milk is marketed informally and does not undergo pasteurization or other treatment; hence there is no processing step during production or distribution. On arriving in urban areas, milk is distributed, with most passing through small-scale traders or hawkers to the end user. Hawkers generally have a regular clientele of households which they supply on a daily basis making door to door deliveries. Households typically buy milk in small quantities, and because they lack facilities to store milk, they consume it shortly after purchase. Most households (96%) drink milk in tea. This is prepared by boiling milk along with water, tea and sugar and it is drunk while still hot.

After constructing the pathway model, we assigned one or more basic processes to each step. *C. parvum* oocysts are shed in cattle faeces, but because of the system of hand milking into an open bucket, the potential for contamination of milk with faecal material, and hence oocysts, is high. We observed that cattle are generally milked in the cattle shed (rather than a special dairy area) and hygiene of cow, shed and person milking is generally poor. Unlike many microbial pathogens, *C. parvum* does not multiply along the pathway. But although this removes one important way of increasing risk, oocysts are much more resistant than most bacteria, enabling them to survive for considerable periods and withstand water chlorination. However, boiling is effective at inactivating oocysts and exposure to light results in some decrease in numbers. Mixing, partitioning and cross-contamination may occur at farm, along the transportation chain or in the consumer household. But, because of the essentially small-scale nature of most production, transport and purchase, the opportunities for widespread dissemination of oocysts are limited.

The next step was to assign mathematical distributions to each of the basic processes (Table 2). This is based on the general principles of microbial dynamics and distribution, available in the literature.

Table 2: Mathematical distribution associated with basic processes of the Modular Process Risk Model for milk consumed in Nairobi

Basic process	Mathematical distribution
Inactivation by UV or heat	The decrease in number of oocysts per unit N is given by: **$\log(N_{out}) = \log(N_{in}) - g(.)$** with g(.) an increasing (!) inactivation function.
Partitioning of milk	The new prevalence after partitioning is given by: **$P^* = P \times (1-(1-1/x)^N)$** Where P* is prevalence after partitioning, P is prevalence before, N is the number of oocysts in the original unit, and x is the number of smaller units. The distribution of oocysts over the x to x_0 smaller units (N_i) is: (**Binomial ($N-x+x_0$, $1/(x-x_0)$) + 1) cells per unit**.
Mixing of milk	The new prevalence after random mixing is given by: **$P^* = 1-(1-P)^N$** The distribution of oocysts after mixing is given by: **$N' = \Sigma_n N_i$.** Where N^i is the number of oocysts in each of the n smaller units.
Removal by detection of faecal contaminated milk	**P*=P.f (1-P+Pf) with 0?f ?1** Where f is measure of removal by detection of contaminated milk: if f=0 all contaminated units are removed, if f=1 none of them are removed.

Discussion

The Modular Process Risk Model provides a clear structure of complex food pathogen transmission pathways. It can help not only predict the level of exposure of consumers to pathogens but also indicate critical control points along the pathway where effective action can be taken to reduce risk. However, application of the model to situations where data are lacking is not straightforward. Information is needed on: oocysts prevalence and concentration and unit sizes; different processes and conditions along the pathway; inactivation of oocysts under different conditions; and variability and uncertainty in all of these. The more this data relies on extrapolation and assumption, the less likely it is to be useful in predicting reality. The model has some underlying assumptions (such as homogeneity of mixing and partitioning and a knowable inactivation function); these assumptions should be validated in the context in which the model will be used. The model is essentially deterministic (i.e. without random aspects) and may not well capture situations where stochastic (i.e. random or probabilistic) processes dominate.

This paper outlines the structure and function of a possible model. The next step would be to obtain the necessary input data and carry out a Monte Carlo simulation based on the model which would provide both a point estimate of exposure and a range of uncertainty[9]. This would then be integrated with a) information on the *C. parvum* dose necessary to produce infection and disease in sub-groups with different susceptibilities, b) the proportion of these different sub-groups

among the population consuming milk c) the ill-effects anticipated from infection and disease in different sub-groups. The final result would be an estimate of both the harm associated with exposure to *C. parvum* in milk and the likelihood of the harm occurring, along with information on how harm could most effectively be reduced. This information helps stakeholders decide the appropriate level of protection and what can best be done to reduce risk to this level.

Using the example of an emerging disease present in Nairobi dairies, our case-study shows how modelling could be used to assess human exposure to zoonotic hazard. Traditional food safety studies look at hazards at unconnected points in the food chain; using a pathway approach; we show how shifting the focus from hazard to risk allows a more comprehensive, systematic and useful assessment of food safety. Risk analysis is an exciting new methodology with great potential for improving public health and the agriculture-based economy in Africa. It has not yet been applied to the informal markets where the majority of poor people buy and sell livestock products but this case-study shows how the methodology could be used to practically improve food safety.

Acknowledgement

This study is being implemented by Dept of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya and supported by ILRI, Kenya and Cornell University, USA. It is funded by IDRC, Canada. Support from USAID is also acknowledged

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Bull. Anim. Hlth. Prod. Afr., (2008), 55, 19-24

A PRELIMINARY INVESTIGATION ON SUSPECTED PLANT POISONING IN THE FACULTY OF VETERINARY MEDICINE FARM, KABETE, KENYA

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ENQUETE PRELIMINAIRE SUR LA SUSPICION D'INTOXICATION PAR DES PLANTES A LA FERME DE LA FACULTE DE MEDECINE VETERINAIRE DE KABETE AU KENYA

Résumé

Une enquête sur la végétation a été conduite dans un enclos de pâturage de l'Université de Nairobi à la Ferme de la Faculté de Médecine vétérinaire pour voir les espèces de plante qui s'y trouvent et vérifier si certaines sont vénéneuses. L'enquête a été menée après que l'on ait signalé des cas de jeunes bovins laitiers avec des signes de toxicité aiguë peu de temps après avoir pâturé dans l'enclos. Une liste complète de la flore sur le site a été dressée et on en a recueilli trente deux espèces de plantes. Cinq des trente deux plantes étaient suspectées d'être la cause probable de l'intoxication parce qu'elles ont déjà été signalées auparavant comme ayant des effets toxiques. Ces plantes étaient botaniquement identifiées comme suit : *Ranunculus multifidus* Forsk, *Cassia didymobotrya* Fres, *Ricinus communis* L., *Datura stramonium* L. et *Momordica foetida* Schum. Il a été conclu que certaines de ces plantes peuvent être à l'origine de l'intoxication, et qu'il faudrait entreprendre des études supplémentaires sur leur taux de toxicité et leur répartition dans la ferme.

Summary

An investigation on vegetation was carried out in one grazing paddock of the University of Nairobi, Veterinary Farm to establish the species of plants present and whether some were known to be poisonous. This investigation was carried out after reported cases of young dairy cattle manifesting signs of acute toxicity shortly after being grazed in this paddock. A check-list of the floristic composition of the site was exhaustively recorded and thirty two plant species were collected from the site. Five out of thirty two plants were suspected to be the possible cause of this poisoning as they have been reported before to have toxic effects. These were botanically identified as *Ranunculus multifidus* Forsk, *Cassia didymobotrya* Fres, *Ricinus communis* L., *Datura stramonium* L. and *Momordica foetida* Schum. It is concluded that some of these plants may be responsible for the poisoning and further studies on their level of toxicity and distribution in the farm needs to be determined.

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Introduction

The animal losses caused by poisonous plants in Eastern Africa is considerable[1]. Many plants contain chemicals or accumulate chemicals which are poisonous to livestock. The key theory on why plants produce toxic compounds is as an evolution in order to deter animals from eating them[2]. The toxicity of a plant is influenced by the plant factors such as stages of growth, wilting or drying or animal factors like species, age and also starvation[3].

Poisoning of livestock can occur when animals are grazing or when fed in zero grazing units. It could be accidental ingestion of material eaten along with grass or willful consumption of poisonous plant especially during drought, when poisonous plants remain green all the year round[1]. Newly introduced animals are also threatened by poisonous plants, as it takes time for the animals to differentiate them in the pastures.

Due to the fast action of some poisons, chances of saving affected animals are slight. Therefore, a good knowledge of these plants is important in order to prevent poisoning of animals. Good pasture management is also vital especially by keeping off undesirable plants in the pastures.

Important poisonous plants can be classified according to the toxic substances they contain. These toxic agents are; *alkaloids , glycosides* (cyanogenic, saponins and mustard oil), nitrates, molybdenum, copper, selenium, ergot and other mycotoxins and coumarone[3]. Other plants may also induce photosensitization and affect milk and its production. The blue water algae is also poisonous to livestock. There are also plants which cause external or internal injury to livestock which may include the gastrointestinal tract and the skin.

Identification of plants in the pastures and areas accessible by animals and their potential to poison is the most effective method of preventing plant poisoning. For the plants that have been accidentally ingested, accurate diagnosis of the potential poisonous plant is critical to treatment[2]. All the plants parts should be sampled to assist in the identification and quality of the samples.

Toxicological studies of some common poisonous plants in Eastern Africa using livestock and laboratory animals have been documented. Some of the poisonous plants which have been studied in East Africa include; *Cestrum aurantiacum*[4], *Burttia prunoides*[5], *Phytolacca dodecandra*[1], *Maesa lanceolata, Crotolaria mauensis Bak, Cassia floribunda Cav.*[1], *Cassia didymobotrya Fres,*[1,6], *Gnidia latifolia Meisn*[7], *Paddie volkensii* and *Scutia myrtina (Burm.F.)Kurz*[9].

The objective of this preliminary study was to identify the poisonous plant species in this farm and later carry out toxicological studies to determine their level of toxicity.

Materials and methods

A general vegetative observation was carried out in one of the paddocks of the University of Nairobi, Faculty of Veterinary Medicine Farm suspected to have poisonous plants. The site vegetation was described in terms of floristic composition *(botanical)* and physiognomic classification. Thirty two plants from the paddock were collected by using a plant press of standard size, secateurs, a knife and a panga. The plant parts collected included; the flowers, seeds or fruiting body, parts of the stem, leaves and root system for the smaller herbs. Each

Table 1. Identified poisonous plants and their poisonous effect

Plant name	English name	Poisonous effects
Ranunculus multifudus Forsk	Butter cup	Vomiting, convulsions, depression in humans
Cassia didymobotrya Fres	Wild Senna	Depression, anorexia, diarrhoea, death
Ricinus communis L.	Castor bean	Dysentry, sanguinous evacuation of bowels, death
Datura stramonium L.	Thorn apple	Paralysis, suspension of secretions, rapid hear beat, death
Momordica foetida Schum.		Death in humans

Table 2. Vegetation description of identified poisonous plants

COVER	TREES AND SHRUBS
6	*Ricinus communis* L.
2	*Ranunculus multifudus* Forsk
3	*Cassia didymobotrya* Fres
5	*Datura stramonium* L.
4	*Momordica foetida* Schum.

Key:

Isolated- 1	Cover values: 4-10% - 4
Scarce- 1	10-25% - 5
Very scattered- 2	5- 50% - 6
Scattered- 3	50-100% -7

plant was described in the field in terms of growth habit, longevity, habitat and location, vegetative characteristic and general morphological features as viewed in the field. The plants were submitted to the herbarium of the Department of Land Resource Management and Agriculture Technology(LARMAT) for botanical identification. The plants were botanically identified and their literature sourced from available textbooks in the herbarium. The plants parts were preserved for future reference. Those that have been reported to be toxic from the literature were identified and the toxic parts, active principles and toxic effects were recorded.

The cover percentage of the trees, shrubs, and grasses were estimated by

ocular methods. The frequency and cover abundance status of each plant was established using step-point method. After recording the floristic composition and using step-point method, a modified Braun-Blanquet cover abundance scale for the percentage cover per plant species was used (Kuchler, 1967)[8].

Results

Thirty two plant species were identified in the area of study. Out of these plants, five were identified as being poisonous (Table1). Table 1. Identified poisonous plants and their poisonous effect

Through the floristic and physiognomic classification, it was established that the five plants were covering a relatively large area of the paddock as seen in the vegetation description (Table 2).

Discussion

Ranunculus multifudus Forsk (Butter cup) is a perennial herb with hairy stems and widely distributed in East Africa[10]. This plant has also medicinal value and its powdered form is used as remedy for coughing among the Zulus, vomiting, diarrhoea and sore throat among the Xhosa. The bruised leaf is used externally for cancer, scabies and mumphs[11].

Cassia didymobotrya Fres, (Wild senna) is a branched shrub, 1.5 – 6m tall and widely distributed in Kenya bushland of moderate rainfall and along the riverines in semi arid areas. The leaf of *Cassia didymobotrya Fres.* is very poisonous. After ingestion, there is intense inflammation of the intestinal canal[11]. The foliage is allegedly poisonous to cattle. Doses of 75gm have been proved to be poisonous to sheep resulting in death with symptoms of gastroenteritis. Anthraquinones have been isolated from the leaves and root. Studies done have reported that the plant causes depression, loss of appetite, diarrhoea and death in cattle[1].

The decoction of the root is used as a strong purgative in Tanzania. The Maasai use the leave as a purgative and also as antimalarial . The plant is also used as a fish poison and a bee repellant[11] .

Ricinus communis L. (Castor bean) is a shrub or small tree that grows up to 6m tall and is widely distributed throughout East Africa. The seeds pressed cake and to a lesser extent the foliage are extremely poisonous when eaten. The poisonous principle is the ricin , a powerful poison that is easily destroyed by the digestive fermentation and heat[10,12]. It also contains a toxic crystalline nitrogenous body called recinine. The seeds are toxic to humans resulting in very acute dysenteries, with sanguinous evacuation of the bowel and death. All parts of the plant are poisonous to human, horse, donkey, cattle, sheep, pig and poultry[11]. The root, leaf and bark has been reported to yield hydrocyanic acid.

The extract of the leaf and also of the plant has been found to inhibit to some extent the growth of *Mycobacterium* tuberculosis. The symptoms of toxicity in cattle include; paralysis, suspension of secretion and rapid heart action resulting in death due to *asphyxia*. Others are general weakness, collapse and bloody diarrhoea[11]. In horses, there is incoordination, profuse swelling, titanic spasms and diarrhoea.

An infusion of the leaf is used as a remedy for stomach-ache among the Zulu and as an enema when administered orally. Others medicinal uses are for cure of rheumatism, abdominal pains, diarrhoea and

skin diseases in human.

Datura stramonium L. (Thorn apple) has stout stems and a foul smell. The toxic principle is in all parts of the plant and particularly the seeds and the leaves. The alkaloids *hyoscyamine, atropine* and *scopolamine* are the poisoning principle. Poisoning in livestock may occur when the animals ingest the plant together with fresh grass or hay. Fresh plant is unpleasantly scented and therefore avoided by stock. Clinical symptoms in cattle include; paralysis, suspension of secretions, rapid heart beat and death due to asphyxia[11].

Various medicinal uses of *Datura stramonium* have been reported. These includes treatment of skin inflammation, rheumatism, gout, wounds, haemorrhoids, dandruff, diarrhoea, toothache and against maggot in humans[11] .

Momordica foetida Schum. is widely distributed in wetter regions of East Africa[12]. The toxic parts are the tuberous root which has been documented to be toxic in humans. Most of the animals avoid it due to the foetid smell. Medicinal uses include; treatment of boils, as a sedative, for irritable stomach, earache remedy, against roundworms, abortaficient and ecbolic. The fruit pulp has been documented to be poisonous to weevils, moths, ants and therefore used as a repellant. The root which contains alkaloids is used as an emetic and purgative, remedy of gout, fever, hemorrhage, epilepsy, difficult child birth and viper–bite[11].

There exists poisonous plants in this farm and there is a need to do more research on the other plants and establish whether some others plants apart from the five are also toxic. These plants are abundant and cover quite a big area of the paddock. There is a need to quantify the level of toxicity of these five plants and others that may be toxic. Such studies have been done by using livestock and laboratory animals and documented by various authors[4,5,6,7,9] .

Control measures aimed at eradicating some of these plants from this farm or preventing animals to access this paddock should be put into place. Investigation in other paddocks in this farm should be carried to check whether there are other toxic plants.

Acknowledgements

We thank the members of Technical staff of both the Veterinary farm and the Department of Land Resource Management and Agriculture Technology for their assistance while carrying out this study.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 25-37

SEROPREVALENCE STUDY OF BOVINE BRUCELLOSIS IN EXTENSIVE MANAGEMENT SYSTEM IN SELECTED SITES OF JIMMA ZONE, WESTERN ETHIOPIA

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ETUDE DE LA SEROPREVALENCE DE LA BRUCELLOSE BOVINE DANS UN SYSTEME D'ELEVAGE EXTENSIF DANS DES SITES SELECTIONNES DE LA REGION DE JIMMA A L'OUEST DE L'ETHIOPIE

Résumé

La prévalence de la brucellose bovine a été évaluée dans une étude transversale dans la région de Jimma à l'ouest de l'Ethiopie à l'aide des tests RBT et CFT pendant la période octobre 2003 - avril 2004. Les animaux utilisés pour l'étude étaient composés de 1305 bovins de race locale en élevage extensif dans cinq districts de la région. Une prévalence globale par animal et une prévalence chez le troupeau de 0,77 % et 2,9 % respectivement ont été enregistrées dans cinq districts. De plus fortes prévalences ont été observées chez le troupeau plus grand (P< 0,001) ; les groupes d'animaux plus âgés étaient plus affectés que ceux plus jeunes (P< 0,05) dans un système d'élevage extensif. Aucune réaction n'a été enregistrée chez les mâles. Une séropositivité de 8 % a été constatée chez les animaux qui avaient avorté auparavant. L'étude montre que la prévalence de la brucellose dans la région est faible ; toutefois, il y a un risque probable de propagation de la maladie chez la population de bétail non affectée puisqu' aucune mesure de protection n'a été prise par les éleveurs dans les régions où ils auraient dû le faire. Comme la prévalence est inférieure à 2 %, le test et l'abattage sont recommandés contre versement d'une compensation aux éleveurs.

Mots-clés: Ethiopie, brucellose bovine, séroprévalence.

Summary

The prevalence of bovine brucellosis was measured in cross sectional study in Jimma zone, Western Ethiopia using Rose Bengal Plate Test (RBT) and CFT from October 2003 to April 2004. The study animals consisted of 1305 local breed found in extensive system in five districts of in the zone. The overall individual animal prevalence and herd prevalence of 0.77% and 2.9% were recorded in five districts, respectively. Higher prevalences were observed in larger herd size (P< 0.001); older age groups were affected more than younger animals (P<0.05) in the extensive management system. No reactions were observed in male. Seropositivities of 8% were observed in animals with previous history of abortion. The study demonstrates that the prevalence of brucellosis in the area is low; however, there is probable risk of spread of the disease in the unaffected cattle population since there are no precaution measures taken in the areas that should have been practiced by farmers. Since the prevalence is below 2%, test and slaughter with compensation payment to farmers is recommendable.

Key words: Ethiopia, Bovine brucellosis, Seroprevalence.

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Introduction

Bovine brucellosis is a contagious disease caused by *Brucella abortus*, a Gram-negative, facultative, intracellular *coccobacilli bacterium*[1]. The disease in cattle is characterized by abortion in late pregnancy and subsequent high rate of infertility in females and varying degree of sterility in the male, leading to a significant economic loss[2]. It affects approximately 5% of the livestock population worldwide and continues to increase in distribution[3]. It is thus one of the most widespread and economically important diseases confronting food production in tropical and subtropical regions of the world[4,5]. The direct loss of meat (as a result of abortion, infertility, and weight loss) in infected herds of cattle was estimated to be 15% while that of milk production was 20% and this can reach up to 40-50% in early abortion[6]. Its occurrence is increasing in developing countries in an even aggravating epidemiological situation. This depends on the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socio-economic situation of the animal holders[5]. In Africa, the disease is considered to be one of the most significant disease problems facing the veterinary profession. The prevalence is often high because of close human-animal contacts and food consumption customs[7].

Brucellosis is known to be an endemic and growing problem in domestic livestock (local and cross breed) herds in Ethiopia. The presence of bovine brucellosis in Ethiopia is well established[8,9,10,11]. Bekele *et al.*[12] found 4.2% positive reactors from zebu cattle. The presence of the disease in different ranches has been reported[13]. Higher prevalence was reported in large herds than smaller herds[14].

Although much work has been done and reports are available, there is no information on the status of bovine brucellosis in extensive management system in Western parts of the country. Therefore, the objectives of this study were to determine the prevalence of brucellosis in the cattle population in extensive management system, and to identify risk factors and quantify their degree of association with brucellosis in cattle in the study area.

Materials and methods

Study area

This study was carried out in Jimma zone, which is located about 335 km southwest of Addis Ababa, the capital of Ethiopia, at 7^0 13' and 8^0 56'N latitude and 35^0 52'and 37^0 37'E longitudes.

