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ORIGINAL ARTICAL Page

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PANAFRICAN STRATEGY FORTHE PROGRESSIVE CONTROL OF PESTE DES PETITS RUMINANTS (PAN AFRICAN PPR STRATEGY)

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Résume

La peste des petits ruminants (PPR) est une contrainte major aux moyens de subsistance et a la sécurité alimentaire des petits producteurs/éleveurs. L'épidémiologie et la biologie du virusde la PPR a beaucoup de choses en commun avec le virus de la peste bovine (PB), qui a étéglobalement éradiqué. Ce document présente une stratégie pour le contrôle progressif de la PPR en s'appuyant sur les enseignements tirés de l'éradication de la peste bovine. Le contrôle progressif de la PPR repose sur une approche modulaire consistant en une série de phases autonomes, chacune des phases ayant son propre ensemble de résultats. Les principaux résultats intermédiaires seront des modèles avérées applicables pour la fourniture de services durables pour le contrôle de la PPR et le renforcement des capacités des institutions de santé animale afin de cibler les services de contrôle pour certains points critiques. Le projet favorisera une méthode de gestion adaptée qui intègre des approches d'apprentissage qui stimuleront l'innovation institutionnel e de la santé animale. Une orientation coordonnée vers des objectifs à long terme en santé animale ajoute de la valeur aux investissements en cours dans la lutte contre les maladies infectieuses.

Mots clés: Peste des petits ruminants, stratégie de contrôle africaine

Summary

Peste des petits ruminants (PPR) is a major constraint to the livelihoods and food security of small scale farmers. The *epidemiology* and biology of PPR virus has much in common with rinderpest virus (RP), an agent that has been global y eradicated. This document presents a strategy for the progressive control of PPR that builds upon the lessons learnt from rinderpest eradication. Progressive control relies upon a modular approach that consists of a series of self-sufficient phases each with its own set of sustainable results. Key intermediate results wilbe proven business models for sustainable PPR control service delivery and enhanced capacity of animal health institutions to target control services to critical control points. The program will foster an adaptive management approach that integrates learning approaches to drive animal health institutional innovation. The coordinated drive towards long term animal health goals wil add value to on-going investments in infectious disease control.

Keys words: Peste des petits ruminants, control strategy, Africa

Introduction

Peste des petits ruminants (PPR), or small ruminant plague, is a viral disease primarily affecting goats and sheep that causes significant economic impact in Africa and Asia. The virus is also known to cause fatal disease in camels and *asymptomatic* infection of cattle and wildlife. PPR was originally recognized in West Africa in 1942 and for many years was seen as a seasonal y epidemic disease in Sahelian regions. The disease was subsequently recognized in Central and Eastern Africa, the Middle East, South Asia in 1970s, 80s 90s respectively (Figure1). Thereafter it appeared in Central Asia and most recently it entered Tibet and now threatens China.

As a disease of small ruminants, PPR is noted for its impact on the livelihoods and foodsecurity of the poor and marginalized segments of society. In recent years, PPR has caused major outbreaks in East Africa and has spread to Morocco. The rest of North Africa, Southern Europe, and Southern Africa are now considered at risk of infection unless coordinated action is taken. The socio-economic losses associated with PPR mainly result from the high case mortality rates that is characteristic of the disease. This negatively affects income from production and value-addition in marketing chains. PPR is a constraint to trade, although this impact is mitigated in local and regional markets due to its wide geographic distribution at present. Small ruminants are recognized as ready sources of food and cash and women and disadvantaged households often rely on smal ruminants. Small ruminants are an important means to rebuild herds after environmental and political shocks. Thus, they are an important component ofpastoral coping mechanism. The main benefits of sustained PPR control wil be enhanced food security, coping *mechanisms* and poverty reduction.

The *etiologic* agent is a member of the *Morbillivirus* genus and a close relative of rinderpest virus, a disease of cattle that hasrecently been globally eradicated. The eradication of RP was aided by features of the disease and available control tools that contributed to successful control. There was only one sero type and live attenuated RP vaccines gave life-long protection against all strains of the virus. There was no carrier state: infection was short lived and resulted in either death or life-long immunity. The virus did not survive for long outside the animal host: it was readily destroyed by heat, sunlight, chemicals and disinfectants. Thus, the virus needed a continuous source of new susceptible animals to survive. Proven diagnostic tests were available. PPR shares all these characteristics.

At the level of animal health institutions,the eradication of RP has created an increased awareness and capacity for co-ordinated control interventions based upon sound epidemiological approaches that are driven by socio-economic incentives. In addition, considerable progress was made to enhance surveillance capacity, regulatory environments as well as private sector and community participation. There is now significant demand for a coherent, long-term strategy for the progressive control of PPR. This document presents a strategy for the progressive control of PPR. The initial objective is to establish sustainable control systems that benefit poor livestock keepers and national economies. The strategy is consistent with a long-term objective of eradication, but does not require a commitment to eradication at the outset. Each phase of the strategy is self-sufficient in that it will provide economical y justified, durable outputs with the resources provided. Further, the strategy consists of a set of regional programs anchored in the Regional Economic Communities (RECs) and integrated in a continental framework.

Objective

The progressive control of PPR through:

- Enabling research to add value to existing tools
- Proven, self-sustaining mechanisms for animal health service delivery
- Critical targeting of field interventions to maximize impact and cost-effectiveness
- Pan African management, coordination and learning systems that maximize the ability to exploit new knowledge

Guiding Principles

The principles guiding the PPR progressive control strategy depend largely on experiences to date in the animal health sector. Some salient principles are:

An adaptive management approach will be taken. The progressive control program will be structured to maximize uptake of lessons learnt during the implementation of the program. The technical and institutional strategy will be updated regularly to ensure maximum relevance to current knowledge and experience.

Surveilance and control interventions will be risk-based and epidemiologically targeted to maximize impact and economic efficiency. Specific epidemiological research will be carried out to identify critical points for control interventions.

The program will continue the tradition of being innovative in surveillance and control through the incorporation of action research within on-going field activities.

Partnership to mobilize the broad resources of animal

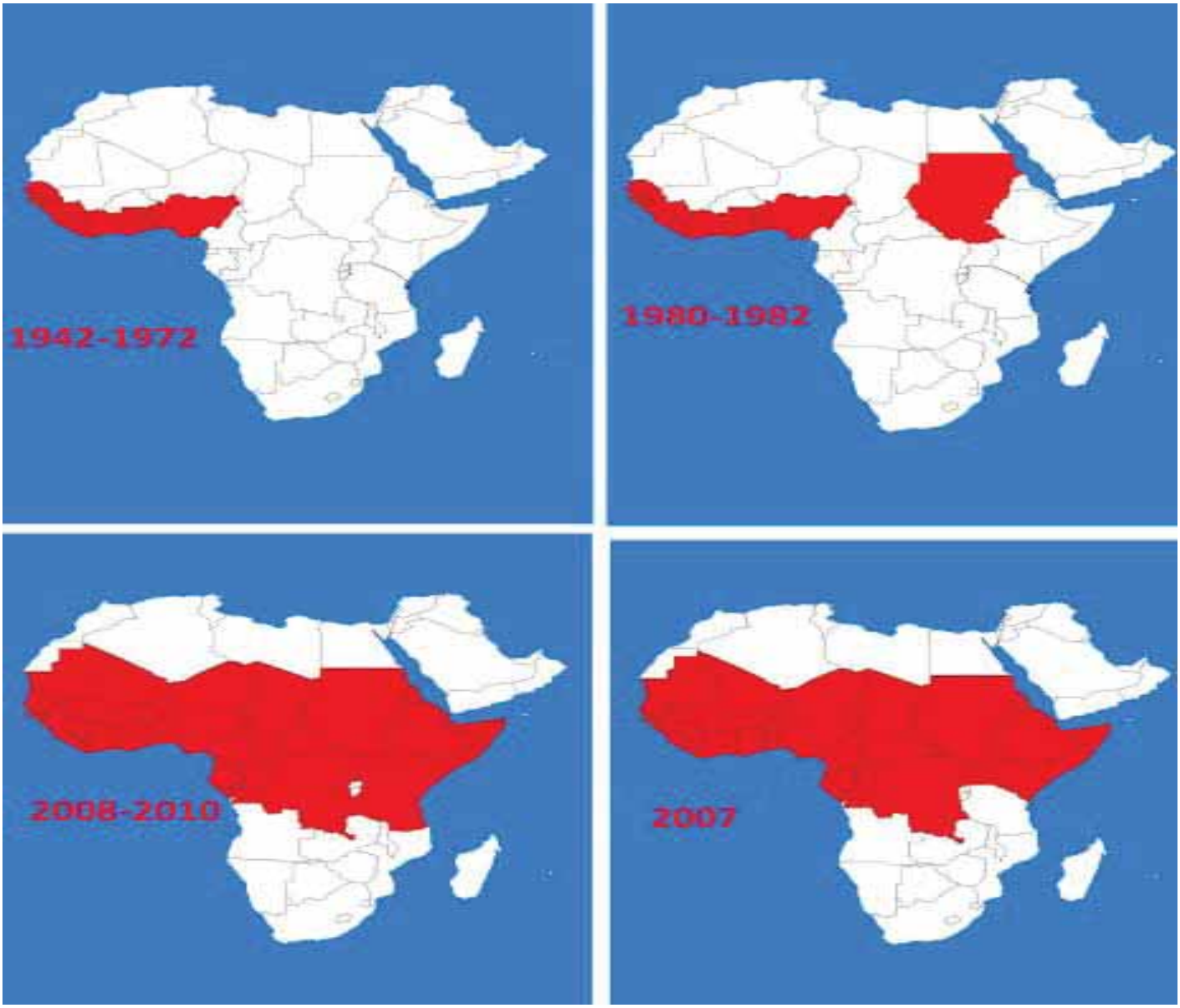

Figure 1: The progressive spread of PPR across Africa. The Southern African Development197 Community is now under threat. Concerted action is urgently needed to turn the tide o PPR in a planned progressive approach to control that maximizes return on investment.