Five districts were selected by clustering sampling. These were Mana, Kersa, Seka-Chokorsa, Limu-kosa and Dedo. All Five districts were located at various km distances from Jimma town.

Study animals

Local zebu cattle in extensive management system in the study area were used as the study animals to determine the prevalence of the disease under investigation. All animals under individual ownership, or management were handled as one herd. The herd was categorized into four classes: cows, calves, bulls, and heifers.

Each class was defined as follows:-
Cow: - female cattle after first calving.
Calf: - cattle of both sexes above 6 months but under one year of age.

Heifer: - female above one year of age but has not calved.
Bulls: - un-castrated male cattle above one year of age.

Study design

A cross-sectional epidemiological study was conducted between October 2003 and April 2004 to estimate the overall prevalence, herd prevalence, and within herd prevalence of brucellosis. The prevalence was determined in respect to risk factors: age, sex, parity, herd size, district, and management system.

Sampling Procedure

Multi stage sampling procedure was followed to select study animals. The numbers of animals to be sampled from each district were determined by the proportion of the cattle population existing in each district. From each district, three peasant associations (PA's) were taken randomly. The number of animals to be sampled from each PA was also determined by the proportion of the livestock population within the PA. From each PA's, two villages were taken which made a total of 30 villages included in the sample. A total of 270 herds with an average herd size of 5 cattle (ranging from 2 to 10), were sampled randomly. All animals above six months of age kept for breeding purpose were sampled from the selected herds. The total sample size was determined using the formula for simple random sampling technique 15 and with an estimated *Brucella* infection rate of roughly 4%, a precision level of 1% and a 95% confidence interval.

Questionnaires

A questionnaire was designed to collect information on factors that are believed to influence the spread and prevalence of *Brucella* infection. These include herd size and composition, management activities, age and sex of the animal, purchase source and stock replacement, lactation status, parity, pregnancy status, and history of previous abortion.

Collecting and handling of blood

Approximately 10 ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tube and needle. Each sample from each animal was labeled by using codes describing the specific animal and herd/ farm. The tubes were set tilted on a table overnight at a room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged to obtain clear serum. The obtained serum was stored at -20^{0}C until they were tested by both Rose Bengal Plate Test and Complement Fixation Test.

Serological Tests

Rose Bengal Plate Test

The RBT antigen was obtained from INSTITUT POURQUIER 325, rue de la galéra 34097 MONTPELLIER CEDEX 5, France. The method prescribed by 16 BgVV Service Laboratory was followed to undertake RBT. The test was undertaken at Faculty of Veterinary Medicine, Department of Microbiology, Debre zeit. Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing; since active materials straight from the refrigerator react poorly. The interpretation was performed as follows:

0 = no agglutination
+ = barely perceptible
++ = fine agglutination, some clearing

+++ = coarse clumping, definite clearing

Those samples identified with no agglutination were recorded as negative those with +, ++, +++, ++++ were recorded as positive.

Complement Fixation Test (CFT)

In the CFT, all reagents were evaluated by titration. The preparation of sheep red blood cells (SRBC), the methods of CFT test, and preparation of reagents were according to the protocol of BGVV Service Laboratory. The CFT test was conducted at the National Veterinary Institute, Department of Immunology, Debre Zeit.

The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read. The working dilution of amboceptor is 4 times MHD[16]. The test was read by recording minimum hemolytic dose of complement (MHD) which was represented by the first well showing complete hemolysis. The next well contains the full hemolytic dose (FHD). The working dilution of complement was then computed: complement dilution= 2FHD/ initial dilution of complement. The last wells with 50 % sedimentation was read and recorded. This was regarded as the right corner value. In this case, the corner value was 1: 25 dilution and was used throughout the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was two units. The interpretation was performed as follows:

Sera with at least 50 % fixation of the complement at a dilution of 1: 10 were taken as positive. A hemolytic reaction of 50 % or less at a dilution of 1: 5 was considered as the minimum sero positive threshold[17].

Data analysis

The total prevalence was calculated based on the RBT+/CFT positive results; by dividing the number of RBT+/CFT positive animals by the total number of animals tested. The within herd prevalence was calculated by dividing the number of RBT+/CFT reactors within a herd by the number of serum samples tested in that herd[15].

The Fisher's exact test was applied to test the existence of associations between seropositivity and risk factors such as age, sex, parity, herd size, district and management system in cattle. In addition, logarithms regression analysis was used to calculate odds ratio (OR) to measure the degree of association between risk factors and the disease in cattle using computer program STATA 7[18].

Results

Questionnaire surveys

Questionnaires were administered to 40 extensive farm owners. The questionnaires addressed a number of aspects of management and husbandry practices, housing conditions, educational

Table 1: Summary of proportional distribution of dairy cattle by reproductive status and abortion Prevalence

Breeding status	Extensive farm
Proportion of lactating cows	316/671 (47.1%)
Proportion of pregnant cows	192/671 (28.6%)
History of abortion	77/393 (19.6%)

n= number of farms

status of the farmers and breeding status of the study animals. History of abortion was taken during blood collection for all cows. About 19.6% of the cows in the system were reported to have had previous abortions (Table 1).

Farm characteristics

Of the 40 farmers studied, 90% of them in the area had only primary education while 10% had formal education above 8th grade (Table 2). It was also found out that all the farmers were dependent on natural mating using bulls. The practices of provision of separate parturition pens, separation of cows during parturition and cleaning and disinfection of contaminated areas was done by few farmers. In addition, most of the farmers in the study area were not disposing afterbirth and abortion materials properly. Half of the farmers were dependent on external sources for replacement stock and culling was practiced by more than half of the farmers in the area.

Table 2: Summary of proportional distribution of educational status of farmers, farm management and husbandry practices in the study areas

	Extensive farms
	(n=40)
Educational status of farmers < 8th grade	
	36 (90%)
> 8th grade	4 (10%)
Total	40 (100%)
Mating practice	
Use of natural mating	40/40 (100%)
Use of AI	-
Use of AI +natural mating	-
Awareness on brucellosis	
Yes	2/40 (5%)
No	38/40 (95%)
Presence of parturition pens	2/40 (5%)
Separation of cows during parturition	5/40 (12.5%)
Cleaning and disinfection of premises	5/40 (12.5%)
Proper disposal of after birth	
Yes	5/40 (12.5%)
No	35/40 (87.5%)
Stock replacement strategy	
Out side source	20/40 (50%)
Own farm	5/40 (12.5%)
Out side source and own farm	15/40 (37.5%)

Table 3: Results of RBT and CFT for brucellosis by study districts

District*	N	RBT	CFT*
		Number (%) positive	Number (%) positive
Kersa	449	3 (0.67%)	0 (%)
Seka chokorsa	309	2 (0.65%)	0 (%)
Limu Kosa	320	9 (2.8%)	8 (2.5%)
Dedo	172	2 (1.16%)	2 (1.16%)
Mana	55	0 (0%)	0 (0%)
Total	**1305**	**16(1.2 %)**	**10 (0.77 %)**

N= number of animals tested, *= Fisher's exact test, P<0.001

Seroprevalence of brucellosis

Of 1305 sera tested using RBT, 16 (1.2 %) animals reacted positively to brucellosis. These reactors were further retested using CFT and 10 (0.77%) animals were confirmed to be seropositive for brucellosis (Table 3). The highest prevalence was found in Limu-Kosa district (2.5%). All male tested were negative for *Brucella* antibody.

Seroprevalence at herd level

The overall herd prevalence, based on RBT+/CFT, was 2.96%. The prevalence were established only in two districts: viz, Limu kosa (12.5%) and Dedo (2.8%) (Table 4). The herd seroprevalence in farms that kept more than five animals were found to be 14.5% (8/55) while it was nil in farms keeping less than five animals. This difference was

statistically significant (p<0.001). The within herd prevalence recorded was ranging from 0% to 33.3%. One or two reactors were recorded in the seropositive herds.

Seroprevalence at individual animal level

The individual animal seropositivity was established by using the RBT positive sera with CFT positive serial serological test results. The overall individual seropositivity in the study area was established at 0.77%. Prevalences were compared between study sites; the prevalence for Dedo, and Limu-kosa were 1.16%, and 2.5% respectively. No reactor animal was detected from Kersa, Mana, and Seka-Chokorsa districts. The difference between districts was statistically significant (P<0.05).

A logistic regression analysis revealed that positive reactors were significantly higher (p<0.05) in older age category than younger ages (Table 5). The seroprevalence was 1.1 % in animals between 3-6 years and 1.6% in animals above 6 years.

In addition, herd size had significant effect on the prevalence of brucellosis in individual animals (p< 0.001). All infected animals were in herds with more than five animals and no infection was established in herds keeping less than five animals (Table 5).

The results of multivariate logistic regression indicated that age (p< 0.05) and herd size (p< 0.01) affected significantly of bovine brucellosis. This result was not different from that of the univariate analyses (Table 6).

Table 5: Seroprevalence of brucellosis according to risk factors (sex, age, herd size) in study areas

		CFT			
Risk factors	**N**	**Number (%) of positive animals**	**CI (95%)**	**p-value**	**OR**
Sex					
Male	273	-	0-1.3	0.1	-
Female	1032	0.97% (10)	0.5-1.8		
Age					
0.5-<3	489	-	0-0.8	0.017**	3 (1.2-7.7)
>3-6	566	1.1% (6)	0.4-2.3		
>6	249	1.6% (4)	0.4-4.1		
Herd size					
1-5	948	0 (0%)	0-0.4	0.000***	6.7 (2.1-9.4)
>5	357	10 (2.8%)	1.4-5		
Total	**1305**	**10 (0.77 %)**			

N= number of animals tested, CI= confidence interval, OR= odds ratio

Table 6: Multivariate logistic regression estimates for risk factors

Risk factors	**CI**	**Odds ratio**	**P-value**
Sex	0.08-6.86	0.75	0.800
Age	1.23-7.40	3	0.016
Herd size	3.98-242.1	31	0.001

Reproductive status and brucellosis infection rates

Odds ratio (OR) was calculated to measure the likely association that could exist between reproductive status and brucellosis. Significant association was found between brucellosis and occurrence of previous abortion ($p< 0.001$). However, factors including pregnancy status, lactation status and parity were not significantly associated with the prevalence of brucellosis. Although no significant association ($P>0.05$) was observed between *Brucella* seropositivity and parity, majority of the positive animals were in the cows with 2nd parity compared to those in their 1st parity (Table 7).

Discussion

All of the respondents (100 %) in this study were using bulls for service for the reason that they had no other options to exercise. In addition, most of the respondents (87.5 %) did not bury afterbirth and aborted fetus, the aborted materials were left them on the ground or given to dogs. Most of the farmers were not also separating cows during parturition. These factors combined with the unawareness of most of the farmers on brucellosis and the poor hygiene practice by farmers could pose a great risk of spread of the disease to unaffected animals. The dependency of most of the farms on outside sources for stock replacement could be one possible way of introduction of the disease into unaffected herds. According to the observation made, out of 77 female animals with abortion history 6(8%) of them were detected positive for brucellosis. It has been reported that numerous infectious and non- infectious agents can cause fetal loss or abortion in cattle[19]. Besides, abortion rate in infected

Table 7: Association between reproductive status and brucellosis prevalence

		CFT			
Risk factors	**N**	Number (%) of positive animals	**CI (95%)**	**p-value**	**OR**
Pregnancy status					
Pregnant	192	1.6% (3)	0.4-1.9	0.8	1.2(0.4-3.4)
Non-pregnant	763	0.92%(7)	0.3-4.5		
Lactation status					
Lactating	316	1.3% (4)	0.4-3.2	0.6	1.4(0.4-4.8)
Non-lactating	639	0.94% (6)	0.3-2		
Parity					
0	318	0	0-1.2	0.06	2.9(1-8.9)
1	138	1.4% (2)	0.2-5		
2	496	1.6% (8)	07-3		
3	2	0	0-8.4		
Previous abortion					
Yes	77	8% (6)	2.95-16.4	0.000***	18.7 (5.2-67.8)
No	878	0.5% (4)	0.1-1.2		

N= number of animals tested, CI= confidence interval, OR= odds ratio

herds is dependent on many factors and varies according to the susceptibility of the pregnant females, management practices, the severity of the challenge, the period for which the herd has been infected and various environmental factors[6].

The overall seroprevalence of brucellosis in individual animals was 0.77 % (n=1305) in the zone in extensive management system. Different findings on serological prevalence of brucellosis have been reported for the last 24 years from different corners of Ethiopia. Most of the works reported so far were from southeastern and central highland and with few reports from northern parts of the country. Much higher seroprevalence were reported by Meyer[8] for cattle owned by the then Institute of Agricultural Research (IAR) (39%), Gebre-mariam[9] from four crossbred dairy farms around Addis Ababa (18.4%) by using RBT and CFT, Zewdu[11] in Sidamo region (15.8%), Molla[10] in different breeds of cattle (indigenous and crossbred) from Arsi (7.62%) by using SAT, Bekele *et al*.[12] from indigenous zebu cattle in central Ethiopia (4.2%), Sintaro[20] from Chaffa State Dairy Farm (Wollo) (22%), and also in ranch animals[13]. Closer values of prevalence were reported by Kebede[21] who found an overall prevalence of 1.8% from Eastern Amhara Region (ranging from 0.2% in the highlands to 3% in the lowlands). The prevalence in this study was in agreement with the findings of Tesfaye[22] who reported an overall prevalence of 0.69% in cattle in extensive and intensive managements in Tigray region and slightly higher than the finding of Yayeh[23] who reported an overall prevalence of 0.14 % in cattle in extensive and intensive managements in selected areas of North Gondar Zone, Ethiopia.

The difference in prevalence observed between the reports from different parts of Ethiopia and the present study may be due to differences in management and husbandry condition in the area. There can also be differences between the study areas regarding conditions that could facilitate the rate of transmission of the disease[19]. Higher prevalence were recorded specially for two districts in Limu-Kosa (2.5%), and Dedo (1.16%). This could be partly related to the existence of a ranch in the area (Limu-Kosa) which had been closed some time before due to health problems encountered in the ranch herd suspected to be brucellosis.

In the present study, males were non-reacting for both tests. This observation was in agreement with the work of Tesfaye[22] and Yayeh[23] who reported only female reactors. Sex has been one of the risk factors affecting susceptibility of cattle to *B. abortus* infection[19]. It is well known that female cattle are more susceptible to *Brucella* infection than males. The probable reason could be the preferential growth of Brucella organism in gravid uterus especially if it is pregnant than in testes[24].

A relatively higher seroprevalence observed, in this study, could be partly explained by the fact that contact between animals increases in communal grazing practices which was the predominant feeding system in the extensive type of management. In such circumstances, cattle of unknown disease status might mix and often grazed together and resulted in spreading and transmission of disease among herds. About 85 % of the farms in the study area shared the communal grazing system. It has also been indicated that free grazing which allows unrestricted contact between animals may have contribution to the spread of brucellosis in extensive management system[25]. In addition, the observation made by Kagumba

and Nandokha[26] indicates that the prevalence of brucellosis was higher in communally grazed large herds of cattle. The same observation made by Maiga[27] indicated that antibodies against *Brucella* were more prevalent among animals in rural concessionary than village herds of communal pen production systems. In general, the patchy distribution of brucellosis in the zone where some herds were free of the disease, while others had a high prevalence of brucellosis as observed in this study could be explained by the fact that some communal herds have been kept closed replacement animals from own herds whereas other cattle owners of the positive reactor herds may have purchased infected animals to replace their stock.

In this study, significantly higher seroprevalence was observed in older age category than younger age category. This observation is in agreement with that of Oloffs *et al*.[28] in which 52% of the seropositive cows were older than 6 years. Bekele *et al.*[13] and Tesfaye[22] also reported similar results that indicate a higher prevalence in animals above six years of age. It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of the individual animal[19]. Younger animals tend to be more resistant to infection and frequently clear infections, although latent infections do occur[29]. Sexually mature and pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex[19].

In this study, seroprevalences were higher in larger herd sizes. This observation is in agreement with the previous findings of other authors. Asfaw *et al.*[14] reported a higher prevalence of brucellosis in larger herds (9.1%) than smaller herds (3.3%). It was also indicated that herd size and animal density are directly related to prevalence of disease and difficulty in controlling infection in a population[29]. In addition, Hellmann *et al.*[30] indicated that herds of bigger size had higher seroprevalence of bovine brucellosis as compared to smaller herds. In larger herd sizes, the disease spreads by several modes of transfer, especially through contact with infected discharges from dam and its fetus. One possible explanation for the high prevalence of the disease in larger herds is that larger herd sizes are often maintained by the introduction replacement stock from outside sources and was proved to be a common practice by this study. It is also fact that the spread of the disease from one herd to another herd from one area to another is almost frequently due to the movement of an infected animal from an infected herd to a non-infected susceptible herd[19]. Abela[31] indicated that large herds and herds with small ruminants were most at risk to brucellosis infection. Thus, brucellosis should never be viewed as the disease of individual animals, but should be considered in the context of herd and also the animal population in the region.

An overall prevalence of 17.6% was recorded for the occurrence of previous abortion in the study area. Cattle with history of abortion were also found to be at higher odds of being sero positive compared to those without history of abortion. These all indicate the very close association between abortion and the prevalence of brucellosis. In highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease[19]. The finding regarding the prevalence of abortion in this study is higher than that of Tesfay[32] who reported a prevalence of 6.1% in Mekele dairy cattle and Yayeh[23] who also reported a prevalence

of 6.7% in North Gondar, Ethiopia.

Conclusion

The results of the present study revealed that bovine brucellosis is prevalent in the Jimma Zone of Oromia Region, Western Ethiopia, even though it is much less than the figures of previous reports from southeastern and central part of the country. The finding of positive serological reactors does not only suggest the presence of the disease in the cattle population in the areas, but also indicates the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and herds. Besides, the study also showed that age and herd size are important risk factors associated with the prevalence of the infection. This emphasizes impact of brucellosis and the need to control and prevent brucellosis in the study areas.

Acknowledgements

The authors thank the staff of Department of Immunology of the National Veterinary Institute and Microbiology Laboratory of Faculty of Veterinary Medicine of Addis Ababa University for their skilful technical assistance. We would like to express our deep appreciation to the Oromia Regional State and Addis Ababa University faculty of veterinary medicine for financial support during the Studies period. The farmers of the study area were also very grateful for their cooperation and provision of sampling animals.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 38-46

PRELIMINARY STUDY OF THE PREVALENCE OF HELMINTHS AND THEIR ASSOCIATED PATHOLOGICAL LESIONS IN FOUR FISH SPECIES FROM RIVER TANA

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ETUDE PRELIMINAIRE DE LA PREVALENCE DES HELMINTHES ET DE LEURS LESIONS PATHOLOGIQUES CHEZ QUATRE ESPECES DE POISSON DE LA RIVIERE TANA

Résumé

Une étude préliminaire a été menée entre janvier et mai 2006 pour enquêter sur la prévalence des helminthes et leurs lésions pathologiques chez quatre espèces de poisson. Au total, 43 poissons frais de la rivière Tana, vendus au marché de Gikomba, ont été achetés et autopsiés. Ces poissons étaient 15/43 (34,9%) de l'espèce *Oreochromis,* 11/43 (25,6%) *Clarias spp.,* 10/43 (23,2%) *Cyprinus carpio* et 7/43 (16,3%) *Barbus spp.* A l'autopsie, on a trouvé plusieurs vers *Contraceacum* au troisième stade larvaire dans la cavité abdominale, les muscles et derrière les branchies. Au total, 91% des poissons-chats *(Clarias spp.)* et 20% des tilapias *(Oreochromis spp.)* avaient une infestation « modérée à grave » de vers *Contraceacum*. L'intensité de l'infestation variait entre 1 et 593 helminthes par poisson, ce qui a provoqué une grave péritonite et l'adhérence sur les organes viscéraux. Au microscope, les helminthes causaient une forte infiltration des hétérophiles, des macrophages, des plasmocytes et la fibrose des organes.

Il y avait une atrophie de la pression de l'épithélium du canal biliaire chez un tilapia, qui a été causée par un ténia migrateur pleurocercoide, un protozoaire *Cryptocotyle* incrusté sur l'arc branchial, et des lésions parasitaires granulomateuses sur les parois des intestins.

Les autres lésions observées étaient des hémorragies, des ulcères et des blessures sur les nageoires, autour de la bouche et sur la peau. Les résultats de cette étude montrent que les poissons de la Rivière Tana sont infestés par les helminthes qui provoquent de graves lésions pathologiques chez les poissons affectés. Ces poissons peuvent aussi servir de réservoirs de ces parasites pour les poissons d'aquaculture.

Mots clés : Etude préliminaire, helminthes, prévalence, lésions pathologiques, Rivière Tana.

Summary

A preliminary study was undertaken between January and May 2006 to investigate the prevalence of helminths and their pathological lesions in four fish species. A total of 43 fresh fish from River Tana, sold at Gikomba market were bought and subjected to postmortem examination. These fish were 15/43 (34.9%) *Oreochromis* species, 11/43

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(25.6%) *Clarias spp.,* 10/43 (23.2%) *Cyprinus carpio* and 7/43 (16.3%) *Barbus spp.* On postmortem examination, numerous third stage *Contraceacum* larval worms were found in the abdominal cavity, muscles and behind the gills. A total of 91% of catfish (*Clarias spp.*) and 20 percent tilapia fish (*Oreochromis spp.*) had moderate to severe *Contraceacum* worm infestation. The intensity of infestation ranged from 1 to 593 helminths per fish which provoked severe peritonitis and adhesions on the visceral organs. On microscopy, the helminths caused severe infiltration of heterophils, macrophages, plasma cells and organ fibrosis.

There was pressure atrophy of bile duct epithelium in one tilapia caused by lodged migratory tapeworm pleurocercoid, a *Cryptocotyle* protozoan parasite embedded on the gill arch and parasitic granulomatous lesions on the wall of the intestines.

Other lesions observed were heamorrhages, ulcers and wounds on the fins, around the mouth and on the skin. Results of this study indicate that riverine fish from River Tana are infested with helminths, which cause severe pathological lesions in affected fish. These fish may also act as reservoirs of these parasites to the farmed fish.

Key words: Preliminary study, helminths, prevalence, pathological lesions, River Tana.

Introduction

The annual fish production in Kenya is approximately 200,000 tonnes that earn the fishermen over Kshs 7 billion (approximately US$90 million), and the country about Kshs 4 billion (approximately US$50 million) in foreign exchange, thus contributing to poverty alleviation in rural Kenya [1, 2].

Wild fish are often infested with many parasites that may cause diseases in them[3]. Monogenean *(Gyrodactylus spp.)* and digenean *(Haplorchis spp.)* trematodes have been reported in farmed fish in Mombasa, with heavy mortalities seen after handling[4] while *Clinostomum* and *Diplostomum spp.* have been observed in various farmed fish in Kirinyaga[3]. Other species of trematodes *Heterophyes Dactylogyrus, Cichlidogyrus* spp., adult *Gymnarchus niloticus, Sanguinicola* and cestodes such as *Bothriocephalidae, Caryophyllidae, Protoecephalidae* and *Amphilinidae spp.* have been reported from various fishes in Lake Victoria[5]. Nematodes *Contraceacum spp*. have been reported in Lakes Victoria, Baringo, Magadi, Nakuru and Naivasha[6, 7]. *Eustrongylides* have been found in East African lakes, including Lake Tanganyika and Victoria [6, 8, 9]. However, these worms have not yet been documented in riverine fish in Kenya. These parasites are reported to cause pathology in highly dense fish populations[10].

Research in fish diseases and parasites in the country is limited, and the little that there is, has mostly been undertaken in large water bodies (lakes and reservoirs). The objective of this study was thus to determine and document the occurrence of helminths and their associated pathological lesions in riverine wild fish in the River Tana basin.

Materials and methods

Study design and fish

Four purposive sampling visits were undertaken between January and May 2006

to Gikomba wholesale fish market to purchase fresh fish. A stratified random sampling, based on species, was used to select fish that were purchased from those sold at the market. For this purpose, the fish were grouped into four species (*Oreochromis, Clarias, Cyprinus* and *Barbus*). These were the commonest fish catches landed at various sites on the River Tana basin[11]. A total of 43 randomly selected wild fresh fish from the River Tana basin and sold at the market were studied. These were of various ages, sexes, sizes and comprised of 15/43 (34.9%) *Oreochromis* (tilapia) species, 11/43 (25.6%) *Clarias* (catfish) spps, 10/43 (23.3%) *Cyprinus carpio* (common carp), and 7/43 (16.3%) *Barbus* spps. They were then transported in coolboxes with ice to the laboratory, at the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi for postmortem examination and other tests.

Postmortem examination

In the laboratory, the fish were subjected to postmortem examination as described below and lesions recorded[12]. Each fish was laid on its side on a paper towel in a dissection tray to prevent slipping, and a midline incision made with a scalpel blade starting at the anterior end of the vent. A lateral incision from the vent side in an arc, on the abdominal wall of the fish upto the upper corner of the operculum, was made to expose the swim bladder and other organs. The body wall was then lifted and the organs observed grossly and in situ. A third incision connecting the two previous incisions (opercular incision) allowed the skin flap to be completely removed. The swim bladder, heart, gills, liver, gastrointestinal tract and spleen were separated and examined. Any lesions and parasites encountered were counted and recorded. A cut was made through the musculature to check for lesions and parasites[10, 11].

Worm identification

Worms collected from the fish body cavity were preserved in 70% ethyl alcohol, manually counted and recorded. Nematodes were identified using the labial parts, presence and shape of esophagus, ventricle and ceacum and the presence of cuticle and genital organs as given elsewhere[13, 14]. Tapeworms were identified using the size and shape of scolexes, number and modification (armed or unarmed) of suckers, the size of segments, cirrus pouch, number of testis and positioning, the shape and positioning of vitellaria, ovaries and uterus, and the position of uterine diverticula[14, 15].

Histopathological examination

Tissue samples were collected from various organs and preserved in 10% formalin, processed for histology by dehydration through various alcohol concentrations and cleared using amylacetate and xylene. They were then impregnated in molten wax, sectioned, mounted on slides and dewaxed. The dewaxed tissues were rehydrated using descending alcohol concentrations, stained with haematoxylin and eosin, dehydrated using ascending alcohol grades, cleared using two changes in xylene and mounted using DPX mountant[16]. They were then examined under the light microscope (X4, X10 and X40 magnification) for any cellular changes.

Data analysis

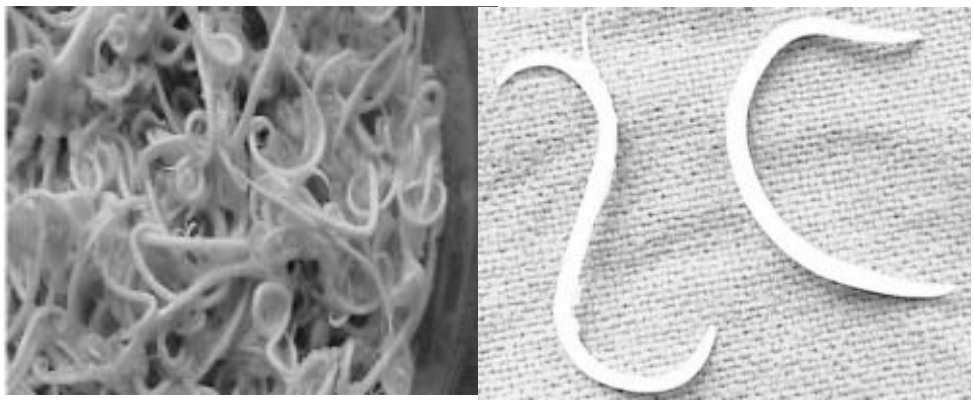
Data was entered in Ms excel, exported to Instat® Statistical package for descriptive statistics[17]. The prevalence was defined as the percent of the total number of fish species infected with helminths divided by the total number of that fish species examined[18].

Results

A total of 43 fish comprising of 11 catfish, 15 tilapia, 10 common carp and 7 *barbus* were examined for lesions and worms. The main worms observed in the fish were 3rd stage *Contraceacum larval* spp., tapeworms (adult and *pleurocercoids*) and trematodes *(Cryptocotyle)*. Parasitic granulomas in intestinal walls were also observed. Of the 43 fish examined, 10/11 (91%) catfish and 3/15 (20%) tilapia had 3rd stage *Contraceacum* larval worms in both abdominal and branchial region. Three out of ten (30%) catfishes and 1/3 (33.3%) tilapia had the *Contraceacum* larvae in the branchial region only, while 7/10 (70%) catfish and 2/3 (66.7%) tilapia had the *Contraceacum* larvae in the abdominal cavity encased in fibrinous material. Catfish had a mean worm count of 169 ± 163 (range 0-593), whereas, the tilapia had a mean of 1± 2 (range 0 – 5) (Fig. 1 and 2). Severe peritonitis characterized by blood stained ascitis, fibrin and adhesions around the larvae and organs in the peritoneal cavity was observed in 10/11 (91%) of the catfish and one tilapia fish.

One (1/11; 9.1%) catfish had in addition an adult *Proteocephalidae* tapeworm in the intestinal tract (Fig. 3). Grossly, the common carp *(Cyprinus carpio)* and *Barbus* spp. did not have worms although they had worm lesions. A total of 5/43 (2/43 tilapia, 2/43 barbus and 1/43 common carp) had swellings around the mouth, while 2 tilapia had grayish lesions on the intestines. Other lesions observed grossly were heamorrhages on the skin, base of fins and eyes in 25/43 (7/43 catfish, 7/15 tilapia, 5/7 common carp and 6/10 *Barbus*) fishes. Muscular heamorrhages in 15/43 (8/11 catfish, 3/15 tilapia, 3/10 *barbus* and 1/7 common carp) fishes and *exophthalmia* in one tilapia. Lesions due to handling were not considered.

Figure 1: Many Contraceacum larvae:



A - Enclosed in fibrin strands: (x2)

B - Two worm separated from the fibrin mesh: (x4)

Plates 1. *Contraceacum* larvae

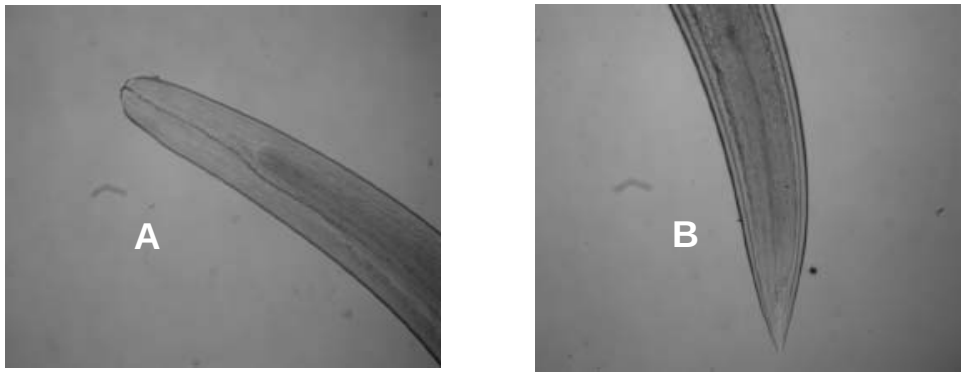


Figure 2: *Contraceacum* 3rd stage larvae showing
A- Head region and
B- Tail region (x10)

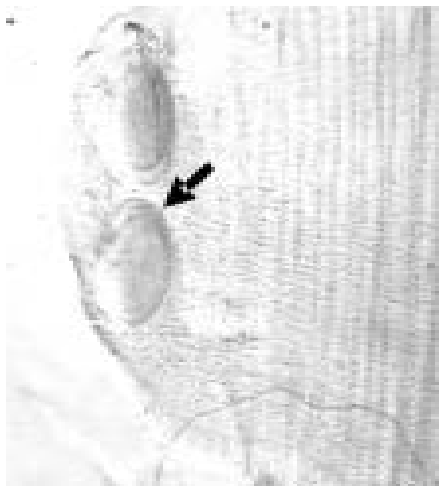


Figure 3: Head region of an adult *Proteocephalus* tapeworm with obvious suckers (arrows) (x40).

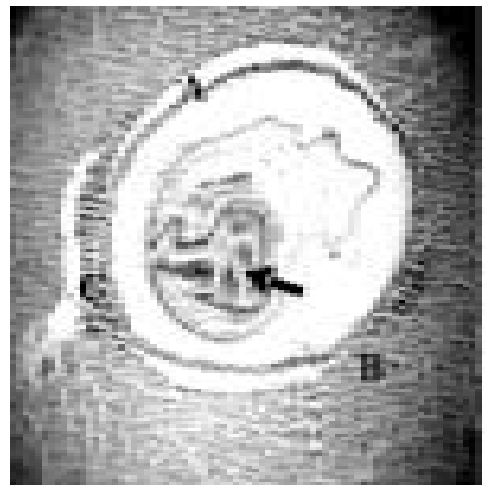


Figure 4: tapeworm pleurocercoid (arrow) in the bile duct of a tilapia causing pressure atrophy of bile necrosis of pancreatic acinar (x40).

On histological examination of the tissues, four (4/15 or 26.7%) more tilapia fish had parasites. One of these had a pleurocercoid tapeworm stage in the bile duct in the liver. This parasite caused bile duct dilatation, pressure atrophy of liver parenchyma and necrosis of adjacent pancreatic acinar cells (Fig. 4). Another one had a Cryptocotyle-like parasite in the gill arches that was surrounded by intensive inflammation (Fig. 5), while parasitic granulomas in the intestines were observed in the other two tilapia fish (Fig. 6).

All *Contraceacum* infested catfish and tilapia showed severe infiltration of mononuclear and polymorphonuclear cells as well as fibroblasts into the mesenteries, intestinal and stomach serosal surfaces. The fish with the swollen mouth areas showed inflammation of the bucco-pharyngeal region with pyogranulomatous lesions, characterized by heterophils, plasma and other mononuclear cells. One *barbus* species had a purulent peritonitis characterized by heterophils and mononuclear cells.

Discussion and Conclusions

These investigations have documented the occurrence of *Contraceacum* larvae, *Proteocephala* spp., Cryptocotyle-like parasites and other parasites in the riverine catfish, tilapia and other fishes. *Contraceacum* in fish in the River Tana basin in Kenya, were more prevalent in omnivorous catfish (91%). Prevalence in tilapia was 20% and the parasites were mainly found encysted in the peritoneal cavity and bucco-pharyngeal cavities unlike in the major lakes, where they were reported to occur in the pericardial cavity with very high prevalence rates[6,7]. Prevalence rates in catfish (91%) were higher than those reported in the lakes, such as Naivasha (85%), Baringo (70%), Magadi (30%) and George (30%)[6,7]. The high prevalence and mean load of *Contraceacum* larvae worms in catfish could be attributed to their feeding habits. Catfish are voracious omnivorous fish that feed on smaller fishes. They also feed on copepods (intermediate hosts of the *Contraceacum* worms) and aquatic plants, some of which may have had

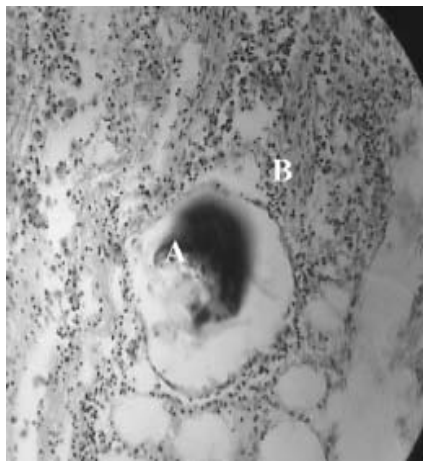


Figure 5: cryptocotyle-like parasite (A) on the gill arch and a ccompanying cellular inflammation (B). (x40)

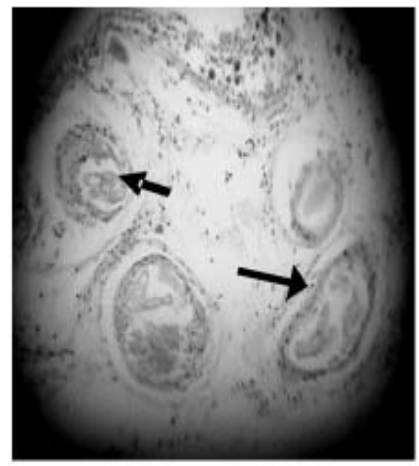


Figure 6: Numerous parasitic granulomas in the intestinal wall of tilapia fish (arrows) (x40)

free-living third stage *Contraceacum* larvae attached. The catfish can therefore accumulate *Contraceacum* larvae more than the other fish. The bigger catfish were found to have very high worm load of upto 590 worms unlike in other studies[19]. The smaller catfish had few worms compared to the larger ones, this may be attributed to the shorter worm accumulation period due to their young age and which may therefore explain the high standard deviation observed in the study. *Contraceacum* larvae caused severe pathological lesions in the peritoneal and bucco-pharyngeal cavities[20]. Large number of these worms in the peritoneal cavity may cause pressure atrophy of the visceral organs while those in the bucco-pharyngeal cavity caused swelling and irritation around the mouth region. The pyogranulomatous inflammation, proliferation of mucus goblet cells and impingement of the buccal cavity, could affect the feeding of the fish and thus growth.

Tapeworms are common in major waters of Africa and demonstrate a high degree of host specificity. *Ptychobothriidae* tapeworm (*Polyonchobothrium clarias*) was found in *Clarias gariepinus* from Lake Victoria[21] whereas a *Proteocephalid, Proteocephalus bivitellatus* was recorded in African cichlid fishes[22]. The catfish in this study was most probably a definitive host.

The pleurocercoid in tilapia bile duct caused damage to the bile ducts, hepato-pancreatic acinar and the liver cells. In severe infections, this may cause reduced production due to disturbed metabolism and even mortalities. Locations of pancreatic tissue in fish vary within and between species[22,23]. The tilapia in this study had hepatopancreas with the pancreatic acinar cells surrounding the portal veins in the liver. Inflammation was reported around *P. clarias* bothria attached to gut mucosa in infected Lake Victoria catfish, where bothridial penetration into gall bladder mucosa caused granulomas[21]. Other parasites were also observed to cause severe pyogranulomatous reaction in affected fish. This may affect their production. Heamorrhages on the skin, fins and eyes and severe purulent peritonitis could be due to parasitic infections or a combination of other causative agents.

Prevention of *Contraceacum* larval infection in riverine natural habitat by control of definitive hosts (piscivorous birds – Pelicans, Cormorants and Herons) or treatment of water or feed is impractical, but may be of value in farmed and aquaria fish, where use of mechanical restraint (mesh-nets, cages, and electrical wiring) and deterrents (shotguns and scarecrows) may keep them off. Treatment with helminthicides (levamisol, mebendazol or ivermectin) in feed or as a bath[24] combined with the control of intermediate copepod hosts by use of ectoparasiticides (Neguvon or Bromex) has been effective in farmed and aquaria fish. General tapeworm control (of adult and migrating pleurocercoid stages) by use of Di-n-butyl tin oxide and Dibutyl tin dilurate 25 and Yomesan[25, 26, 27] coupled with copepod control is recommended in farmed and aquaria fish.

Results from this study indicate that wild riverine fish from Tana River are infested with helminths, which cause severe pathological lesions in the affected fish. These fishes may also act as reservoirs of these parasites to the farmed fish.

Acknowledgements

The staff of the Department of Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University

of Nairobi are acknowledged for their technical assistance.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 47-54

EVALUATION OF ANTIBODY ELISA, COPROSCOPY AND SERUM ENZYME ANALYSIS IN THE DIAGNOSIS OF BOVINE FASCIOLOSIS

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EVALUATION DE L'ELISA ANTICORPS, COPROSCOPIE ET ANALYSE DE L'ENZYME DU SERUM DANS LE DIAGNOSTIC DE LA DISTOMIASE BOVINE

Résumé

Le titrage avec immunoadsorbant lié à une enzyme (ELISA), la sédimentation fécale et les tests de l'action de l'enzyme du sérum ont été faits sur des échantillons de fèces et de sérum recueillis de 134 bovins (55 positifs et 79 négatifs pour les lésions dues à la douve du foie) lors de l'inspection de viande en Ethiopie. Dans l'ensemble, 29% des animaux étaient positifs pour les œufs de *Fasciola,* alors que 75% des échantillons étaient positifs pour les anticorps de *Fasciola* et ce, à l'aide de l'ELISA. Tous les bovins qui étaient positifs à l'autopsie (54 des 134 bovins) étaient sérologiquement positifs pour les anticorps contre les antigènes F2 de *Fasciola hepatica.* Un test de concordance relativement fiable a été effectué entre l'analyse des fèces et l'ELISA (kappa=0,236), et entre l'ELISA et les conclusions de l'autopsie des lésions du foie dues à *Fasciola* (kappa=0,373). Une forte concordance a été observée entre la coproscopie et les lésions à l'autopsie (kappa =0,758). Les différences moyennes quant à la densité optique de l'ELISA parmi les divers types de lésions du foie étaient notables (P<0,05). La GGT (la gamma-glutamyl transférase) et LDH (la lacticodéshydrogénase) dont la concentration chez les animaux était de 56 (41,8%) et de 100 (74,6%) respectivement, révélaient des lésions du foie ou une action accrue du foie. Par rapport à l'autopsie, on a obtenu respectivement une concordance relativement fiable et une absence de concordance avec les tests GGT et LDH.

Mots-clés : Coproscopie, ELISA, *Fasciola,* enzymes du foie, ruminants.