health institutions at national, regional and international levels

Regional strategies will be tailored to local smal ruminant health priorities. PPR control will be combined with other activities such as vaccination against contagious caprine pleuropneumoia and/or sheep and goat pox, provision of therapeutic services for the control of ecto and endo-parasites and other endemic diseases impacting on small ruminant production and productivity etc. to increase efficiency, broaden impact and encourage fuller participation.

The Pan African program will be implemented in the context of global PPR progressive control programs and OIE principles.

Risk-basedTargeting of Surveillance and Control Interventions

It is a recognised principle that the probability of disease transmission is not uniform across national populations. There are often a number of risk factors that contribute to the overall risk of disease transmission in a particular community, production system or value chain. These risk factors are often quite simple attributes of the sub-population such as the amount of movement, exchange of animals, distance from services and inter-species contact or interaction with wildlife.

When the nature and distribution of risk factors for trans-

mission and maintenance of an agent are known, it becomes possible to target surveil ance and control measures to high risk settings. This maximizes impact and minimizes cost. Effective targeting of high risk communities through participatory disease surveillance was one of the factors in the success of rinderpest eradication, but can also make control programs more efficient where the goal is sustained suppression of disease and disease impact rather than eradication.

The risk factors for transmission and maintenance of PPR are partially understood, but more information on the interaction of wildlife and livestock as well as on the role of specific production systems/activities would contribute to effective targeting. The tools that can contribute to more effective targeting are

- Epidemiological studies on testable hypotheses
- Longitudinal studies to elucidate transmission dynamics
- Participatory epidemiological assessments
- Risk analysis
- Surveillance

Networks for standardized diagnostics were a significant contributor to the success of rinderpest eradication. Networking can promote the use of bench-marked tests that allows data to be compared with confidence across diverse ecological zones and production systems. This adds value to surveillance data and facilitates risk-based targeting. Targeting strategies should be annually reviewed in light of epidemiological intel igence on disease outbreaks and the risk of disease outbreaks. This is an integral part of the adaptive management approach of the Pan African PPR Strategy.

Animal Health Service Delivery

Animal health service delivery includes a range of activities to prevent, detect and mitigate disease. From this perspective, service delivery for PPR includes surveillance and diagnostic services, vaccination and biosecurity actions to reduce the risk of outbreaks, vaccination and biosecurity actions taken to contain outbreaks, as well as treatment of secondary bacterial pneumonias resulting from PPR infection. The global eradication of rinderpest provided a tangible goal that helped to drive innovations in the delivery of animal health services. These innovations included new partnerships to deliver surveil ance and control services under the overall management and supervision of veterinary authorities.

This process of animal health institutional change will continue as part of the progressive control of PPR. The Pan African PPR Strategy includes action to understand optimal bundling of control and surveillance interventions from both an epidemiological and socio-economic perspective. An evidence-based approach will be taken that captures synergies with on-going initiatives for the evaluation of services, improvement of governance in the sector, and action research initiated by the PPR progressive control activity to test specific solutions.

Vaccination programs will utilize vaccine produced from the Nigeria 75/1 strain as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010. This vaccine has been shown to be safe and ef ective. It is not thermostable. Adoption of thermostable vaccine manufacturing technology will enable more intensive coverage. Only vaccine that has been certified by the Pan African Veterinary Vaccine Center (PANVAC) will be used.

The large size of small ruminant populations in Africa and the rates at which these populations replace themselves place high demands on vaccination programs. As about 50% of a small ruminant population is new each year, flock immunity is unlikely to increase using a strategy of repeated annual vaccination campaigns. Large numbers of vaccinations may be required even in targeted programs and these vaccinations may need to be delivered in a concise time period to achieve high flock immunity.

From the outset, the campaign must address an important policy issue. The scale of vaccination demanded indicates that publicly funded vaccination campaigns will be difficult to sustain. This suggests that a coordinated commercial approach that mobilizes private sector delivery agents and investment may be needed. This suggests that service delivery models that include profit-oriented, payment- for-services options wil be needed to generate suf icient financing. This is essential y the current model in many countries: industry-driven, coordinated control programs. On the other hand, the epidemiology of PPR may require intensive focal vaccination to achieve sufficient flock immunity to interrupt transmission at critical control points. Intensive vaccination can be more challenging to achieve in service delivery systems that include payment for services. Thus, a range of proven service delivery models are needed as well as guidance on the conditions under which different models may be appropriate to conditions and objectives.

The Pan-African PPR Strategy advocates for a period of experimentation where a series of service delivery options are evaluated under different conditions. This wil build an evidence base for making informed policy decisions. The issues to be explored are:

- Cost sharing and commercialized approaches
- Options for service delivery partnerships
- Business models and animal health institutions
- Governance approaches

The delivery options should be evaluated from the perspective of:

- Epidemiological impact on PPR consistent with program goals
- Financial sustainability
- Quality and accessibility of animal health services
- Contribution to strong animal health institutions

Research

In line with the adaptive management approach, a number of learning and research activities will be undertaken to enhance the institutional capacity, technical tools and ability to target interventions. Underpinning this is the need for a clear and up-to-date understanding of the socio-economic context in which PPR progressive control is being undertaken so that interventions are delivered in a manner that al ows socio-economic forces to ef ectively drive the program to a successful, sustainable outcome.

Targeted research will be carried out in the following areas:

- Economic analysis of the impacts, benefit - cost of progressive control, cost-effectiveness of control options, and incentives for economic contribution and participation
- Epidemiologic research to better understand transmission dynamics, the dif erent roles of wildlife and livestock species, production systems, ecosystems and viral lineages with the goal of identifying critical points and optimal methods of intervention at critical control points.
- Action research and policy dialogue on public-private-community partnerships to deliver control and surveillance services. Questions include the best use of community animal health workers, gender issues, and the role of producers' associations, non-governmental organizations or other civil society actors in service delivery. The goal is to develop and test new business models for the sustained, commercialized delivery of disease control services
- Good diagnostic tools exist. However, refinement and elaboration of diagnostics will add value to the range of existing tools. Work to define minimal performance characteristics of diagnostic assays and establish bench marking procedures for diagnostic networks is needed. Standardization of tools should include tests for confirming outbreaks, tracking molecular epidemiology, supporting diagnostics for the field (pen-side tests) and sero-monitoring of vaccinated flocks.
- Currently recognized vaccines based on the Nigeria 75/1 strain of attenuated PPR virus have been found to be safe and effective in both research trials and during widespread field use. This technology is more than sufficient for the initiation of progressive control activities. However, improvements in vaccine thermostability and the ability to distinguish between animals immune through vaccination and those that are immune due to recovery from natural infection would be advantageous.

1. Several approaches to thermostable vaccines have been described to the level of proof of concept. More work is needed to compare alternative approaches and to develop a full database on thermostability as an evidence base to support the confident roll out of a thermostable vaccine on a broad scale.
2. Research to develop a marked vaccine and complementary serological tests as part of a differentiating infected from vaccinated animals (DIVA) strategy for vaccines based on the Nigeria 75/1 strain will be supported.

The Pan PPR strategy will support research as an integral part of the coordination activity. As was the experience with RP eradication, optimal impact of research resulted from research embedded in the action program. Independent research will also be encouraged. Key research stakeholders in PPR and morbil ivirus research are the reference laboratories recognized by the World Organization for Animal Health (OIE) and the UN Food and Agriculture Organization (FAO), the International Livestock Research Institute (ILRI), the Joint Division of FAO and the International Atomic Energy Agency (IAEA), National Diagnostic Laboratories and National Agricultural Research Services (NARS) and academic institutions where appropriate. As in the past, the role of non-governmental organizations (NGOs) as a source of innovation and a valuable partner for action research and field validation of new approaches wil continue.

Coordination and Partnership

One of the lessons learnt from the global eradication of rinderpest was that effective coordination adds value to animal health investment by channel ing otherwise divergent activities towards a coherent and sustainable objective. A sense of ownership among stakeholders contributes to the success of coordinated programs. The role of coordination is to convene inclusive dialogue to define and refine strategies, to harmonize approaches across regions and the continent, to assist in the process of governance including the development of policy, regulations and legislation. Coordination means knowledge

Figure 2: The Pan African PPR Strategy will be anchored in the regional economic communities in order to ensure strong ownership and local relevance. Control interventions that address local smal ruminant health priorities wil be bundled to ensure maximum impact at the household level and strong producer participation.

management and information exchange. Guidance on monitoring and evaluation activities is considered an important coordination task. Coordination includes strong action to advocate for program support in technical, political and financial terms at all levels.

AU-IBAR is best placed to coordinate the Pan African PPR Strategy due to the recognition of their:

- Continental mandate as the organization of African states for the coordination of the utilization of animal resources
- Proven leadership in RP eradication
- African ownership and strong commitment
- Convening authority in Africa

It is the policy of the African Union that programs are implemented through the regional economic communities (RECs) (Figure2). Following this policy, the Pan African PPR Strategy can develop locally appropriate strategies that address regional smal ruminant health problems thus assuring greater participation and impact. Working through the RECs, will also enhance ownership.