Summary

Enzyme linked Immuno Sorbent Assay (ELISA), faecal sedimentation, serum enzyme activity tests were made on faecal and serum samples collected from 134 cattle (55 positive and 79 negative for liver fluke lesions) at meat inspection in Ethiopia. Over all, 29% of the animals were positive for *Fasciola* eggs, while 75% of the samples were positive for antibodies of *Fasciola* using ELISA. All cattle which were positive at postmortem examination (54 out of the 134 cattle) were serologically positive for antibodies against F2 antigens of *Fasciola hepatica.* A relatively fair test of agreement was found between the faecal examination and ELISA (kappa =0.236) and between ELISA and postmortem findings of fasciola-liver lesions (kappa =0.373). A substantial agreement was found between coproscopy and postmortem lesions (kappa =0.758). The mean differences in ELISA optical density among the different levels of liver lesions was significant (P<0.05). GGT (gamma-glutamyl transferase) and LDH (lactate

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dehydrogenase) concentration of animals 56 (41.8%) and 100 (74.6%) respectively indicated the liver lesions or increased activity of liver. Compared with postmortem examination, a relatively fair and absence of agreement was obtained respectively, with GGT and LDH tests.

Key words: Coproscopy, ELISA, *Fasciola,* Liver enzymes, Ruminants.

Introduction

The economic losses due to fasciolosis throughout the world are enormous and these losses are associated with mortality, morbidity, reduced growth rate, condemnation of fluky liver, increased susceptibility to secondary infections and expense due to control measures. Proper and early diagnosis of the disease is therefore important in prescribing effective drugs and assisting any control programme. Diagnosis of fasciolosis is based primarily on clinical signs, seasonal occurrence, history of fasciolosis in the area or the identification of snail habitats; postmortem examination and examination of faeces for fluke eggs. These may be supplemented by two other laboratory tests. The first is the estimation of plasma enzymes level released by damaged liver cells and the second is the detection of antibodies against components of flukes; the ELISA and passive haemoagglutination tests being the most reliable[1].

However, the different diagnostic techniques used today to confirm the presence of fasciolosis in ruminants have their own limitations. Coprological analysis is still commonly employed to diagnose bovine fasciolosis despite the overwhelming consensus that this method is not wholly reliable. Using this method, eggs are not detected until the latent period of infection when much of the liver damage has already occurred[2]. On the other hand, low infections cannot be detected and becoming the source of new infection[3]. The most direct and reliable technique for the diagnosis of fasciolosis is liver examination at slaughter or necropsy. But using this diagnostic technique it is impossible to detect fasciolosis in live animals. Serum activities of lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) may be used as markers of the different stages of fasciola infection in sheep, indicating the presence of necrosis of the liver cells caused by juvenile migrating flukes and bile duct lesions associated with mature helminths, respectively[4]. Different immunodiagnostic tests have been used in the early immune diagnosis of fasciolosis, but they have some disadvantages, such as cross-reactions with other trematodes, leading to false positive results[5]. This paper attempts to evaluate the effectiveness of different direct and indirect diagnostic techniques in the diagnosis and follow up of bovine fasciolosis in naturally infected animals at meat inspection.

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Materials and methods

Study animals

A total of 134 cattle slaughtered at Elfora export abattoir of Debre Zeit were used in this study. Out of these animals, 55 were positive for lesions of liver fluke at meat inspection and 79 were apparently free from liver fluke lesions and were considered as negative animals.

Coprological examination

Faecal samples were collected from the study animals directly from the rectum during antemortem examination. The collected samples were taken to the laboratory with tightly closed universal bottles and examined for *fasciola* eggs according to the method described by Antonia *et al* [6].

Postmortem examination

The liver of each animal was removed and examined for gross pathological lesions. The bile duct and the gall bladder were also incised. Identification of the fluke species was made by using technique described by Soulsby[7]. Categorization of the pathological lesions observed in affected livers was based on the approach by Ogunrinade and Ogunrinade[8] as follows; i) lightly affected; if small portion of the organ is affected and only one bile duct is enlarged on the visceral surface of the liver; ii) moderately affected; if half of the organ is affected and two or more bile ducts are enlarged and iii) severely affected; if most portion of the organ is involved and the liver is cirrhotic.

Blood collection

Blood samples were collected using sterile vacutainers from the jugular vein of the study animals. The serum separated for serological and enzymological activity and kept at -20^{0}C until tested.

ELISA for detection of Fasciola antibodies

The *fasciola* antigen coated ELISA diagnostic kit produced by the Institute Pourquier of France was used to detect fasciola antibodies against the specific "F2" antigens. It has been validated for bovine and ovine sera and on bovine milk. The sample was tested in duplicate on antigen pre-coated polystyrene microplates with a total of 45 samples per plate. Serum samples and control sera were diluted 1:20 in the ELISA buffer. Negative and positive sera were tested in duplicate. The optical density (OD) for each sample was read at 450nm. The corrected OD 450 was calculated for each serum by subtracting the OD value obtained from the uncoated well from the OD value of the coated well. The test result was considered reliable when the positive control serum has a minimum uncorrected OD value of 0.350 and a ratio between the corrected OD value of the positive control and uncorrected OD value of the negative control is greater than or equal to 3.5.

Enzyme activity determination

The enzyme level of LDH and GGT was colorimetrically estimated using photometer on serum samples as per the protocol indicated in the manufacturers' commercial kits. Reading was made at 0 and 340 nm.

Data analysis

Microsoft Access and Microsoft Excel were used for data management. Statistical software SPSS 11.5 versions for windows were used for data analysis. Level of rejection was set at $p>0.05$. Test agreement between the different tests was compared using kappa statistic where kappa value above 0.81 denotes perfect agreement, 0.61-0.80 substantial agreement, 0.41-0.60 moderate agreement, 0.21-0.40 fair agreement and 0-0.20 slight and 0 poor agreement and negative values indicate agreement less than is expected by chance[9].

Results

Coproscopy findings

Out of the 134 cattle examined for Fasciola eggs, 41(29.9%) were positive and 93(70.1%) were negative. Of the 54 animals

Table 1: Comparison of the different diagnostic methods in relation to the results of coproscopy and postmortem examination

Factor		ELISA		GGT		LDH		Liver Exam	
		-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Coproscopy examination	Negative (-ve)	33	60	64	29	22	71	79	14
	Positive (+ve)	1	40	15	26	10	31	1	40
	Kappa test	P=0.236		P=0.291		P=-0.011		P=0.758	
Liver examination	Negative (-ve)	34	46	58	22	17	63		
	Positive (+ve)	0	54	21	33	15	39		
	Kappa test	P=0.373		P=0.332		P=-0.066			

with known positive liver lesions, coproscopy was able to detect only 41 animals (75%) with a fair test of agreement (kappa=0.373)(Table 1).

Serological examination (ELISA)

The results of the indirect ELISA method unveiled that 75% of the examined serum samples were positive for antibodies against *Fasciola* specific 'F2' antigens at various levels of infection. Out of these positive samples, 23%, 48% and 1.5% of the samples were suggestive of having low, medium and severe level of *Fasciola* infestation, respectively. All the serum samples collected from positive animals at meat inspection were positive to antibodies of *Fasciola* indicating the 100% sensitivity of the test. While some animals, which were negative for liver lesions were also found to harbour antibodies of *Fasciola*. The mean differences in OD readings of ELISA (Table 2) among the different levels of liver lesions was significant (P<0.05). However, the least significance difference (LSD) test indicated the absence of difference between lightly infected and apparently normal livers (P>0.05).

Table 2: Summary of univariate statistical test on ELISA and enzyme values according to the intensity of liver lesions

Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
LDH 2	Contrast	124104.367	1	124104.367	.510	.476
	Error	31381918.653	134	243270.687		
GGT 2	Contrast	39993.010	1	39993.010	3.497	.064
	Error	1475487.537	134	11437.888		
ELISA c	Contrast	74311.924	1	74311.924	35.604	.000
	Error	269246.119	134	2087.179		

Serum enzyme analysis

Out of the 134 cattle serum samples, 56(41.8%) animals had an increase in the level of concentration of GGT while 100 (74.6%) of the animals had an increase above the normal value of serum LDH activity suggesting the possible presence of liver lesions or increased activity of liver. However, the mean differences in both LDH and GGT readings (Table 2) among the different levels of liver lesions is not significant (P<0.05).

Intensity of liver lesions

Out of the 54 livers of cattle known to harbour lesions of fasciolosis, 31(23%) were lightly infected, 20(15%) moderately infected and 3 (2.2%) were severely damaged. The summary of the results of the univariate statistical test of different diagnostic test employed on 134 cattle based on the severity of liver lesions.

Discussion

A prevalence rate of 30% was found in slaughtered cattle at Elfora Abattoir, Debre Zeit which is in the range of 11.5% to 87% as reported by various authors for Ethiopian cattle[10,11,12,13]. Most reports of bovine fasciolosis in Ethiopia are based on either routine faecal examination or at meat inspection. However, both these tests do not reflect the actual prevalence of the disease as they are subjected to various limitations. Accurate detection of *Fasciola* infections in cattle is difficult due to the poor sensitivity of methods to estimate the number of *Fasciola* eggs in the voluminous faeces of large ruminants. Though liver lesions suggestive of bovine faciolosis are found, there are at least a quarter of those lesions (25%) with no detectable *Fasciola* eggs in the faeces further substantiating the limitation of coproscopy. The present finding further substantiates the above statement as the test of agreement between coproscopy and postmortem liver lesions is only moderate (P=0.758). The recent study[14] made on slaughtered cattle in Switzerland unveiled the diagnostic sensitivity of coproscopy to be 69.0% (57.3-79.7%) supports the present findings. In contrast to coproscopy results, the probability of identifying infected cattle by a positive ELISA, detecting antibodies to 'F2' antigen of *Fasciola* spp was 100% making the overall sero-prevalence of bovine fascioloisis in the area to be 75%, more than double that for coproscopy. This finding is substantiated by the fair test of agreement obtained between coproscopy and ELISA (P= 0.373). The higher sero-prevalence of bovine fasciolosis in ELISA compared to the routine faecal examination technique obtained might suggest the possible presence of immature flukes migration from the gut to the bile ducts. As the ELISA can detect the presence of flukes in cattle as early as two weeks after infection[15,16], thus it would be the preferred method for monitoring fluke infections in cattle populations.

Certain liver tissue cells contain characteristic enzymes, which enter the blood only when these cells are damaged or destroyed. The presence of significant quantities of these specific enzymes in blood indicates the probable site of tissue damage. GGT is especially useful in assessing liver function associated with liver diseases[17,18]. In the present study, out of the 54 cattle with known liver lesions of fasciola, 33 (61%) were having elevated levels of GGT than normal value for cattle indicating as well a fair test of agreement between GGT and presence of liver lesions. Contrary to the GGT, the level of LDH has shown to be

elevated both in negative as well as positive animals for liver lesions indicating the absence of agreement (kappa =-0.66). It was noted that there is an absence of significant mean difference in enzymic activity among the different intensities of liver lesions where only 2.2% of lesions are severely damaged and the majority lightly infected, indicated that enzyme tests is not always associated with intensity of liver fluke infection. Serum concentrations of liver-specific enzymes are generally higher in acute than in chronic liver diseases. They may be within normal limits in the later stages of sub acute or chronic hepatic disease[18]. Increased LDH activity indicates mostly a cell necrosis during migration of young flukes through the liver *parenchyma,* while GGT would be more associated with bile duct damages. GGT increase indicates penetration of the bile ducts by flukes, causing a hyperplastic cholangitis[19,20,21,22].

In the present study, a significant variation ($P<0.05$) in ELISA was obtained in relation to the different intensities of liver lesion except between lightly infected and apparently normal livers. The presence of antibodies does not always correlate to active fasciolosis because antibody levels diminish slowly after cure[23,24].

It can be concluded that the use of immunoenzymatic techniques such as ELISA using F2 antigens is an important adjunct for the diagnosis of fasciolosis because of its better sensitivity than coproscopy. On the other hand, interpretations of enzyme assay as an aid in the diagnosis of fasciolosis require careful consideration of the clinical status of the animal and laboratory test (coproscopy and or immunoassay) results of fasciolosis.

Acknowledgements

The authors would like to thank the Office of Research and Graduate Studies of the Addis Ababa University for financial support to undertake the study. We thank the Institute Pourquier of France for providing us the *Fasciola* ELISA kit free of charge. We are grateful to the Elfora export abattoir for its technical support.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 56-66

GENETIC BASIS, TRANSFERABILITY AND LINKAGE OF STREPTOMYCIN AND SULPHONAMIDE RESISTANCE GENES IN *ESCHERICHIA COLI* FROM FOOD OF ANIMAL ORIGIN IN KENYA

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BASE GENETIQUE, TRANSFERABILITE ET LIAISON DES GENES DE RESISTANCE A LA STREPTOMYCINE ET AU SULFAMIDE CHEZ *ESCHERICHIA COLI* ISSUS DES ALIMENTS D'ORIGINE ANIMALE AU KENYA

Résumé

La base génétique et la transférabilité de la résistance à la streptomycine et au sulfamide ont été étudiées chez 23 souches de *Escherichia coli* issues d'aliments d'origine animale au Kenya. Le lien physique du gène *strA* de résistance à la streptomycine avec le gène *sul2* de résistance au sulfamide a été examiné par PCR et confirmé par séquençage. Deux petits plasmides de 6 kb (pSSGK1) et de 8 kb (pSSTGK1) identifiés par transformation pour modifier la résistance au moins à la streptomycine et au sulfamide étaient restreints en vue d'établir leur lien. Leurs cartes de restriction étaient comparées les unes aux autres ainsi qu'avec les cartes des autres plasmides issus de *E. coli* pour modifier leurs propriétés de résistance.

La résistance à la streptomycine était fondée sur l'expression des gènes *strA, strB* et/ou *aadA1,* alors que la résistance au sulfamide était codée par le gène *sul2* ou *sul1*. Les gènes *strA, strB* et *sul2* étaient transférables par le biais de conjugaison et de transformation. Les gènes *sul2* et *strA* physiquement liés étaient présents dans les deux plasmides pSSGK1 et pSSTGK1. Les plasmides pSSGK1 et pSSTGK1 étaient différents l'un de l'autre, mais respectivement semblables aux plasmides de résistance au sulfamide/streptomycine et sulfamide/streptomycine/tétracycline décrits auparavant dans *E. coli* uropathogènes issus des humains.

La conjugaison des plasmides codant la résistance à la streptomycine et au sulfamide peut être un mécanisme pour la large diffusion et la persistance de ces résistances dans *E. coli* issus des aliments d'origine animale au Kenya. Le lien physique des gènes *strA* et *sul2* transportés par le plasmide pourrait faciliter la propagation de ces gènes par une co-sélection au cours de la pression sélective imposée par l'utilisation de l'un ou l'autre des deux agents antimicrobiens, et souligne la nécessité de recourir à l'usage judicieux de la streptomycine ou du sulfamide dans l'élevage.

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Summary

The genetic basis and transferability of streptomycin and sulphonamide resistance was studied in 23 *Escherichia coli* isolates from food of animal origin in Kenya. Physical linkage of the streptomycin resistance gene *strA* with sulphonamide resistance gene *sul2* was investigated by PCR and confirmed by sequencing. Two small plasmids of 6 kb (pSSGK1) and 8 kb, (pSSTGK1) identified by transformation to mediate resistance to at least streptomycin and sulphonamide were restricted in order to define their relatedness. Their restriction maps were compared to one another and with the maps of other plasmids from *E. coli* known to mediate these resistance properties.

Streptomycin resistance was based on the expression of the *strA, strB* and/or *aadA1* genes, while sulphonamide resistance was encoded by the *sul2* or *sul1* gene. The *strA, strB* and *sul2* genes were transferable via conjugation and transformation. Physically linked *sul2* and *strA* genes were present in both plasmids pSSGK1 and pSSTGK1. The plasmids pSSGK1 and pSSTGK1 were different from each other, but similar respectively to sulphonamide/streptomycin and sulphonamide/streptomycin/tetracycline resistance plasmids described previously in uropathogenic *E. coli* from humans.

Conjugation of plasmids encoding streptomycin and sulphonamide resistance may be one mechanism for the wide dissemination and persistence of these resistances among food animal *E. coli* in Kenya. Physical linkage of the plasmid-borne *strA* and *sul2* genes would facilitate the spread of these genes by co-selection during selective pressure imposed by the use either of the two antimicrobials and highlights the need for the prudent use of streptomycin or sulphonamides in animal husbandry.

Introduction

Antimicrobial resistance among pathogenic and commensal bacteria has become a serious problem worldwide that affects treatment of infectious diseases both in humans and in animals[1]. Resistance genes are often located on mobile genetic elements like plasmids, transposons, or gene cassettes in integrons[2, 3]. The determination of the genetic location and the potential linkage of antimicrobial resistance genes are crucial in predicting the risk of spread and persistence of resistance[4]. Plasmids, transposons and integrons are spread vertically during the division of the host cell, but can also be transferred horizontally between bacteria of the same or different species and genera via transduction, conjugation/mobilization or transformation and thus contribute to the increase in multiresistant bacteria[2]. Conjugation and mobilization are the most important in the spread of resistance genes between bacteria of different species and genera[4] while transformation is the major way of introducing plasmids into new host bacteria under *in vitro* conditions[5].

In *E. coli*, resistance to streptomycin can be mediated by ribosomal mutation in the chromosomal gene rpsl or by enzymatic modification of the drug. The phosphotransferase aph(3'')-Ib and aph(6)-Id genes (also known as *strA* and *strB*, respectively) and the adenyltransferase gene ant(3´´)-Ia (also designated *aadA1*) are the most frequently encountered genes that code for streptomycin modifying enzymes in *E. coli*[6,7]. Acquired resistance to sulphonamides can result either from

mutations in the chromosomal dihydropteroate synthase (DHPS) gene (folP), which decreases DHPS affinity for the sulphonamide inhibitors or, more frequently, from the acquisition of the sul-type genes that encode DHPSs with reduced affinity for sulphonamides[8].

Plasmid mediated streptomycin and sulphonamide resistance has previously been reported in clinical isolates of Yersinia pestis[9] in humans and animal faecal *E. coli*[10]. Several studies in Kenya[11, 12, 13] have reported diverse plasmids to be associated with streptomycin and/or sulphonamide resistance in *E. coli* of animal origin but none of them has analysed in depth the genetic basis of these resistances. In a recent study in Kenya[14] high incidences of resistance to streptomycin and sulphonamide were observed in multidrug resistant *E. coli* strains of food animal origin indicating possible genetic linkage. The aims of this study were to (i) investigate the genetic basis of streptomycin and sulphonamide resistance among resistant *E. coli* isolates, (ii) determine the transferability of the resistant genes and (iii) investigate the possibility of a physical linkage between the streptomycin resistance gene *strA* and the sulphonamide resistance gene *sul*[2].

Materials and methods

Bacterial strains

Twenty three *E. coli* isolates previously obtained from food animals in Kenya[14] and found to be resistant to both streptomycin and sulphonamide were used. Of the 23 *E. coli* isolates, 18 were from pigs, 4 from chicken and 1 from cattle (Table 1).

Plasmid analysis

Plasmids were isolated from all 23 *E. coli* isolates by a modification of the alkaline lysis procedure[15] and subsequently purified by affinity chromatography on Qiagen Midi columns (Qiagen, Hilden, Germany). Plasmids in *E. coli* V5[16] (2.1-54.0 kb)[17], the *Klebsiella pneumoniae* plasmid R55 (150 kb), and the 90 kb plasmid of *Salmonella Typhimurium* LT2 were used as size markers[16]. The plasmids were separated on a 0.8% agarose gel and plasmid sizes estimated according to Rochelle *et al*[18] and Wand *et al*[19].

Transfer of resistance

Conjugation studies were carried out by the plate-mating procedure[20] with the nalidixic acid resistant (NalR) mutant of *E. coli* K12 7118N Lac- as the recipient strain. These experiments were carried out only on isolates with plasmids of size >30 kb since these are the plasmids which are likely to be transferred by conjugation. Transconjugants were selected on Luria-Bertani (LB) agar plates containing 30 µg/ml streptomycin or 500 µg/ml sulphamethoxazole and 60 µg/ml nalidixic acid. In order to investigate the possibility of the occurrence of streptomycin or sulphonamide resistance genes on small non-conjugative plasmids, plasmids were transformed into the *E. coli* strain JM109 by the $CaCl_2$ method[21] and the transformants selected on LB agar supplemented with 30 µg streptomycin or 500 µg sulphamethoxazole. The transconjugants or transformants were screened for the presence of plasmids as previously described by Kehnrenberg and Schawrz[22]. In addition the transconjugants or transformants were also tested for resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, tetracycline, streptomycin, sulphonamide or sulphamethoxazole/trimethoprim by disc diffusion to determine

whether the resistance plasmids transferred mediate further resistance properties.

Restriction endonuclease analysis of plasmids

Restriction mapping was performed on three streptomycin/sulphonamide resistance plasmids of 6 kb in size and one 8 kb streptomycin/sulphonamide/ tetracycline resistance plasmid identified by transformation using *BclI, BglII, DraI, EcoRI, EcoRV, HpaI, KpnI, PstI, PvuII, SacI, HindIII,* XbaI and SmaI endonucleases as described earlier by[22] in order to define their relatedness. The restriction maps were compared to one another and with the maps of other plasmids from *E. coli* known to mediate these resistance properties. Comparative restriction analysis of the three streptomycin/sulphonamide resistance plasmids showed indistinguishable fragment patterns with *DraI, EcoRI, EcoRV, HpaI, KpnI, PstI, PvuII, SacI,* and *SmaI. Endonucleases HindIII, XbaI BclI* and *BglII* did not cut any of these plasmids. Therefore a common designation, pSSGK1 was chosen for these plasmids while the streptomycin/sulphonamide/tetracycline resistance plasmid was designated as pSSTGK1.

PCR detection of resistance genes

Whole cell DNA was isolated from all 23 *E. coli* isolates[22] and used for the detection of the streptomycin resistance genes *strA, strB, aadA1* and sulphonamide resistance genes, *sul2* and *sul1* using PCR assays. The primers used are shown in Table 2. PCR assays followed previously described protocols[21]. The PCR products were detected by electrophoresis in 1.5% agarose gels. PCR analysis for the physical linkage of the streptomycin resistance gene *strA,* with the sulphonamide resistance gene *sul2* was performed with primer pairs *sul2*-forward-*strA* reverse and *strA*-forward-*sul2*-reverse and confirmed by sequencing. Plasmids obtained after conjugation or transformation were screened for the presence and linkage of streptomycin and sulphonamide genes

Cloning and sequencing

The PCR products obtained with primers *sul*2-forward and strA-reverse, were cloned into pCR® BluntII TOPO® (InvitrogenTM Groningen, The Netherlands) and sequenced (MWG-Biotech, Ebersberg, Germany)[22]. Homology searches for comparative analysis of nucleotide sequences were performed with the BLAST programme at the National Centre for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

Occurrence of plasmids in E. coli isolates

Nineteen of the 23 *E. coli* isolates investigated carried plasmids (Table 1). The number of plasmids per isolate ranged from 1 to 4 while the size of plasmids ranged from 2 to 106 kb.

Transferability of streptomycin and sulphonamide resistance

Streptomycin and sulphonamide resistance was conjugally transferable in five (41.6%) of 12 isolates studied. The conjugational resistance transfer frequency ranged from 4.2×10^{-9} to 3.7×10^{-6}. Three of the isolates co-transferred resistance to ampicillin, tetracycline, chloramphenicol and sulphamethoxazole/trimethoprim with both streptomycin and sulphonamide resistance. Additionally, streptomycin and sulphonamide

Table 1: Characteristics of the 23 *E. coli* isolates used in this study

Animal species	Strain ID.	Resistance phenotype	Streptomycin and sulphonamide resistance genes detected	Approximate size(s) of plasmids in kb	Plasmids transferred by conjugation or transformation*	Conjugation transfer frequency
Pig	12C	$Amp^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	90	-	-
	35F	$SxT^{R}Sm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	31	-	-
	3C	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	72	72	3.7×10^{-6}
	3F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Gm^{R}Km^{R}Cm^{R}Sul^{R}$	*strA, strB, sul*2	95	95	
	70C	$Amp^{R}SxT^{R}Sm^{R}Cm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	2	-	-
	72C	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Cm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*1	106	106	2.0×10^{-7}
	33F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	9	-	-
	7F[a]	$Amp^{I}Sm^{R}Sul^{R}$	*sul*2	6	-	-
	27F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Km^{I}Sul^{R}$	*strA, strB, sul*2	85, 62	85, 62	2.1×10^{-6}
	65F	$Tet^{R}SxT^{R}Sm^{R}Km^{R}Cm^{R}Sul^{R}$	*strA, strB, sul*2	100, 58	100, 58	2.1×10^{-8}
	20C	$Amp^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	10, 3	-	-
	4F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	18, 6, 4	6*	-
	80F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Km^{R}Cm^{R}Sul^{R}$	*strA,, strB, sul*2	106, 10, 5	106, 10, 5	$4.3 \times 2.1 \times 10^{-8}$
	8C	$Tet^{R}Sm^{R}Sul^{R}$	*strA, strB, sul*2	8, 6, 4	8*	
	66C	$Amp^{I}Sm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	106, 16, 6, 4	106, 16, 6*	2.9×10^{-6}
	74C	$Tet^{R}Sm^{R}Sul^{R}$	*strA, strB, sul*2	106, 6, 5, 4	106, 6*	
	21C	$Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	none	-	-
	29F	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	none	-	-
Chicken	70P	$Tet^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	3	-	-
	45D	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Cm^{R}Sul^{R}$	*strA, strB, sul*2	62, 9, 4	62, 9	4.2×10^{-9}
	85P	$SxT^{R}Sm^{R}Sul^{R}$	*strA , strB, aadA*1, *sul*2	106, 10, 4	106, 10, 4	2.8×10^{-7}
	83K	$Amp^{R}Tet^{R}SxT^{R}Sm^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*1	none	-	-
Cattle	19C1	$Amp^{R}Sm^{R}SxT^{R}Sul^{R}$	*strA, strB, aadA*1, *sul*2	none	-	-

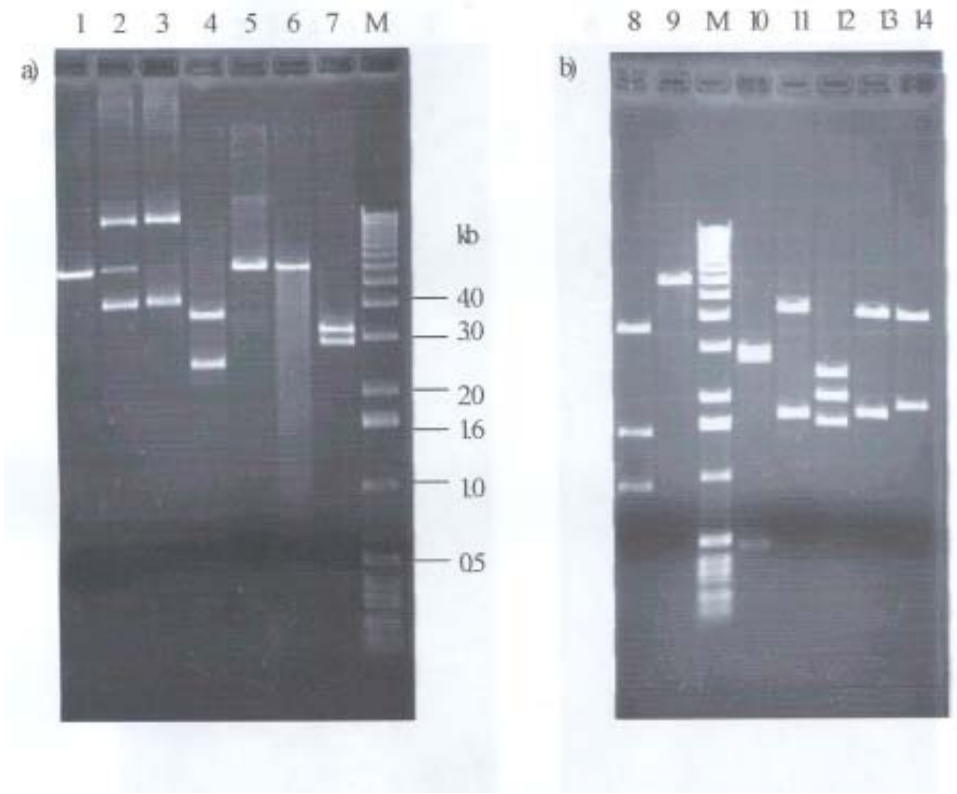


Fig 1: Agarose gel electrophoresis of some restriction digests of pSSGK1 plasmid DNA after (a) Single digets: lanes 1 *Pvu*II; 2 *Bam*HI; 3 *Hind*III, 4 *Eco*RV, 5 SacI, 6 *Dra*I and 7 *Pst*I. (b) Double digests: lanes 8 *Dra*I/ *Eco*RV, 9 *Eco*RI/HpaI, 10 *Dra*I/PstI,11*Kpn*I/*Dra*I, 12 KspI/*Dra*I, 13 *Pvu*II/EcoRI, and 14 *Sac*I/KpnI Lane M contains the DNA size standard (1 kb ladder, Gibco-BRL) of which the sizes of some fragments are given on the right hand of figure (a)

resistance was also co-transferred with resistance to sulphamethoxazole/ trimethoprim, ampicillin, and tetracycline in one isolate and to tetracycline and sulphamethoxazole/trimethoprim in another. Streptomycin and sulphonamide resistance was also transferable by transformation in 4 of the 19 isolates carrying plasmids. In three of the 4 isolates a 6 kb plasmid mediated resistance to streptomycin/sulphonamide while an 8 kb plasmid mediated resistance to sulphonamide/streptomycin/tetracycline in the fourth isolate.

Restriction profiles of resistance plasmids

The restriction profiles of the plasmids pSSGK1 and pSSTGK1 are shown in Figures 1 and 2 respectively. The restriction maps of these resistance plasmids revealed that the plasmids were structurally different from each other but similar to the previously described[23] sulphonamide/streptomycin and sulphonamide/streptomycin/tetracycline resistance plasmids pSSOJO1 and pTOJO1 respectively (Fig. 3)[23].

Table 2: Sequences of oligonucleotides used as primers and annealing temperature for the detection of resistance genes

Target gene	Oligonucleotide sequence (5´ - 3´)	Amplicon size (bp)	Annealing temp (^{o}C)	Reference sequence {Genbank accession number (s)}
aadA1	f:- GTGGATGGCGGCCTGAAGCC	527	56	*E. coli*
	r:- ATTGCCCAGTCGGCAGCG			(M10241, X02340)
strA	f:- GACTGGTTGCCTGTCAGAGG	646	64	Plasmid RSF 1010
	r:- CAGTTGTCTTCGGCGTTAGCA			M28829
strB	f:- ATCGTCAAGGGATTGAAACC	510	56	*E. coli*
	r:- GGATCGTAGAACATATTGGC			(NC_005324)
sul1	f:- ATGGTGACGGTGTTCGGCATTCTG	418	64	Plasmid R388
	r:- CTAGGGATGATCTAACCCTCGGTC			(X12869)
sul2	f:- ACAGTTTCTCCGATGGAGGCCG	704	64	*E. coli* plasmid p9123
	r:- CTCGTGTGTGCGGATGAAGTCA			(AY360321)

f, forward primer; r, reverse primer

PCR detection of the resistance genes

The streptomycin and sulphonamide resistance genes detected in each of the 23 *E. coli* isolates studied are shown in Table 1. All the 23 *E. coli* isolates were positive for at least one of the two sulphonamide resistance genes tested. Twenty one (91.3%) of the isolates were positive for *sul2* while the remaining two were positive for *sul1*. Out of the 23 *E. coli* isolates, twenty two were positive for at least two of the three streptomycin resistance genes tested. Fifteen (65.2%) isolates were positive for all three streptomycin resistance genes tested. Only one among the streptomycin resistant isolates was negative for all the three streptomycin resistance genes tested in this study. Streptomycin resistance genes *strA*, strB and sulphonamide resistance gene *sul2* were detected in plasmids transferable via conjugation or transformation.

Identification of the physical linkage of the resistance genes

Both plasmids pSSGK1 and pSSTGK1 yielded PCR amplicons of approximately 1.5 kb with the primer pair *sul2*-forward-*strA* reverse (Fig. 4) while the primer pair *strA*-forward-*sul2*-reverse yielded no amplicons suggesting that physically linked *sul2* and *strA* genes were present in these plasmids and were arranged in the orientation *sul2-strA*. Plasmids from transconjugants yielded no amplicons. Nucleotide sequence analysis revealed that the *sul2-strA*, 1514 bp amplicons consisted of 770 bp of the *sul2*

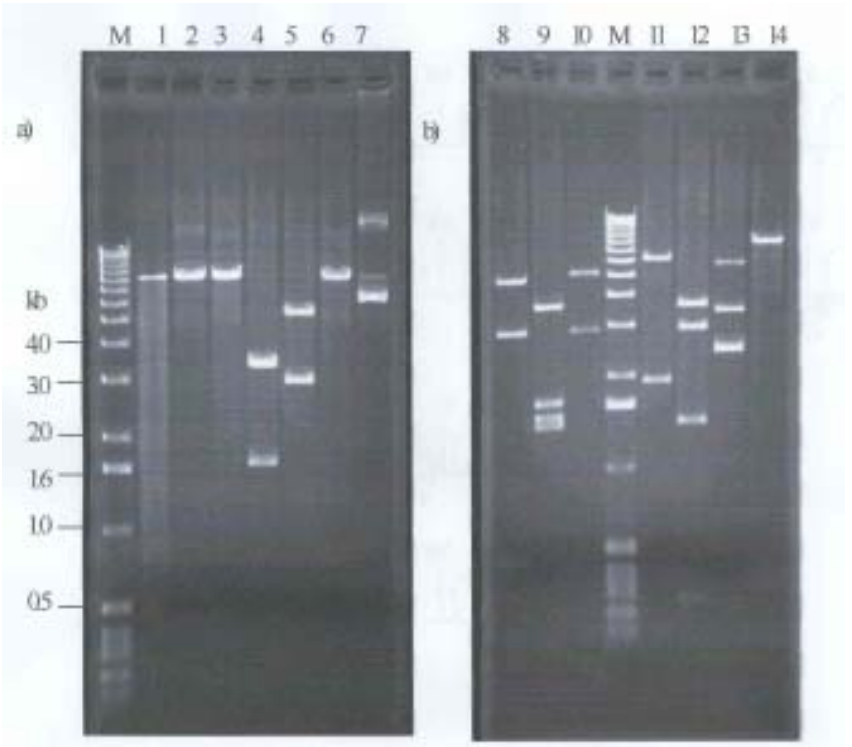
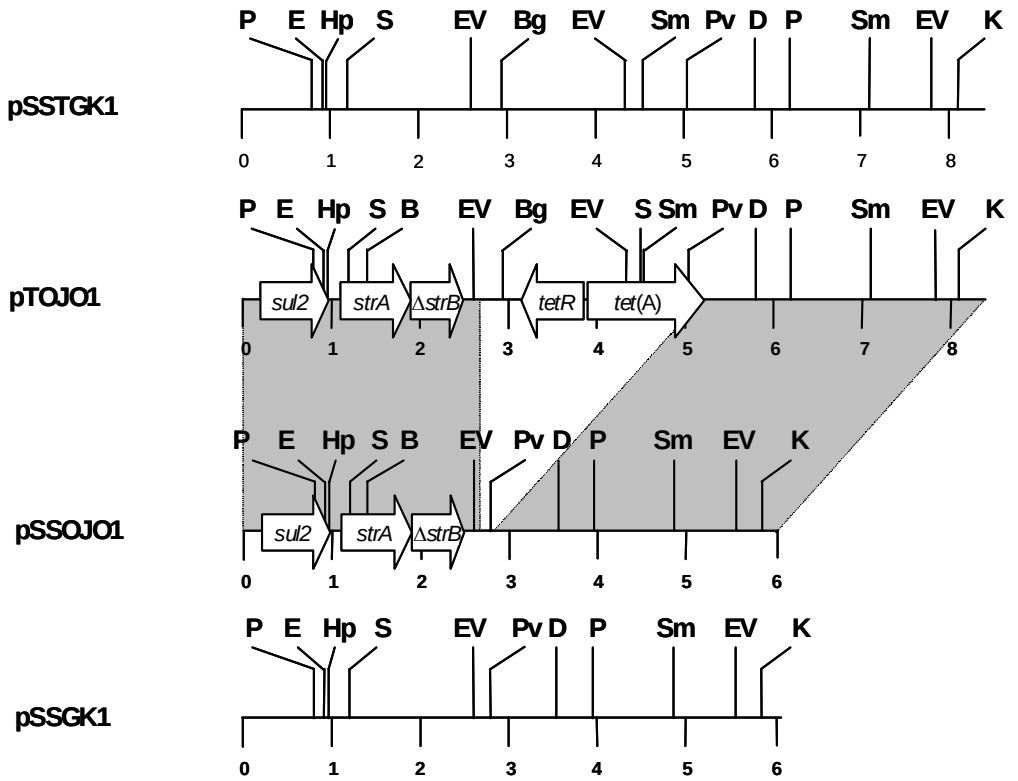


Fig 2: Agarose gel electrophoresis of pSSTGK1 plasmid DNA after digestion with restriction endonucleases. (a) Single digests; lanes, DraI, SacI, 3 *Kpn*I, 4 KspI, 5 PstI, 6 *Eco*RI and 7 BclI. (b) Double digests; lanes 8 *Dral*/PstI, 9 *Dra*I/EcoRV, 10 PstI/EcoRI, 11 *Sac*I/KpnI, 12 *Eco*RV/Bg/II, 13 *Sma*I/*Sac*I and 14 *Eco*RI/HpaI. Lane M contains the DNA size standard (1 kb ladder, Gibco-BRL) of which the sizes of some fragments are given on the left hand side of figure (a).

gene, 684 bp of the *strA* gene and a spacer of 60 bp. Comparisons of the nucleotide sequence of the *sul2* and *strA* amplicons corresponded exactly to the respective segments of the *sul2* and the *strA* genes of RSF1010[24].

In this study, approximately eighty three percent (83.3%) of the *E. coli* isolates harboured 1 to 4 plasmids of different sizes. Large plasmids ranging in size from 30 to 106 kb were detected in 58.3% of the isolates. This is in line with results from previous studies of *E. coli* isolates from chickens[11], rats[13] and cow milk[12] in Kenya. Resistance was transferable via conjugation and this suggests that conjugation of plasmids encoding streptomycin and/or sulphonamide resistance may be one mechanism for the wide dissemination of this resistance among *E. coli* found in food animals. Co-transfer of resistance to chloramphenicol, sulphamethoxazole/ trimethoprim, ampicillin and tetracycline was observed.

Fig 3: Comparative analysis of the restriction maps of resistance plasmids pSSGK! and STGK 1 (this study) as well as pTOJO1 and pSSOJO1[23]. Restriction endonucleases: B (Bcl1), Bg (*Bg*/II); D (*dra*I); E (*Eco*RI); EV (*EcoRV*); Hp (*Hpa*I); K (*Kpn*I); P (*Pst*I); Pv (*Pvu*II) S (*sac*I) and Sm (*Sma*I)



Two small structurally different plasmids of 6 kb (designated pSSGK1) and 8 kb (designated pSSGK1) were identified by transformation to mediate resistance to streptomycin/sulphonamide, and to streptomycin/sulphonamide/tetracycline, respectively. Restriction enzyme analysis of these plasmids revealed that the 6 kb plasmid pSSGK1 is very closely related to the streptomycin/sulphonamide resistance plasmid, pSSOJO1 detected in uropathogenic *E. coli* from humans in Nigeria[23]. Other streptomycin/sulphonamide resistance plasmids have also been reported including, p9123, which was recently found to enhance host fitness in the absence of specific antimicrobial selective pressure[25] and pBP1, which was prevalent in the *E. coli* population in the 1970s and 1980s[26]. The 8 kb plasmid pSSTGK1 is similar to the streptomycin/sulphonamide/tetracycline resistance plasmid, pTOJO1, found in human uropathogenic *E. coli* in Nigeria[23]. The streptomycin resistance genes *strA,*

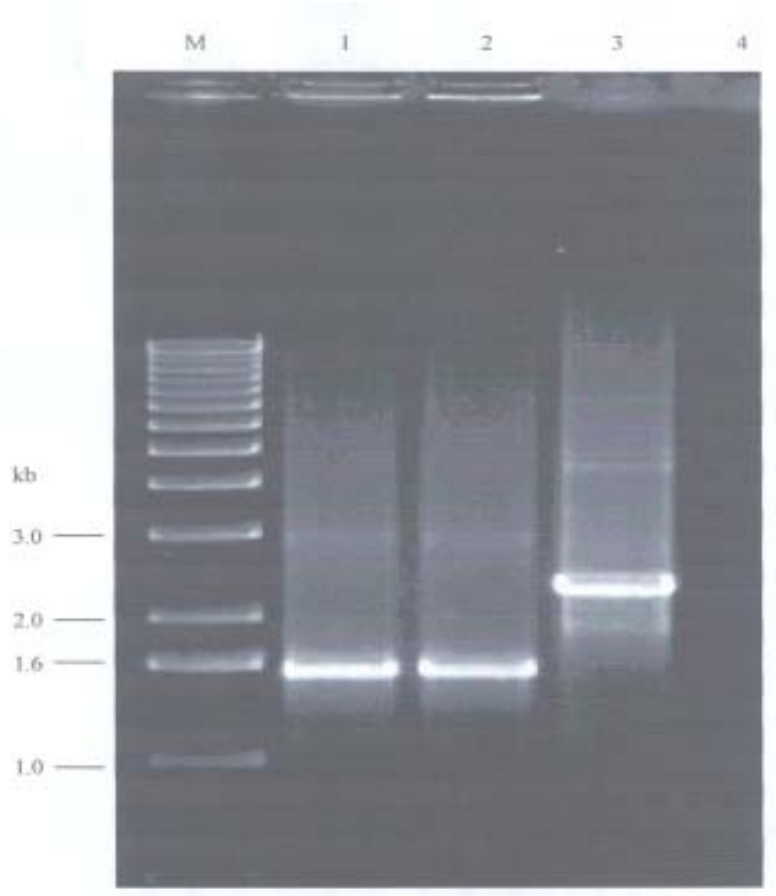


Fig. 4. Amplicons obtained from plasmids pSSGK1 and pSSTGK1 with primer pair sul2-*strA* (lanes 1-2), positive control; the plasmid pMHSCS1 carrying physically linked *sul2*-*strA* in a 2231 bp resistance gene cluster *sul2*-catA3-*strA*[22] (lane 3), negative control (H_2O) (lane 4) and molecular size marker (lane M)

strB, and *aadA1* as well as the sulphonamide resistance genes *sul1* and *sul2* were responsible for resistance in the *E. coli* strains examined. The *strA, strB* and *sul2* genes were found to be located on plasmids and transferable via conjugation or transformation. The one strain not possessing any of three streptomycin resistance tested genes may harbour other genes or the resistance may be conferred via chromosomal mutations that alter the ribosomal binding site of streptomycin[27]. Failure to detect either the streptomycin resistance gene *aadA1* or sulphonamide resistance gene *sul1* gene in plasmids following transformation or conjugation experiments suggests chromosomal localization of the genes, although location on large non-conjugative plasmids, cannot be excluded.