At the national level, veterinary services wil lead program activities. It is anticipated that veterinary services will act in a manner consistent with the principles of good governance and seek to facilitate and manage activities by creating an enabling environment for broad stakeholder participation. It is anticipated that national services wil work with private practitioners, veterinary associations, community-based organizations/programs, producers and producer associations, non- governmental organizations (NGOs) as wel as value chain stakeholders and trading partners to implement PPR progressive control.

Key partners for research and diagnostics service networks are the OIE and FAO Morbil ivirus Reference Centers, ILRI, national diagnostic laboratories, IAEA and NARS. In terms of vaccine, AU-PANVAC and vaccine producers are key partners. The program will undertake to facilitate the production of high quality vaccine as an essential input.

International organizations such as the OIE and FAO are essential partners. It is anticipated that the Pan African Strategy will be implemented in the context of a global program facilitated by the international organizations. The OIE's leadership in terms of establishing standards for participation in trade and achievable pathways to national freedom from disease will play a key role in shaping the strategy.

One of the lessons from rinderpest was that the NGOs played several key roles in facilitating eradication. In fact, eradication would not have been accomplished without them. They often stepped forward to create service delivery systems in some of the most daunting and dangerous environments. The NGOs also took the lead in the animal health institutional enhancements that were conditions for the success of rinderpest eradication as well as being positive outcomes in their own right. Finally, the NGOs have been key partners in the validation of new approaches and the empowerment of stakeholders to advocate for animal health institutional change.

Communication and Knowledge Management

Effective knowledge management will be an important component of the coordination strategy. AU-IBAR will act as the host organization in terms of collating information on the disease situation and disease impact.

National reporting through the ARIS-2 system will be strengthened with appropriate attention to digital reporting technologies for field use. In this manner, progressive control of PPR will

have knock-on benefits in terms of better information

exchange. Every ef ort wil be made to harmonize disease reporting systems across the region and globally.

AU-IBAR will host forums for sharing of knowledge on epidemiology, vaccines and diagnostics, and animal health institutions that bring together diverse professionals specialized in action, learning and discovery. The knowledge management unit will seek to develop new applications for information exchange that take advantage of the revolution in social networking technologies. The goal will be to maximize adaptive learning and to promote progressive evolution in practices and policies. To this end, the knowledge management unit will maintain up to date guidance documents on strategy, technical tools, and policy on the
web.

Capturing Lessons

The foundation of the adaptive management approach is a complete study of the lessons from RP eradication. To this end, the initial stages of the Pan African PPR Strategy call for objective assessments of the interventions implemented as part of RP eradication. These studies should look at institutional, economic, environmental and epidemiological impact of the global eradication.

One salient lesson from RP eradication was that not enough was done to measure impact. The Pan African PPR Strategy proposes that action should be taken to maximize learning in order to institutionalize adaptive management from the outset. To accomplish this, the program will gather baseline information and establish sets of process and performance indicators, impact indicators and desired outcomes.

Animal health institutional change and capacity development is critical to the success of progressive control. In order to maximize learning in this area, a more systematic approach to understanding animal health institutions and institutional change will be undertaken. This will include:

- Institutional mapping
- Documenting service delivery systems and their performance
- Documenting surveillance systems and their performance
- Analysis of incentives and drivers for participation as they relate to the above

Conclusion

PPR is an important constraint to food security and the livelihoods of poor farmers. Existing knowledge, experience and technology provide a solid platform for embarking on program of progressive control of PPR across Africa. The progressive control program will take an adaptive approach that seeks to learn from program experiences to continuously enhance impact and efficiency. The coordination of efforts to control PPR will add value to current investments to mitigate epidemics or activities seeking to promote food security. Responsible coordination and programming of inputs that reflect economic and epidemiological realities of PPR are needed.

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TYPING OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM MILK COWS WITH SUBCLINICAL MASTITIS IN DAKAR, SENEGAL

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TYPOLOGIES DES SOUCHES DE STAPHYLOCOCCUS AURES ISOLEES DU LAIT DES VACHES ATTEINTES DE MAMMITES SUB-CLINIQUES A DAKAR, SENEGAL

Résumé

Des enquêtes menées au Sénégal ont montré la forte correlation des staphylocoques aux mammites subcliniques chez les vaches laitières. La présente étude avait pour but de caractériser les souches de Staphylococcus aureus identifiés dans les fermes laitières à Dakar. Sur un total de 244 isolats de Staphylococcus spp isolés de 135 vaches laitières à mammites subcliniques dans six troupeaux de vaches laitières en zone péri-urbaine de Dakar, 109 souches de S. aureus ontété isolées et identifiées par des méthodes phénotypiques.
Par PCR, les gènes de la thermonucléase et des antigènes capsulaires majeurs 5 et 8 ont été identifiés respectivement chez 98,17%, 93.58% et 1,84% des souches de S. aureus. Parmi les gènes de virulence recherchés, les gènes spa, Luk D et Luk S ont été retrouvés respectivement chez 68,81%, 62,39% et 0,92% des souches de S. aureus. En revanche, les gènes lukF, lukM et tst1 étaient absents. Parmi les six entérotoxines recherchées, sec et seh étaient absentes, seb, sei et sej ont été retrouvées respectivement chez 2,75%, 3,67% et de 26,6% des souches. Enfin, le gène de résistance à la méthicil ine a été retrouvé dans 2,75% des souches isolées. L'analyse de l'association entre les gènes de virulence et la présence de mammites subcliniques a montré l'importance des gènes spa, lukD et sej. Une bonne sensibilité de S. aureus a été notée à la plupart des antibiotiques testés. Cette étude a montré la faible virulence des souches de
S. aureus isolées.