The occurrence of the streptomycin resistance genes *strA, strB* and *aadA1* genes in the same *E. coli* strain has been documented previously[8]. The *strA, strB* and *sul2* genes were originally described in the small, non-conjugative, broad-host-range IncQ plasmid RSF1010[24]. The *strA* gene has subsequently been found as part of transposon Tn5393 and related elements in *phytopathogenic Erwinia amylovora, Pseudomonas syringae pathovar papulans*, and *Xanthomonas campestris pathovar vesicatoria*[28]. Tn5393 carrying *strA* is typically plasmid encoded but may also be chromosomally inserted[29]. Additionally, the strA with a truncated Tn5393 tnpR gene was detected on a transferable streptomycin resistance plasmid in a clinical isolate of the Yersinia pestis[9]. The *strB* gene has been widely detected in both pathogenic[23] and commensal *E. coli* strains[6]. The *sul2* gene has been reported as the most frequently acquired sulphonamide resistance gene in human and animal faecal *E. coli* and was found to be mediated by plasmids of various types[10]. An 8.4 kb plasmid carrying *sul2, strA* and *strB* genes has been described previously in uropathogenic *E. coli* from humans[25].

Physically linked sulphonamide resistance gene *sul2* and streptomycin resistance gene strA were present in both the 6 kb plasmid pSSGK1 and 8 kb plasmid pSSTGK1 and were arranged in the orientation *sul2-strA*. Plasmids carrying physically linked *sul2* and *strA* gene in the same orientation have previously only been reported in bacteria of the genera *Pasteurella* and *Mannheimia*[22]. Comparisons of the nucleotide sequence of the *sul2* and *strA* amplicons corresponded exactly to the respective segments of the *sul2* and the *strA* genes of RSF1010[25]. The 60 bp spacer region between the physically linked *sul2* and *strA* genes identified in this study was within the previously reported range of 25 to 152 bp for linked *sul2-strA* genes in bacteria of the genera *Pasteurella* and *Mannheimia*[23]. With regard to transcription of these genes, it is likely that the linked resistance genes *sul2-strA* are co-transcripted from the promoter upstream from *sul2* gene since Scholz *et al.* [24] did not detect any specific promoter structures for *strA*.

Linkage of genes encoding resistance to antimicrobials is important in maintaining resistance to any of the antimicrobials represented in the gene cluster without specific selective pressure as has been reported for chloramphenicol[30]. The detection of the gene cluster *sul2-strA* on structurally different plasmids underlines their mobility and the significance of plasmids in the spread and persistence of streptomycin and sulphonamide resistance in food animals in Kenya. The physical linkage of streptomycin

resistance gene *strA* and sulphonamide resistance gene *sul2* offers the possibility of co-selection of either of the genes during selective pressure imposed by the use of either of the antimicrobials and highlights the need for their prudent use in animal husbandry.

Acknowledgements

This study was funded by the German Academic Exchange Service (DAAD). We thank Roswitha Becker and Jane Kamau for their technical assistance.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56,67-73

INFLUENCE OF TIGERNUT *(CYPERUS ESCULENTUS L.)* ON THE SEMEN CHARACTERISTICS AND TESTICULAR PARAMETERS OF RABBITS

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INFLUENCE DE L'AMANDE DE TERRE *(CYPERUS ESCULENTUS L.)* SUR LES CARACTERISTIQUES DU SPERME ET LES PARAMETRES TESTICULAIRES DES LAPINS

Résumé

Douze lapins hollandais sexuellement mûrs ont été utilisés pour déterminer l'effet de l'amande de terre *(Cyperus esculentus)* sur les caractéristiques de reproduction des lapins. Le groupe expérimental (T_2) a été comparé à un groupe non-traité (T_1). Les résultats ont montré que le volume de sperme de T_1 (0,30±0,03ml) était beaucoup plus élevé (P<0,05) que celui de T_2 (0,21±0,1ml). La concentration du sperme de T_2 (345,28±46,60 x10^6 /ml) était nettement plus forte (P<0,05) que celle de T_1 (153,33±27,78 x10^6 /ml). Il n'y avait pas de différence significative (P>0,05) quant à la motilité des spermatozoïdes vivants/morts et au temps de réponse entre les deux groupes. Les paramètres testiculaires n'ont montré aucune différence notable (P>0,05) entre les deux groupes pour ce qui est du poids des testicules, du canal déférent, de la longueur du testicule, de la circonférence du testicule droit, de la longueur de l'épididyme, du poids des deux testicules ainsi que du poids des organes reproducteurs. Toutefois, il y avait de grandes différences (P<0,05) entre les groupes quant à la circonférence du testicule gauche (CTG) et au poids de l'épididyme gauche (PEG). Bien que le volume de sperme de T_2 ait baissé, sa concentration de spermatozoïdes était plus élevée que celle de T_1 ; ce qui montre qu'il y a de plus fortes chances de fécondité chez le groupe T_2 , et cela pourrait être exploité dans les programmes d'insémination artificielle (IA).

Mots-clés: Lapin, mâle, sperme, testicule, amande de terre *(Cyperus esculentus L.)*

Summary

Twelve sexually mature Dutch rabbits were used to evaluate the influence of tigernut *(Cyperus esculentus)* on the reproductive characteristics of rabbits. The treatment group (T_2) was compared to an untreated group (T_1). Results showed that semen volume of T_1 (0.30±0.03ml) was significantly higher (P<0.05) than the T_2 (0.21±0.1ml). The sperm concentration of T_2 (345.28±46.60 x106 /ml) was significantly higher (P<0.05) than T_1 (153.33±27.78 x106 /ml). There were no significant differences (P>0.05) in sperm motility, live/dead and reaction time between the two groups. The testicular parameters revealed no significant differences (P>0.05) in the testes weight, vas deferens, testis length, right testis circumference, epididymal length, paired testis weights and weight of reproductive tract between the groups. However, significant differences (P<0.05) existed between the groups in the left testis circumference (LTC) and weight of left epididymis (LEW) between groups. Although semen volume of the T_2 was depressed its spermatozoa concentration was higher than that of T_1. This is indicative of higher chances of fertility of T_2 group and could be processed for further usage in artificial insemination (AI) programs.

Key words: Rabbit, Buck, Semen, Testicle, Tigernut *(Cyperus esculentus L)*

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Introduction

One of the prerequisites for the improvement of reproductive performance is the knowledge of semen characteristics of breeding males. The quality of semen plays a major role in determining the fertility and reproductive efficiency of livestock. *Libido* or sexual desire is an important aspect of male reproductive function. Lack of libido *(impotentia coeundi)* may be hereditary or may originate from psychogenic disturbances, endocrine imbalance or environmental factors. Although seminal characteristics may be satisfactory, fertility may be adversely affected due to poor *libido*[1]. Hughes and Varley[2] reported that poor *libido* is one of the causes of reduced fertility in farm animals. It can be measured by the reaction time of the animal. *Libido* can also be assessed by the number of ejaculates per buck per day[3].

Furthermore, the role played by the buck in rabbit production/reproduction has not been properly investigated. Litter sizes at birth of 8 to 10 are common in rabbits. However, in many tropical environments, litter sizes fall much below this. This could be attributed to various factors, ranging from environment to physiology. Among physiological components that contribute to low reproductive performance are the semen quality and its principal constituents, spermatozoa. Hafez[4] stated that testicular size is among the factors influencing the semen characteristics. Ovuru and Nodu[5] reported mean weight of the testes of rabbits to be 5.13±0.17g while Herbert *et al*[6] reported mean testes weight of 6.7±0.2g for bucks. However, testicular characteristics of farm animals of some species may vary according to the system of management[7]. It is expected that the higher the semen quality the greater the number of sperm cells available to fertilize the ova and this in turn could result to large litter size.

The aphrodisiac effect of *Myristica fragrans Houtt.,* popularly known as nutmeg on sexual function has been reported[8]. *Cyperus. esculentus L.* commonly known as tigernut, has been reported to be an aphrodisiac[9]. *Cyperus esculentus* also known as chufa, yellow nutsedge, zulu nut, ground almond is considered an important food source for migratory birds[10]. Tigernut has been cultivated since early times (South Europe and West Africa) for its small, tuberous rhizomes which are eaten raw or roasted, used for hog feed, and pressed for the juice to make a beverage[11]. Tigernut (*C. esculentus*) is reputed to have high total anti-oxidant capacity[12] and can also be used as (bio-diesel) fuel[13]. Phytochemical studies reveal that the tigernut tubers contain protein, carbohydrates, sugars, and lots of oil and fiber. Eteshola and Oraedu[12] reported that tigernut is good for human health, containing high levels of iron and potassium. The milky-looking aqueous extract of tigernut has a pleasant and characteristic flavour of vanilla and almonds. Coskuner *et al*[14] reported that chufa lines contain on average (gkg^{-1}) 932.8 dry matter, 245.0 crude lipid, 256.8 starch, 14.3 ash, 50.5 protein, 89.1 crude fibre, 17.1 reducing sugar, 154.3 total sugar and 130.4 sucrose. Its fatty acid composition included (gkg^{-1}) 689.20 to 732.90 oleic acid, 125.5 to 141.2 palmitic acid and 99.6 to 154.6 linoleic acid, which is comparable with that of olive oil. Eteshola and Oraedu[12] in chromatographic and chemical studies found that the dominant saturated fatty acid was the myristinic acid and the dominant fatty acids were oleic acid. The value of linoleic acid was found as 8.8 %. Fatty acids have been reported to have effect on sexual

reproduction.

Nutrition has great effect on the production and reproductive performance of farm animals as have been reported by Oyedipe *et al.*[15] and Vincent *et al*.[16]. Jainudeen and Hafez[1] stated that nutritional deficiency delay the onset of puberty and depresses production and characteristics of semen in the male animal. In addition, nutrition affects the endocrine rather than the spermatogenic function of the testis. Tigernut could be considered potential feedstuff that will influence the reproductive abilities in bucks.

Objectives of the study are to evaluate the effects of tigernuts *(C. esculentus)* on the semen characteristics and testicular dimensions or rabbits.

Materials and methods

Location of experiment

The experiment was conducted in the Rabbitry Unit of the Teaching and Research Farm, as well as the laboratories of the College of Animal Science and Animal Health, Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria.

Experimental animals and procedure

Twelve Dutch-belted bucks aged 8 months purchased from a Rabbit Farm situated in Mbieri, in Imo state, Nigeria were used for the study. They were quarantined (in the University Rabbitry Quarantine Unit) for two weeks, during which they were vaccinated against mange. Thereafter, the rabbit were divided into two groups of 6 each designated T_1 and T_2, (control, treatment group respectively). They were housed singly per pen in a three tier hutches. The treatment group (T_2) was placed on a concentrate diet containing tigernut while the control (T_1) diet contained no tigernut. They were also supplied water and forage ad libitum. Table 1 presents the composition of the experimental concentrate diet.

Data collection and evaluation

Semen was collected using an artificial vagina[17] Collection of semen samples was done twice weekly between hours of 0900

Table 1: Composition of experimental diets.

Ingredients	**T_1 (%)**	**T_2 (%)**
Maize offal	57.00	37.00
PKC	30.00	30.00
SBM	10.00	10.00
Bone meal	2.00	2.00
Salt	1.00	1.00
Tigernut	0.00	20.00
Total	**100**	**100**

and 1000. Several teaser does were used during the collection days. This was done to maintain sexual novelty and response of the buck. Following brief exposure of the female to the male, he was allowed to mount the teaser and the already-prepared artificial vagina (AV) unit was introduced tactfully and semen collected from the buck. Upon collection the teaser was immediately removed from the buck's cage.

Reaction time

This was assessed over a period of 60 seconds using a stopwatch. If male did not evince interest within the 60 seconds period the teaser was exchanged. This was based on the assumption of animal receptivity differences, such as a breed or age of teaser used.

Semen volume

The volume of semen collected was recorded from the graduated collection tube.

Sperm motility

A droplet of the identified semen samples was dropped on slide using a dropper and then covered with cover slip and viewed under the microscope on a warm stage. Percentage motility was estimated subjectively under several fields of view.

Live:dead ratio

This parameter was determined by staining with eosin/negrosin stain. A droplet of semen sample was dropped on slide and then a drop of the stain was added to it, mixed properly before smearing with another glass slide already labelled. The dead cells picked up the stain and appeared violet in colour. Counts of both dead and live cells were recorded from different fields of view.

Sperm concentration

Prior to counting, Processing of the semen was done using the procedures described by Herbert[18] 10% formal phosphate buffer was used to fix sperm cells in the sample drawn and this added no organic moiety to both the sperm cells and the seminal plasma components of the semen. Sperm counts were carried out on the fixed semen using an improved Neubauer chamber haemacytometer.

Testicular measurements: At the end of semen evaluation, rabbits from each of

Table 2: Seminal Parameters of the experimental rabbits.

Parameters	**T_1**	**T_2**
Semen volume (ml)	$0.30^{a} \pm 0.03$	$0.21^{b} \pm 0.01$
Sperm motility (%)	57.78 ± 3.69	67.64 ± 3.59
Sperm conc (x 10^6 /ml)	$153.33^{b} \pm 27.78$	$345.28^{a} \pm 46.60$
Live/dead (%)	71.67 ± 2.11	77.50 ± 2.79
Reaction time (seconds)	14.86 ± 1.74	18.06 ± 3.19

a, b, means in the row with different superscript are significantly different ($P<0.05$)

Table 3: Testicular dimension of experimental rabbits

Parameters	T_1	T_2
Left Testis Weight (g)	1.85±0.20	1.85±0.15
Left Vas Deferens (cm)	10.05±0.15	8.80±1.20
Left Testis Length (cm)	3.15±0.15	3.00±0.00
Left Testis Circumference (cm)	7.95^{a}±0.05	7.05^{b} ±0.05
Left Epididymis Weight (g)	$0.55±0.02^{a}$	$0.28±0.11^{b}$
Left Epididymis Length (cm)	1.75±0.05	1.25±0.25
Right Testis Weight (g)	1.70±0.05	1.90±0.10
Right Vas Deferens (cm)	10.00±0.00	8.30±1.30
Right Testis Length (cm)	3.00±0.20	3.15±0.05
Right Testis Circumference (cm)	7.80±0.10	7.10±0.20
Right Epididymis Weight (g)	0.63^{a} ±0.08	0.30^{b} ±0.00
Right Epididymis Length (cm)	1.60±0.10	1.35±0.15

a,b, means in the row with different superscript are significantly different (P<0.05)

the groups were sacrificed for evaluation of testicular parameters. The whole reproductive system was excised and properly trimmed of adhering fats and muscles. The different testicular measurements taken were: whole reproductive tract weight, individual and paired testes weights, epididymal weights, lengths of Vas deferens, testes, epididymis and circumference of testes. The different testicular weights were obtained using a sensitive electronic weighing scale. Lengths as well as circumferences were measured by using thread, which dimensions were later read from a meter rule.

Statistical analysis

The data obtained were subjected to statistical analysis using the students' 't' test. Significant means were separated using LSD. All statistical analyses were done in accordance with the methods of Steel and Torrie[19].

Results and discussion

The results on the seminal and testicular characteristics of the T_1 and T_2 are presented in Tables 2 and 3, respectively. The mean semen volume was significantly higher (P<0.05) in T1 (0.30+0.03ml) than T_2 (0.21+0.01ml. The semen volumes recorded in the present study were less than volumes of 0.6mls and 0.71mls recorded by Fielding[20] and Herbert[18], respectively. The depressed volume of the T_2 may be attributed to the test ingredient, thus confirming the reports of Mann[21] that nutrition affects the secretory functions of the accessory sex glands. The larger semen volume of T_1 could be attributed

to the massive secretion of their accessory glands.

Sperm motility, live/dead ratio and reaction time between the two groups showed no significant differences (P>0.05), but T_2 tended to have higher numerical values than T_1. The percentage gross sperm motility of both groups in this study (57.78% and 67.64 % for T_1 and T_2, respectively) was below records of 70 %[18] and 71.0 to 76.7%[22] for rabbits. However the sperm motility recorded in the present study compares favourably with the range 58.13 to 62.50%[23] and 60.50 to 67.50%[6].

Reaction time between the two groups did not differ significantly although the treated group showed a longer reaction time. This is contrary to what was expected with respect to the aphrodisiac function of the tigernut (*C. esculentus*) as it was expected that the T_2 group should have a shorter reaction time. However, there could be other unknown factors responsible for longer reaction time of T_2.

The sperm concentration was a significantly different (P<0.05) between T_1 (153.33±27.78x106/ml) and T_2, (345.28±46.60x106/ml). The sperm concentration obtained in the present study fall within range 150 to 500 x106/ml recorded by Lebas[24] for rabbits. Banerjee[25] reported that apart from individual variation, factors such as age, climatic conditions, nutrition, and frequency of ejaculation affect the quantity and quality of semen. However, in this study, the only varying factor was the tigernut in the diets.

The left and right epididymal weights of the T_1 were significantly higher than those of T_2. Also the left epididymal length followed the same trend Table 3.

The testes weight of T_1 (3.60±0.30 g) and T_2 (3.85±0.40 g) as well as their respective reproductive tract weight, (14.7+2.55g) and (13.55+0.6) showed no significant differences (P>0.05).

The significant differences in the testis circumference and weight of epididymis could be attributed to individual differences, and/or rate of maturity of the testicular segments which was mainly due to the marked growth of the length of the tubules combined with growth of the interstitial cells as observed by Kuenzel *et al.*[26] and Kirby[27].

Conclusion

In this study, the volume of semen produced by the treated group T_2 was less than the control although the concentration of spermatozoa was significantly higher for this group. This indicated that the test ingredient, tigernut *(Cyperus esculentus)* could have had a depressive effect on the volume of semen but increased the concentration of sperm cells. Furthermore, higher concentration of spermatozoa indicates higher chances of fertilization of ova in the doe, and is more likely to result in increased litter size. Semen from such treatment could be processed for further usage in artificial insemination (AI) programs.