Mots-clés: vaches laitières - mammites subcliniques - Staphylococcus aureus - PCR - Dakar

Summary

Surveys conducted in Senegal have shown a strong association of staphylococci with subclinical mastitis in dairy cows. This study aimed to characterise Staphylococcus aureus strains identified in the dairy farms in Dakar. Of a total of 244 Staphylococcus spp isolates col ected from 135 lactating cows with subclinical mastitis at six dairy herds in peri-urban region of Dakar, 109 S. aureus strains were isolated and identified using phenotypic methods.
Using PCR, genes of thermonuclease and major capsular type 5 and 8 antigens were identified respectively in 98.17%, 93.58% and 1.84% of S. aureus strains. Similarly, the spa gene was found in 68.81% of S. aureus strains, luk D (62.39%) and luk S (0.92%). Among the virulence genes sought, spa, Luk S and Luk D genes were found respectively in 68.81%, 62.39% and 0.92% strains of S. aureus. However, the lukF, lukM and tst1 genes were absent in al isolated S. aureus. Among the six enterotoxins analysed, none of the S. aureus harboured the genes sea, sec and seh. Only the seb, sei and sej were found respectively at rates of 2.75%, 3.67% and 26.6%. Final y, the gene for resistance to methicil in was found in 2.75% of the strains isolated. The analysis of the association between virulence genes and the presence of subclinical mastitis showed the importance of genes Spa lukD and SEJ. Antibiotic resistance testing revealed a good sensitivity of S. aureus to most of the antibiotics tested. This study showed the low virulence of the S aureus strains isolated.

Keywords: Dairy cows - Subclinical mastitis - Staphylococcus aureus - PCR - Dakar

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Introduction

Staphylococci are responsible for several human and animal infections. They represent the main bacterial pathogens involved in (sub) clinical mastitis in dairy farming and are therefore responsible of significant economic losses in dairy industry. *Staphylococcus aureus* is considered as the most *pathogenic* and the major pathogen causing mastitis in dairy cows (Tollersrud *et al.,* 2000a; Nagase *et al*., 2002 ; Fueyo *et al*., 2005 ; Kaloret *et al*., 2007). Studies conducted in Senegal have shown its high prevalence in cases of subclinical bovine mastitis (Konte, 2003; Kadja *et al*., 2006). The pathogenicity of S. aureus is related to many factors including surface adhesins, capsular polysaccharides, exoenzymes and exotoxins that enhance its infectivity and survival in the mammary epithelial cells and neutrophiles. For instance, mammary isolates of S. aureus can secrete one or more leucotoxins, like the Panton-Valentine leukocidins (lukS-PV and lukF-PV) and the leucotoxins D (lukD) and M (lukM). The Panton- Valentine leukocidins (PVL) cause leucocyte destruction and tissue necrosis (Rankin *et al*., 2005). It is a leukotoxin associated with human clinical diseases and more recently to bovine mastitis in Europe (Fueyo *et al*., 2005 Schubert et al., 2001; Rainard *et al*., 2003). Mammary isolates of S. aureus can also secrete superantigens, like the enterotoxins (sea and its variants), seb, sec4ce12, she, sei, sej), the toxic shock toxin 1 (TSST-1) and exfoliating enzymes, that may play an important role in the initiation and exacerbation of mastitis (Schubert et al., 2001; Rainard et al., 2003; Dingues et al., 2000). A correlation has moreover been reported between the clinical or subclinical evolution of mastitis and the ability of the causative strains to produce enterotoxins and TSST-1 (Jone and Wieneke, 1986; Matsunaga et al., 1993).

Due to the economic and hygienic importance of S. aureus-associated mastitis, the purpose of this study is to characterize phenotypically and genotypically S. aureus strains isolated from subclinical mastitis cases circulating in dairy cattle farms in Dakar.

Material and methods

2.1. Bacterial isolates

A total number 244 Staphylococcus spp isolates were col ected from 135 lactating cows with subclinical mastitis of six dairy herds in peri-urban region of Dakar (Senegal). The milk samples were tested by the California mastitis test (CMT) for subclinical *mastitis*, and were scored according to the National *Mastitis* Council guidelines (1999). Isolation of *Staphylococcus* was performed on CMT-positive milk samples (score =2). For each cow, milk from all positive quarters was pooled and mixed at the Microbiology laboratory of Veterinary School of Dakar (E.I.S.M.V). Milk mixture samples were analysed by standard bacteriological isolation and identification method according to the National Mastitis Council guidelines (1999). The isolates of *Staphylococcus* were obtained using various cultural (haemolysis on Columbia agar with 5% sheep blood, growth on Chapman agar) and biochemical tests (oxidase and catalase). Putative *S. aureus* isolates were further identified at the Bacteriology laboratory of the Faculty of Veterinary Medicine of Liege (Belgium).

2.2. Identification to S. aureus

Staphylococcal isolates were identified as S. aureus on the basis of the haemolytic activity on sheep blood agar, the positive reaction at the Pastorex Staph-plus latex agglutination assay (Bio-Rad, France), the positive reaction at the coagulase production assay with rabbit blood (Merck KGaA, Germany) and the positive PCR detection of the nuc gene coding for a specific *thermonuclease*.

2.3. Genotypic characterization (DNA isolation and PCR procedures)

- DNA preparation.

The genomic DNA was purified fol owing the protocol described by Ünal *et al.* (1992). For the rapid lysis procedure, bacteria were harvested from either agar plates (one loopful using a 1- µl loop) or from 100 µl of an overnight liquid culture (~10^8 bacteria). The bacteria were collected, centrifuged at 16000 rpm for 2 minutes. The supernatant was discarded and the pellet was re-suspended in 50 µl of lysostaphin (Sigma, Belgium) and incubated at 37°C for 10 minutes. 50 µl of a solution of Proteinase K (Eurogentec, Liège, Belgium) at 100µgml-1 and 150µl of buffer (0.1 M Tris, pH 7.5) were added and the suspension was further incubated at 37°C for 10 minutes. Finally, tubes were placed in boiling water at 95°C for 5 minutes in order to inactivate the proteinase K. The final product was directly used for PCR or stored at -20 ° C until further use.

- PCR amplification.

The virulence-associated traits were investigated with specific PCR targeting the following genes: cap5H and cap8H (capsular polysaccharide 5 and 8), the IgG binding region of the protein A (spa2), lukD, lukF-PV, lukM, lukS-PV (coding for *leucotoxins* sub-units), the toxic shock syndrome toxin coding gene (tst-1) and *enterotoxins* genes

Table 1: Primers for amplification of genes of *S. aureus*

		Primer sequence (5'-3')	PCR* programs	Size of amplified products (pb)
Genes designated				
nuc		F :ATGAAGTCAAATAAATCGCT	1	422
		R :TCCACTAATTCCTTATTGTA		
cap 5H		F: ATGAGGATAGCGATTGAAAA	2	518
		R : CGCTTCTTAATCACTTTTGC		
cap 8H		F :ATCGAAGAACATATCCAAGG	3	867
		R :TTCATCACCAATACCTTTTA		
cap 8HK		F :ATCGAAGAACATATCCAAGG		
		R :TTCATCACCAATACCTTTTA		
	luk S-PV	F: GTCGTTAGGAATAATCACTCC		
		R: CCTGTTGATGGACCACTATTAA	1	834
luk				
	luk M	F :AACGTGTTTTAATAGCGTCATC		
		R: CACTTCTTACTAATGCTGGGTA	1	423
	luk D	F: CTTATCAGGTGGATTGAATG		
		R : CTATACTCCAGGATTAGTTTCT	6	792
	luk F PV	F :TAGATAAAAGAACTAATTTCAA		
		R :TGAGTTGCTCTATTTTCATC	6	526
tst-1		F : GGATTTGCTAGACTGGTATAGTAGTGG		364
		R :TTTTTTATCGTAAGCCCTTTGTTGC	5	
spa-2		F :TATATCTGGTGGCGTAACAC	4	229
		R : GTTGAAGTTATTTTGTTGCG		
MecA147-F		F: GTGAAGATATACCAAGTGATT	6	147
		R:ATGCGCTATAGATTGAAAGGAT		
	sea	F :GAAAAAAGTCTGAATTGCAGGGAACA	1	561
		R :CAAATAAATCGTAATTAACCGAAGGTTC		
	seb	F :TCGCATCAAACTGACAAACG	6	477
		R : GCAGGTACTCTATAAGTGCC		
	sec4	F : GACATAAAAGCTAGGAATTT	5	1018
		R :AAATCGGATTAACATTATCC		
se	seh	F : CAATCACATCATATGCGAAAGCAG	1	375
		R : CATCTACCCAAACATTAGCACC		
	sei	F : CTCAAGGTGATATTGGTGTAGG	6	577
		R :AAAAAACTTACAGGCAGTCCATCTC		
	sej	F : CATCAGAACTGTTGTTCCGCTAG	6	142
		R : CTGAATTTTACCATCAAAGGTAC		