Acknowledgements

The authors are in debited to the Dean of the College of Animal Science and Animal Health, Michael Okpara University of Agriculture Umudike for allowing the use of the College facilities. We also thank Mrs. A. Ekpo the University Rabbitry attendant for the help rendered to them during the period of data collection.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 78-89

MEASURES OF HEALTH AND PRODUCTION IN PREWEANED KIDS IN PASTORAL HERDS IN NORTHERN KENYA

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MESURES SANITAIRES ET PRODUCTION CHEZ DES CHEVREAUX NON SEVRES DANS DES TROUPEAUX PASTORAUX AU NORD DU KENYA

Résumé

Une étude a été conduite dans six sous-locations situées dans le Nord du Kenya pour évaluer les mesures sanitaires et la production des chevreaux non sevrés dans des troupeaux pastoraux. On a conduit une évaluation auprès des pasteurs, afin de connaître leur avis et aussi pour savoir dans quelle mesure les maladies constituent une contrainte à la performance des chevreaux non sevrés, évaluation suivie d'études transversales et longitudinales pour une triangulation. Le taux moyen de morbidité était de 33% et il était fortement associé aux sous-locations ($p<0,05$). Sur la base du diagnostic de laboratoire, le taux moyen de prévalence apparente de nématodose et de coccidiose était respectivement de 2,8 et 23,2%. Le taux brut annuel de morbidité chez les chevreaux était de 19,7%. Les tout premiers cas de nématodose et de coccidiose ont été diagnostiqués chez les chevreaux âgés de 1,3 et 2 mois respectivement. Les taux annuels de morbidité de nématodose et de coccidiose étaient respectivement de 15,8 et de 56,8%. Le taux brut annuel de mortalité pour les chevreaux était de 10,5%.

Dans l'ensemble, le gain pondéral moyen quotidien (GMQ) à l'âge de 240 jours était de $52,9\pm0,3 gd^{-1}$, tandis que la croissance des chevreaux en bonne santé était beaucoup ($p<0,05$) plus forte ($58,3\pm1 gd^{-1}$) que celle des chevreaux en mauvaise santé ($51,8\pm8 gd^{-1}$). L'état sanitaire et la sous-location étaient liés au GMQ. Il y avait une interaction sous-location/saison et état de santé/saison. Les résultats sur la performance ont montré que les taux de morbidité et de mortalité élevés ont affecté les gains de poids.

Summary

A study was conducted in six sub locations in Northern Kenya to establish measures of health and production in preweaned kids in pastoral herds. A pastoralists' appraisal was carried out to assess pastoral perceptions on diseases constraining performance of preweaned kids followed by cross sectional and longitudinal studies for triangulation. Mean morbidity prevalence rate was 33.0% and was significantly associated with sublocations ($p<0.05$). On basis of laboratory diagnosis mean apparent point prevalence rates of nematodosis and coccidosis were 2.8 and 23.2%, respectively. The annual crude morbidity incidence rate in kids was 19.7%. The earliest cases of nematodosis and coccidiosis were diagnosed in 1.3 and 2.0 months old kids respectively. The annual morbidity incidence rates of nematodosis and coccidiosis were 15.8 and 56.8% respectively. The annual crude mortality incidence rate for the kids was 10.5%.

The overall mean daily weight gain (MDWG) at 240 days of age was 52.9±0.3gd^{-1} where growth of healthy kids was significantly ($p<0.05$) higher (58.3±1.0gd^{-1}) than that of the unhealthy ones (51.8±1.8gd^{-1}). Health status and sub location were associated with MDGW. There was an interaction term on sub location and season and also health and season. Results on performance showed that high crude morbidity and mortality rates compromised the weight gains.

Introduction

Overall, 90 per cent of all goats in the world are found in developing countries. Tropical Africa is home to one-third of the world's total goat population[1,2]. The increasing world importance of meat and milk from goats is indicated by the increase in number of animals in the developing world[1,3]. Of the 222.9 millions goats in Africa[4], 10.3 millions are found in Kenya[5]. Marsabit District, part of the Arid and semi-arid lands (ASALs) of northern Kenya has about 442000 goats reared on natural vegetation mainly under pastoral livestock production (PLP) systems[6].

Animal health management through blanket veterinary packages in pastoral systems have been advocated in the past and were always welcome by goat keepers[7,8]. Padhila *et al.*,[9] reported improved veterinary care as the most rewarding intervention in kids in Brazil. Carles[10] reported that the effect of a health programme was not realized in goats in Korr while Njanja[11] recorded higher growth rates in treated than non-treated kids in Turkana PLP system. Also, Bosman and Ayeni[12] Ba and Udo[13], observed that veterinary intervention packages did not have a significant effect on kid mortality in Mali where 50 per cent of the losses were due to malnutrition, trauma and loss by straying. Furthermore, experiences from PLP systems indicated that many losses of kids were due to poor husbandry practices management. These contrasting results imply that there is need to develop performance-related strategies on basis of measures of health and production in goats

The young animals are more vulnerable to causal agents of diseases than adults[14]. The initial studies also reported high mortalities in kids among East Africa's pastoral societies[15,16,17]. Pastoral systems in ASALs of Africa are experiencing a shift in production goals from subsistence to commercial and therefore efficiency and profitability of all outputs will have practical consequences in planning[18,19]. In PLP systems the immature class of animals are a critical proportions of the flocks[20] and considering the fact that natural recruitment is one of the major goals,[8,21], there is need therefore to address diseases/agents constraining their performance.

The main objective of the study was to determine morbidity and mortality constraining growth rate of preweaned kids and major associated factors in pastoral herds as a prerequisite for improved productivity. The specific diseases/casual agents were identified as basis for formulating appropriate guidelines for improved performance-related health management.

Materials and Method

The study area

This study was carried out in pastoral herds in six sub locations in Southwestern Marsabit District, Northern Kenya lying

between latitude 01o 15' N and 04^0 27' N and longitude 36^0 03' and 38^0 59'E .The sub locations were namely Olturot, Ilaut, and Ngurunit in the arid agro-ecological zone (AEZ) V, and Kargi, Korr and Loglogo in the very arid AEZ VI).

AEZ V covers approximately 28 per cent of the total area of the District The dominant vegetation is bushed grassland with tree and shrub coverage of less than 10 per cent. AEZ VI covers approximately 69 per cent of the District's total area lying below 700m see level and is dominated by dwarf-shrub/ annual grassland vegetation[23]. The average daily temperature is 30^{0}C. Rainfall is bimodal in distribution with a long rainy season from March to May and a short rainy season from October to November. Median annual rainfall is 150 to 300 mm and is erratic, sparse and unpredictable, often interrupted by drought periods and the potential annual evaporation is about 250mm[20,23].

Rapid rural appraisal

A rapid rural appraisal (RRA) was conducted in six settlements which were also the only market centers in the respective sub locations to establish pastoralists' perceptions on diseases/causal agents constraining production. It was carried out in focused group discussions using semi-structured questionnaires (SSQ) as a checklist. The participants were pastoralists who owned livestock in addition to any occupation or communal responsibilities as teachers, civil servants, community workers and elders. They were briefed on their roles in the RRA meetings and the need to contribute fully in all discussions. Illustrations were carried out using the locally available materials like sand, stones, faecal pellets and sticks at the sites in the community halls or open grounds. The various attributes were listed on the flip charts as a guide to the discussions and for records. The participants agreed to cooperate in the follow-up cross-sectional and longitudinal studies.

Cross-sectional survey

After the RRA a cross-sectional survey was conducted where 60 households, 10 from each of the six sublocations were randomly selected from a list frame and interviewed using a structured questionnaire. Each household presented four kids below eight months old for examination and samples collected for corpological examination. From the clinical examination and the managers information sick animals were diagnosed and number of the dead recorded. The morbidity/mortality prevalence rates in the preweaned kids were determined from these responses.

Longitudinal survey

A longitudinal survey was carried out after the cross-section study for 12 months in 11 and 15 households, in Olturot and Korr sub locations, respectively. Monthly visits were made to carry out clinical examination and take production measurements in the flocks. Structured questionnaire were administered during these visits. All kids below eight months were recruited at the initiation of the study and new ones progressively included. Birth weights of individual preweaned kids were taken within 12 hours and thereafter live weights at monthly intervals until weaning at eight months. Individual kids were eartagged for permanent identification. The study was conducted at the prevailing pastoral animal health management levels. The herds were mainly composed of the East African Goat (EAG) and a few Galla (G) with their cross

breeds (EAG*G).

All the preweaned kids during the study period were considered at risk, the withdrawals were those slaughtered, given-out or lost before attaining the weaning age. The mean birth weight was calculated from the total birth weights taken from kids born and the mean daily weight gains (MDWG) determined using the formula:

MDWG = (xt2-xt1)/t2- t1

Where, x= liveweight and t= time (age in days).

Laboratory anaylsis

Faecal samples were obtained from the rectum of individual animals and stored in

Disease or agent	Market centres (ranking serially from 1st to 10th positions)						
	Kargi	Olturot	Ilaut	Ngurnit	Korr	Loglogo	%R.F**
CCPP	6	2	3	*	2	1	100
Lice & Fleas	5	7	6	*	3	3	100
Diarrhoea	3	3	5	*	4	4	100
Orf	1	4	2	*	5	7	100
Konkoro[1]	4	-	9	*	6	8	83.3
Lkang[1]	2	1	1	*	1	-	83.3
Ticks	8	-	4	*	-	2	66.7
Worms	7	6	8	-	-	5	66.7
Foot rot	9	-	-	*	-	6	50.0
Pox	-	5	-	-	-	-	16.7
Coneurosis	-	-	-	*	-	-	16.7
Cough	-	-	-	*	-	-	16.7
Ndis[1]	-	-	7	-	-	-	16.7

*Not ranked because the participants were unable to reach a consensus ** %R.F. = Percentage Relative Frequency

(-)Not perceived to occur

[1] Local name in Rendille or Samburu vernacular; *konkoro* (central nervous disorder), *lkang (*rabies like disease*), ndis* (yellow liver)

plastic bags and later examined for the presence of gastrointestinal worm eggs and *Coccidia oocyts.* In the very young kids, faecal dropping were collected around the anal surface. Faecal examinations were carried out in the field within 12 hours. Thereafter, the faecal samples were pooled, prepared and transported to the major laboratory at KARI-NVRC Muguga for culturing. The cultures were established and larvae harvested for identification using standard helminthological laboratory techniques[24].

Data recording and analysis

Data from the field activities and laboratory results were recorded in designed forms. The information was coded and separate tables developed as templates of relational database using the Access® programme[25] . Data verification was carried out by rechecking against the original data records on the forms and corrections made on the template.

A descriptive analysis was carried out and proportional frequencies calculated using the Windows Excel®[25] (Microsoft inc. 2001) while prevalence and incidence rates were estimated using the Stata®[26] and Genstat®[27] softweare. Live weights were analysed to determine means and daily weight gains (MDWG) and analysis of variances calculated. Significant similarities and differences were generated in morbidity/ mortality prevalences and incidences or MDWG using the t, F, and χ^2 tests. The SAS®[28] programme was used for regression analysis using the generalised linear models (GLM) and generalised estimating equations (GEE) procedures to determine association with the independent variables. Significant differences in all cases were accepted at $p<0.05$.

Results

The participants listed 14 diseases and agents affecting preweaned kids. The

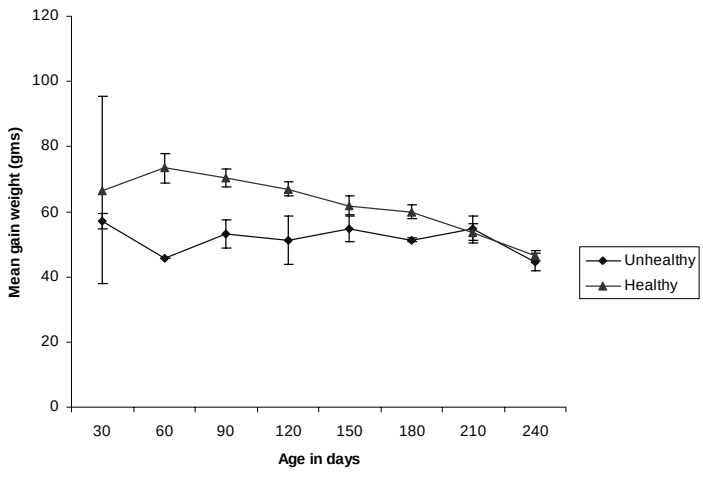

Figure1: Mean ±CI daily weight gain curves (gm) of kids by age and health status from longitudinal study

ranking of each disease or agent by sub location and percentage relative frequency of occurrences are presented in Table 1. Contagious caprine pleuropneumonia (CCPP), Lice and fleas, orf and diarrhea causing agents were ranked as the most common diseases/casual agents causing poor performance and death in kids. A rabies-like disease (lkang) and ticks were also ranked highly except in Loglogo sub location. Highly ranked diseases/casual agents were those associated with causing deaths in kids. Predators were also reported in 83.3% of the area and were ranked between 4th and 7th positions in importance as general constraints to pastoral livestock production.

In all sub locations, pastoralists claimed that they practiced traditional mobility and quarantines, cleaning of bomas and wells, avoidance of known poisonous plants and parasite-infested areas, use of veterinary drugs ("antibiotic-terramycin") to alleviate the perceived constraints. However, they also expressed their limitations in applying these practices as due to reduced frequency of mobility, inadequate water and pastures, insecurity, poor animal health delivery systems and marketing and also drought.

A total of 1244 kids were studied during the cross-sectional phase. Samples for examination were taken from 216 kids. Overall, mean morbidity prevalence rate was 33.0%. Mean morbidity prevalence rates were significantly associated ($p<0.05$) with the sub location, the highest was in Olturot (56.3%) and the lowest in Kargi sublocation (16.4%). The interaction terms between sex and AEZ or sub-locations were not significant ($p>0.05$). Three types of clinical cases were observed and the highest overall mean apparent infection prevalence rate was in worms (2.3%) followed by orf (1.9%) and pox

Table2: The distribution of morbidity cases by clinical manifestations, cause-specific and age-specific incidences in preweaned kids in Korr and Olturot sub locations

Disease/Condition	Age (months)							
	0 to 1		2 to 3		4 to 8		Total	
	No.Sick	*IR(%)	No.Sick	IR(%)	No.Sick	IR(%)	No.Sick	IR%)
Pneumonia	1	0.4	25	3.5	27	4.4	53	6.2
Orf	0	0.0	2	0.3	26	4.3	28	3.3
Cough	0	0.0	2	0.3	22	3.6	24	2.8
Diarrhoea	4	1.6	9	1.3	5	0.8	18	2.1
Corneal opacity	0	0.0	9	1.3	0	0	9	1.1
Abomasal obstruction	0	0.0	0	0	7	1.2	7	0.8
Pox	0	0.0	3	0.4	4	0.7	7	0.8
Warts	0	0.0	3	0.4	1	0.2	4	0.5
Ringworm	0	0.0	3	0.4	0	0	3	0.4
Hereditary deformity	1	0.4	0	0	1	0.2	2	0.2
Cold stress	0	0.0	2	0.3	0	0	2	0.2
Worms	0	0.0	0	0	2	0.3	2	0.2
Cocciodiosis	0	0.0	0	0	2	0.3	2	0.2
Wounds	0	0.0	1	0.1	1	0.2	2	0.2
Lkang (rabies like)	0	0.0	0	0	1	0.2	1	0.1
Moniezia segments	0	0.0	0	0	1	0.2	1	0.1
Snake bites	0	0.0	0	0	1	0.2	1	0.1
Umbilical hernia	1	0.4	0	0	0	0	1	0.1
Trauma	0	0.0	0	0	1	0.2	1	0.1
Total	7	2.7	59	8.4	102	16.8	168	19.7
*IR = Incidence rate								
Number at risk	270		713		636		902	
½ Withdrawals	14.5		6.5		27.5		48.5	

(0.5%). Overall, mean apparent prevalence rates of nematodes and coccidiosis on basis of laboratory diagnosis were 2.8 and 23.2%, respectively. From the results of generalised linear models (GLM) there was no association of the infections with AEZ, sex, sub-location or previous treatment ($p>0.05$). Mean mortality prevalence rate of kids was 50.3%. The sub locations were significantly associated with mortality of kids ($p<0.05$) with the highest in Korr sub-location (61.8%) and lowest in Ilaut (35.1%).

The annual crude morbidity incidence rate for the kids was 19.9% (Table 2). It was significantly higher ($p<0.05$) in Korr (29.4%) than in Olturot flocks (14%). There was no significant difference ($p>0.05$) of crude morbidity incidence rate of female (19.5%) and male kids (19.9%).

The cause-specific morbidity incidence rate from birth up to eight months of age was highest for pneumonia and cough followed by orf and diarrheoa causing agents. The age-specific morbidity incidence rate was highest in the four to eight months old age group followed by two to three months old and least in the less than one month old. The highest cause-specific morbidity incidence rates by age were; Diarrhoea causing agents in the up to one month old and pneumonia in the two and three months old and four to eight months age group. Postmortems carried out on the carcasses of kids dying of rabies-like disease (Lkang) confirmed that the so called mysterious disease was abomasal impaction due to physical blockage of the gastrointestinal tract resulting in death. It occurred during the dry season after the kids were watered. The poor dry forage expanded upon absorption of water thus causing physical blockage of the gut. Clinical Cases occurred and were distributed throughout the year with the highest proportions during the early dry season (July, 19%) followed by the wet season (December, 16.1%; April, 13.6 %).

The faecal samples collected from eight month old kids with diarrhoea and claimed to be worm infested did not have nematode eggs on examination. These kids with worm manifestations were only positive for coccidia oocysts. The earliest cases of nematodosis and coccidiosis diagnosed on basis of EPGs and coccidia oocysts were in 1.3 and 2.0 months old kids respectively. Overall crude morbidity incidence rates of nematodosis and coccidiosis in kids on basis of EPGs and coccidia oocysts counts were 15.8 and 56.8 per cent, respectively.

The crude mortality incidence rate for the kids was 10.5% (Table 3). The causes-specific mortality incidence from birth up to eight months of age was highest for predation followed by abomasal obstruction (Lkang) and pneumonia. The age-specific mortality incidence rate was highest in the four to eight months old followed by up to month and two to three group of kids. The causes of death of 6.8 per cent of the kids were not known.

The crude mortality incidence rate of kids within sexes or birth orders was not significantly different ($p>0.05$). Like in prevalence, from the generalized linear regression models, sub location was the only significant factors associated with incidence rate of mortality of kids ($p<0.05$) and it was significantly higher ($p< 0.05$) in Korr (14.6%) than that in Olturot flocks (8.2%).

The mean birth weight of 44 kids whose births were observed during the monthly visits in the longitudinal study was 2.2 ± 0.8 kg. There was no significant difference of mean birth weights within sex, season or sub location ($p>0.05$).The overall mean daily

weight gain (MDWG) of kids in the longitudinal study and explanatory factors are presented in Table 4. The overall MDWG of kids at 240 days of age was 52.9±0.3gd-1. The MDWG of healthy kids was significantly higher than that of the unhealthy ones ($p<0.05$) at 240 days. Also, the overall MDWG of kids within sexes, breeds or litter size were significantly different ($p<0.05$). All twin births were EAG kids. The MDWG of kids born during (1st dry season) DS1 was higher than that of kids born in the other seasons ($p<0.05$) while kids born during 2nd dry season (DS2) (40.4±0.5 gd-1) had lower MDWG than those born in the wet seasons ($p< 0.05$).

The MDWG curves of kids by age and health status fitted with confidence limits (95%) are presented in Figure 1. The mean MDWG of healthy and unhealthy kids increased from birth up to 60 days of age and thereafter dropped until weaning. The

Table 3: The distribution of mortality cases by clinical manifestation, causal-specific and age–specific incicideces for preweaned kids in Korr and Olturot sublocations

Disease/Condition	Age (months)							
	0 to 1		2 to 3		4 to 8		Total	
	No. dead	*IR(%)	No. dead	IR(%)	No. dead	IR(%)	No. dead	IR(%)
Predation	0	0	5	0.7	11	1.8	16	1.9
Abomasal obstruction	0	0	0	0	7	1.2	7	0.8
Pneumonia	0	0	3	0.4	2	0.3	5	0.6
Cold stress	0	0	2	0.3	0	0	2	0.2
Diarrhoea	0	0	0	0	1	0.2	1	0.1
Moniezia segments	0	0	0	0	1	0.2	1	0.1
Unknown	13	5.1	15	2.1	30	4.9	58	6.8
Total	13	5.1	25	3.5	52	8.5	90	10.5
*IR = Incidence rate								
Number at risk	270		713		636		902	
½ Withdrawals	14.5		6.5		27.5		48.5	

healthy kids had significantly higher ($p<0.05$) MDWG than the unhealthy ones from 60 to 180 day of age. Kids born during WS1 had significantly the highest MDWG during the early age (up to 60 days), which dropped to equal that of DS2 kids at weaning age. The mean live weights of WS1 and DS2 kids at 240 days of age were 11.9±0.1 and 11.9±0.2 kg respectively. Kids born in the DS1 and WS2 had significantly similar ($p>0.05$) and moderate MDWG in the early age (up to 60days) and thereafter, MDWG of kids of DS1 increased steadily and were significantly ($p<0.05$) the highest at 120 and 150 days of age. The MDWG of DS1 kids thereafter dropped from the age of 180 days up to weaning. The MDWG of WS2 increased gradually throughout the growing period with a drop at 120 and 150 days of age but was significantly ($p<0.05$) the highest at weaning. Mean live weights of DS1 and WS2 kids at 240 days of age were 14.1±0.8 and 15.8±0.6 kg respectively.

From the results of GLM for kids on

Table 4: Mean±SE daily weight gains (gms) of kids from birth to 240 days of age and explanatory factors from the longitudinal study

Factor	Level	N	Mean ± SE	t	p	F	p
Sublocation	Korr	877	53.4±0.7 [a]				
	Olturot	2221	52.7±0.4 [a]	1.973	0.331		
	Total	3098	52.9±0.3				
Breed	EAG	3057	53.1±0.3 [a]				
	EAG*G	41	37.9±2.1 [b]	5.000	0.000		
Litter size	Single	2944	53.3±0.4 [a]				
	Twin	154	43.8±1.5 [b]	5.998	0.000		
Sex	Female	1490	49.8±0.5 [a]				
	Male	1608	55.7±0.5 [b]	-8.572	0.000		
Health	Unhealthy	341	51.8±1.0 [a]				
	Healthy	86	58.3±1.8 [b]	-2.918	0.004		
[1]Season	Dry Season -1	344	60.0±1.1 [a]				
	Wet Season-1	1649	56.7±0.5 [b]				
	Dry Season -2	741	40.4±0.5 [bc]				
	Wet Season-2	364	54.2±1.0 [b]			162.721	0.000

[1]Different superscripts within factor levels indicate significance: $p<0.05$

MDGW, the explanatory factors that were significantly associated with MDGW in the analysis were: sub locations and health. Olturot was associated with kids with higher MDGW than those in Korr ($p<0.05$) while healthy kids (58.3 ± 1.8 gd^{-1}) had higher MDWG than the unhealthy ones (51.8 ± 1.0 gd^{-1}) ($p<0.05$). There was an interaction term on sub location and season. Kids born in Olturot during DS1 ($59.1+0.5$ gd^{-1}) and WS1 (63.2 ± 1.3 gd-1) had significantly higher MDWG than those in Korr (DS1 = 55.0 ± 1.8; WS1=52.6 ± 0.8 gd^{-1}). The seasons and health interaction were also significant for kids born during WS1 ($p<0.05$). Healthy kids (52.5 ± 1.8 gd^{-1}) born during this season had higher MDWG than the unhealthy ones (48.1 ± 1.1 gd^{-1})

Discussion

On the basis of these findings, highest prevalence and incidence rates of diseases were worms and pneumonia respectively while coccidiosis was rated the highest subclinical disease. Past studies in PLP system did not record annual morbidity rates of preweaned kids as described in this work[10,15,17]. Overall, diseases and casual agents of importance perceived by pastoralists were the same as those recorded in cross section and longitudinal phases of the study . During the course of this study there was no outbreak of (CCPP) an important endemic and notifiable disease although the pastoralists were aware of it economic importance. The causes of deaths in most neonates up to the age of one month were not known since it was also not practically possible to conduct postmortem. Most of the pastoralists tend to ignore these losses and it was only after insistence that such information were given for records. This omission often results to underestimation of the perinatal losses. Since nonparasitic diarrhoea was the major disease in this age group, one may suggest that bacterial or viral gastroenteritis may have been the most probable cause of death. Recent work by Munyua[29] working in similar environment showed that *E. coli* gastroenteritis with or without septicemia was an important cause of preweaned kid mortality.

Prevalence mortality rate in this study was higher while annual incidence mortality rate was lower than those recorded by other researchers in the past[11,16,18]. Most of the information was from interviews but this study gave an accurate record of events as they occurred. The high prevalence mortality rate was within the range expected during the drought periods[11,29]. During the study period a minor drought occurred in 1999/ 2000 while average normal rains were received in 2001/2002. The low incidence mortality rate was partly due to this favourable environment probably with interactions of increased animal health practices. Also, failure of the pastoralists to lay importance on death of the neonates may have contributed to underestimation of the situation. Non-infectious cause (predation) was recorded as the most important cause of death and it was claimed to be on the increase. These new findings on changing trend of infectious and non-infectious causation of morbidity and mortality show that there is need to review husbandry management strategies with emphasis on predation to improve productivity.

The fact that healthy kids had higher MDWG than the unhealthy kids was a good indication of the benefits of using veterinary drugs in PLP systems. However, such

interventions should be strategised in order to avoid contrasting results as those recorded in the past[9,12,29] In addition, the interaction of health and season as manifested should be considered during such veterinary interventions with emphasis on unhealthy kids born during 1st wet season. Furthermore, that there was an interaction of health and sub location also demonstrated the complex compounding effects of other factors i.e. nutrition status and husbandry practices. These factors have been reported to have effects on mortality and performance in ASALs systems[31] and probably contributed to the phenomenon described in this study. Hitherto, recognition of indigenous knowledge has led to acceptance of positive traditional management practices in improved animal husbandry strategies in the ASALs[31,32,33]. Therefore, a performance-related health management of preweaned kids in pastoral herds should be integrated with these additional practices for any anticipated increase in productivity to be achieved.

On the basis of this study it is concluded that a veterinary disease control regimes can improve preweaned kid productivity in PLP systems. It was revealed that such veterinary health interventions should include control of coccidiosis. Also, the interventions should target the known diseases demonstrated within the ranks and measures established. Further studies should be carried out to determine the unknown conditions experienced and cost-effective levels of applications of veterinary drugs in relation to environmental dictates as observed in this study.

Acknowledgements

The authors acknowledge the support of pastoral communities, staff of the Ministry of Agriculture and Rural Development, ILRI and KARI. The financial support of European Union/Agricultural Research Support Programme II (EU/ARSPII) and KARI is highly acknowledged.

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Bull. Anim. Hlth. Prod. Afr., (2008), 56, 84-

EVALUATION OF THE EFFECT OF EXOGENOUS ENZYMES IN CATTLE DIET

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EVALUATION DE L'EFFET DES ENZYMES EXOGENES DANS L'ALIMENTATION DU BETAIL

Résumé

Une étude a été menée en vue de déterminer l'effet de la préparation d'une enzyme brute sur les fanes de Gamba *(Andropogon gayanus)*. Douze génisses croisées Bororo blanc x Bunaji étaient soumises de façon aléatoire à trois rations, à savoir : des fanes de Gamba non traitées (Groupe A), des fanes de Gamba traitées avec un faible taux d'enzyme (Groupe B) et des fanes de Gamba traitées avec un taux élevé d'enzyme (C) en plus d'un supplément de concentré pendant 8 semaines. Il n'y avait pas de différence significative (P>0,05) quant à la consommation alimentaire pour les différentes rations. A la fin de l'expérience, les animaux du groupe C avaient le gain de poids (P<0,05) le plus élevé (7,91 Kg par rapport à 2,9 Kg et 3,36 Kg pour les groupes A et B respectivement). L'efficience alimentaire et les taux de rétention de l'azote étaient beaucoup plus élevés (p<0,05) chez les animaux du groupe C. L'absence de différence notable quant à la consommation alimentaire pour les trois rations dans la présente étude et la documentation disponible semblent indiquer que l'effet des enzymes exogènes a été exercé après rumination. On peut conclure que les fanes de Gamba avec un taux élevé d'enzyme ont un énorme potentiel pour accroître le gain pondéral et l'efficience alimentaire des génisses nourries de fourrage à faible valeur nutritive, notamment pendant la longue saison sèche. Il faudrait, toutefois, mener d'autres études sur les mécanismes par lesquels les enzymes exogènes influent sur les gains pondéraux.

Mots clés : Enzymes exogènes, alimentation du bétail.

Summary

In a study to evaluate the effect of a crude enzyme preparation on Gamba hay *(Andropogon gayanus)*, 12 yearling Fulani white x Bunaji heifer crosses were randomly allotted to three dietary treatment groups comprising untreated Gamba hay (A), low level enzyme treated Gamba hay (B) and high level enzyme treated Gamba hay (C) in addition to a concentrate supplement for 8 weeks. There was no significant difference (P>0.05) between the feed intake in the different diet groups. At the end of the experiment, animals in group C had the highest (P<0.05) weight gain (7.91kg compared to 2.9kg and 3.36 kg for group A and B respectively). Feed efficiency and nitrogen retention values was significantly (p<0.05) higher in animals of group C. The absence of significant difference in the feed intake across the treatment in this study and evidence from literature suggests that the effect of exogenous was exerted post-ruminal. It can be concluded that the high level enzyme treated Gamba hay has great potential in increasing the weight gains and fed efficiency of heifers fed poor quality forage particularly during the long dry season. But the mechanism through which exogenous enzymes influence live weight gains needs to be further investigated.

Keywords: Exogenous enzymes; Cattle diet.

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Introduction

Adu and Adamu[1] demonstrated that tropical animals do not consume enough poor quality roughage to meet body maintenance requirements. Tropical forages are characterized by cell walls rich in silica, lignin, and cutin, are deficient in essential nutrients and have limited energy value. These factors limit fermentation of structural carbohydrates and production of volatile fatty acids (VFA) and microbial mass growth is compromised[2]. Consequently digestion in the rumen is low and a prolonged forage retention time in the rumen limits intake.

Various methods of improving fibre digestion and efficiency of utilization focus on enhancing rumen microbiological degradation either through physical, chemical or biological treatment or through supplementation with a protein or readily available carbohydrate source[3] but all these methods have been met with limited success due to unavailability of inputs or complexity of the process. Recently supplementation with exogenous enzymes has shown a lot of promise as a means of increasing forage utilization and improving the productive efficiency of ruminants. Studies have shown that adding exogenous fibrolytic enzymes to ruminant[4] diets increased milk production[5,6,].and average daily gain[7,8], in some cases. These increases in animal performance are attributed to increased feed digestion and utilization. Numerous other studies reported increased digestion of dry matter (DM) and fibre measured in situ or in vitro[9,10,11,6]. A variety of mechanisms have been put forward to explain the changes which range from increased ruminal hydrolysis of fibre[12] increased number and synergy between degrading bacteria[13] enhanced binding and penetration of the substrate[4]. McAllister et al8 reported that possible modes of action of exogenous fibrolytic enzymes may be through (i) digesting or weakening structural barriers that impede microbial digestion in the rumen prior to consumption or (ii) it might hydrolyze feed directly or work synergistically with ruminal microorganisms to enhance feed digestion (iii) exogenous enzymes may improve nutrient absorption by reducing intestinal viscosity or by hydrolyzing substrates that escape ruminal digestion (iv) exogenous enzymes increase the rate of feces decomposition. It is generally accepted that production responses to enzyme supplementation are greatest in situations where energy is the limiting nutrient[14] or where fibre digestion is compromised. This study was aimed at evaluating the effect of feeding yearling cattle with different levels of crude enzyme treated Gamba hay supplemented with concentrate.

Materials and methods

Twelve yearling heifers were randomly allotted to three dietary treatment groups and each group balanced for live weight. The animals were All the treatment groups had access to a concentrate supplement fed at 1% of their body weight; the supplement was compounded by mixing 25% cottonseed cake, 63% maize offal and 12% maize (table 1 shows chemical composition of the concentrate). Animals in group A (the control group) were fed untreated Gamba hay, group B animals were fed low level enzyme treated Gamba hay and group C animals were fed high level enzyme treated Gamba hay. The animals were completely confined during the experiment which lasted for 8 weeks.

Crude Enzyme Preparation and Assay

A spore suspension (2 x 10^5) of Aspergillus niger SL1 was used to inoculate mineral salt cellulosic media containing (g/l) KH_2PO_4, 10.0$(NH_4)_2$ SO_4 10.5 Mg $SO_4 7H_2O$ 0.83, CaCl2 0.5, $FeSO_4$.$7H_2O$ 0.013 Mn SO_4 H_2O 0.00$_4$ $ZnSO_4$ $7H_2O$, 0.004$CaCl_2$.$6H_2O$, 0.0067 yeast extract 0.5 and 40 g of a cellulosic sample (wheat bran). The medium was incubated for 72 hours at 30^0C.and centrifuged for 15mins at 2500rpm to separate the mycelia and supernatant fluid. The supernatant fluid obtained is referred to as crude enzyme extract, and was used for cellulose enzyme assay. The cellulase activity was determined calorimetrically by measuring the changes in reducing sugar groups by hydrolysis of carboxymethyl cellulase (CMC) substrate as described by Ali *e t al*[15]. Cellulase activity was determined as 4.0 units per ml in the crude extract. The crude enzyme extract obtained is thus a mixture containing xylanase, cellulose, hemicellulase enzymes of undetermined proportions. The enzyme solution obtained was diluted with tap water in ratio 1ml crude enzyme solution to 25ml tap water to obtain the high level enzyme treatment solution, which had 1.6 x 10^{-1}units/ml enzyme activity. The low level enzyme solution was obtained by diluting 1ml crude enzyme solution with 50ml tap water which had 8 x 10^{-2} units /ml enzyme activity.

Gamba hay treatment and feeding

The Gamba hay was treated using a knap sack sprayer to spray appropriately diluted crude enzyme solution on to hay that had been spread evenly on a polythene sheet on the floor. Before spreading the Gamba hay each bale was weighed to ensure 5kg of Gamba hay was sprayed with 2 liters of appropriately diluted enzyme solution. The Gamba hay (treated or untreated) offered at 20% of voluntary intake. In order to ensure that animals had access to both feed stuffs, Gamba hay fed at 2% body weight was offered with concentrate at 7.00 am.

Animal Preparation

Animals (white Fulani x Bunaji heifer crosses) were allowed to acclimatize to the new environment in the holding pen for one week prior to data collection. All animals had earlier on been dewormed, deticked using albendazole 10ml/20kg, steladone and given a prophylactic dose of oxytetracycline. Animals were individually housed in pens measuring 3.5m x 2.5m. Feed intake was measured daily and live weight gain was measured fortnightly.

Table 1: Chemical Composition of Gamba and Concentrate offered to the Experimental Animals

	Gamba	**Concentrate**
Dry matter	98.3	92.3
Crude protein	3.5	12.56
NDF	67.9	43.02
ADF	39.4	14.63
Ash	7.85	6.787
EE	1.69	6.61

Sample collection

The final weighing coincided with the beginning of a nitrogen balance study, which was conducted in metabolic cages measuring 2.5m length and 1.5m breath. The floor of the cages had slats that allowed feaces to pass through to collecting trays placed beneath the cages; urine was collected in a lower tray fixed to a container via a funnel. The heifers were allowed two weeks adjustment period followed by 7-day total feaces and urine collection. Procedure for collection and sampling of faeces and urine were as outlined by Adu and Adamu[1], while data analysis was as described by Steel and Torrie[16].

Results

Feed Intake and dry matter digestibility Table 2 shows that there was no significant (P>0.05) difference in average daily total dry matter feed intake between the three groups although the group fed the control diet was numerically higher intake. Dry matter digestibility was significantly (P<0.05) higher with enzyme treated Gamba hay diets compared to the control, but the difference between the Gamba treated diets was not significant although higher with the high level enzyme treatment. A similar trend was observed in the digestibility of crude protein, crude fiber, neutral detergent fibre and acid detergent fibre as the enzyme treated Gamba diets were significantly higher than that of the control diet. There was no significant difference in the digestibility of ether extract across the different treatment group.

Live weight gains

Group C animals (fed high level enzyme treated Gamba) had the higher (p<0.05) daily weight gain (0.14 kg) compared to animals in groups A (0.05) and B (0.06 Table 2). Animals fed the low enzyme treatment Gamba hay also had significantly (P<0.05) higher live weight gains compared to the animals fed the control diet.

Nitrogen retention

The nitrogen intake figures (table3) for the animals fed high level enzyme treated Gamba hay was significantly higher

Table 2: Weight gains and feed intake

	Control Group A	Low level Group B	High level Group C	SEM
Average initial weight (kg)	78.0	77.40	77.08	NS
Average final weight (kg)	80.90	80.76	85	
Average daily gain (g/day)	51,7	60	141	**
Ave. daily DM intake from concentrate (kg)	0.782	0.792	0.799	NS
Ave. daily DM intake from Gamba hay (kg)	1.72	1.59	1.597	NS
Average daily DM intake (kg)	2.503	2.382	2.39	NS
Feed Efficiency (gain/intake)	0.0206 [c]	0.025 [b]	0.0589 [a]	*

[abc]Means with different superscripts along the row differ significantly (P<0.05)
ns= not significant

* = significant 5% level ** = Significant 1% level

Table 3: Nitrogen metabolism and digestibility of untreated and treated Gamba hay.

Parameter	Control	Low level	High level	SEM
	Group A	Group B	Group C	
N intake g/day	158.54	155.15	156.35	14.05ns
Fecal N g/day	87.19a	76.022b	57.85b	4.32**
Urinary N g/day	19.02a	15.74b	14.14c	1.45**
N excreted g/day	106.21a	91.76b	71.99c	5.12**
N retained g/day	52.32c	63.38b	84.36a	4.86**
% N retained of N intake	33.00^{c}	40.08^{b}	54.01^{a}	4.43**
Nutrient Digestibility%				
Dry matter	57.32b	59.52a	61.17a	4.82**
Crude Protein	62.25b	65.72a	67.43a	3.77*
Crude Fibre	45.35b	57.75a	58.66a	3.68**
Organic Matter	58.00a	61.35a	63.45a	1.77*
Ether extract	62.74	62.40	62.0	1.97ns
Neutral detergent fibre	51.34b	59.76a	62.44a	2.69**
Acid detergent fibre	43.21b	46.55a	48.33a	3.28*

abc Means with different superscripts along the row differ significantly (P<0.05)
* = significant 5% level, ** = Significant 1% level

(P<0.05), compared to the other groups. The animals fed the high level enzyme treated hay (group C) had significantly (P<0.05) lower urinary and fecal nitrogen contents (57.85g/day and 14.14g/day respectively) compared to the other treatment groups and consequently had significantly higher Nitrogen retention percentage (54 %). The animals fed the low level enzyme treated Gamba hay also have significantly higher nitrogen retention compared to the control group.

Discussion

Feed Intake and dry matter digestibility

The non-significant differences (P>0.05) in the dry matter intake of all the animals suggests that the enzyme treatment has not affected palatability and rate of fibre degradation in the rumen. Evidence from literature shows that enzymes affect feedstuff before they are ingested as well as post ruminal during passage through the intestinal tract[18, 19]. Most likely the enzyme treatment exerted its effect post ruminal during passage along the intestinal tract. Beauchemin *et al*[20]. had a similar experience using cows with rumen and duodenal cannulae, they noted that while an enzyme product applied to a TMR had little effect on ruminal fiber digestion, total tract digestibility was increased suggesting that at least part of the mechanism of action of the enzymes was associated with post-ruminal digestion. Iwasa *et al*[7], also reported a non significant increase in dry matter intake due to exogenous enzyme treatment;

they concluded that enzyme treatment might not necessarily increase forage digestibility in the rumen but influence the amount of nutrients absorbed and utilized post ruminal. McAllister *et al*[8] reported that exogenous enzymes might improve nutrient absorption and subsequently live weight gains by reducing intestinal viscosity or by hydrolyzing substrates that escape ruminal digestion.

Live weight gains

In this study the difference in average daily gains (ADG) from animals fed the untreated Gamba hay and those fed the high level enzyme treated Gamba hay is about 135.7% which comparatively shows an increase in the results obtained from a similar study in which ADG of cattle fed alfalfa hay increased by 24 to 30% with lower levels of added enzyme[20]. This variability in results may likely be due to enzyme-substrate specificity particularly as different feedstuffs were used. The fact that the dry matter intake was not significantly affected by the enzyme treatment suggests that the enzyme exerted it effect post ruminal. The higher digestibility of crude protein, crude fibre, NDF and ADF in enzyme treated Gamba hay might have improved nutrient absorption by reducing intestinal viscosity or by hydrolyzing substrates that escape ruminal digestion. It has been postulated[21] that applying enzymes to feed could create a slow releasing mechanism by which the enzyme is released into the rumen fluid as the feed is digested. They may also remain active in the lower digestive tract contributing to the ruminal digestion of fibre or they could indirectly improve nutrient absorption in the lower tract by reducing viscosity of intestinal digesta[8].

Nitrogen retention

The significantly higher levels of Nitrogen retention in animals fed enzyme treated hay observed in this study most likely resulted from the exogenous enzymes improving nutrient digestion judging by data from the digestibility study (Table 3) which showed higher digestibility for crude protein, crude fibre, ADF and NDF. Consequently they probably had better absorption through reduced intestinal viscosity or a better level of substrate hydrolysis.

Conclusion

It can be concluded from this study that exogenous enzymes have great potential to increase weight gains in cattle fed poor quality Gamba hay. Further studies would be needed to determine the exact mechanism through which the weight gain is achieved.

Acknowledgement

The efforts of the Provost, Staff and Students of College of Agriculture and Animal Science Ahmadu Bello University Mando Kaduna towards the success of this research is very much appreciated.

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