PCR 1: 35 cycles 94°C-30sec ; 51, 4°C- 30sec ; 72°C-45sec. PCR2 : 35 cycles 94°C -30sec ; 50°C -30sec ; 72°C-45sec. PCR 3 : 35 cycles 94°C -30sec ; 46,5°C -30sec ; 72°C-45sec. PCR 3c : 35 cycles 94°C -30sec ; 52°C -30sec ; 72°C-90sec. PCR 5: 35 cycles 94°C -30sec ; 46,4°C – 30sec ;72°C – 30 sec; PCR 6: 30 cycles 94°C -15sec; 55°C-15sec; 72°C-30 sect 35 cycles 94°C-30 sec; 48°C-30 sec; 72°C-30sec. 55°C – 15sec ; 72°C – 30sec et 35 cycles 94°C -30sec ; 48°C – 30sec ; 72°C – 30sec.

PCRs were carried out in a 50 µl reaction mixture with 10 µl DNA, 1 µl of each 2 primers (20µM, Eurogentec/Liege Belgium), 1µl DNTP Mix (Thermo Fisher Scientific, Abgene House, Blenheim Road), 5 µl 10X ThermoPol buffer (New England Biolabs R Inc.), 0.12 µl Taq 1U DNA polymerase (New England Biolabs R Inc.) and 31.88µl sterile water.

The amplification parameters (temperature programs, size of amplicons) and primers sequences were used according to Taminiau et al. (2007) 17 (Table 1). The PCRs were carried out in thermocycler machine (ep AG-22331 Eppendorf, Hamburg-Germany).

At first the amplification products were separated by electrophoresis in Agarose 96- well plates (E-Gel® 96 with SYBR® Safe) and visualised with a photo imager with 1D gel analyzer Kodack program. Putative positive amplification results were subsequently confirmed by individual electrophoresis in 1% agarose gel. Sensitivity of S. aureus isolates was determined using the agar diffusion test technique (Bauer et al., 1966) on Mueller- Hinton agar using the following antibiotic impregnated disks: oxacillin (5µg), penicillin (10µg), enrofloxacin (5µg), tetracyclin (30µg), gentamicin (10µg), erythromycin (15µg), cefuroxim (30µg) and neomycin (30µg). Antimicrobial disks were purchased from Sensidisk (Becton Dickinson, Heidelberg Germany). Diameters of inhibition were read after a 24 hour-long incubation at 37° C. The oxacil in sensitivity was evaluated after 24 hours at 30°C. Zones of growth inhibition were evaluated according to recommendations of Antibiogram Comity of French Society for Microbiology (CA -SFM).

Results

Of the 244 *staphylococci* isolates analysed, 109 strains were identified as *S. aureus* on the agglutination assay (Pastorex Staph-plus) and at the coagulation test of rabbit blood. The PCR for the thermonuclease (nuc) was positive for 108 strains (99%) and beta haemolysis on sheep blood agar was observed with 70 strains (64%) (Table 2). The specific PCRs also identified the genes coding for the capsular antigens 5 (cap5) and 8 (cap8) in respectively 102 strains (94%) and 2 strains (2%), and for the protein A (spa) in 104 strains (95,4%).

The presence of different toxin-encoding genes was also investigated in the 109 strains identified as *S. aureus*. Of the 244 *staphylococci* isolates analysed, 109 strains were identified as *S. aureus* on the basis of the positive results at the latex agglutination assay (Pastorex Staph-plus) and at the coagulation test of rabbit blood. The PCR for the thermonuclease (nuc) was positive for 108 strains (99%) and beta *haemolysis* on sheep blood agar was observed

Table 2: Origin, hemolysis and other characteristics of S. aureus isolates from milk samples from cows with bovine mastitis

N. of strains	Hemolyse beta	Thermonuclease (nuc)	Capsular polysaccharide		Protein A (spa2	Mec A MRSA	Leukotoxins				Toxic shock syndrome	Enterotoxins					
			Cap 5	Cap 8HK			LukD	lukM	lukF-PV	lukS-PV	toxin (tst-2)	sea	seb	sec	seh	sei	sei
Total : 109 (%)positives	70 (64,22)	107 (98,17)	102 (93,58)	2 (1,83)	104 (95,41)	3 (2,75)	68 (62,38)	0 (0)	0 (0)	1 (0,92)	0 (0)	0 (0)	3 (2,75)	0 (0)	0 (0)	4 (3,67)	29 (26,61)

Table 3. Production of toxins and their association in *S. aureus* stains isolated from bovine subclinical mastitis milk from Dakar

Toxins	N°. of positive isolates	% of positive isolates
Protein A (spa)	75	68,81
lukD	68	62,38
seb	3	2,75
sei	4	3,67
sej	29	26,61
spa + luk D	40	36,70
sej + spa	23	21,10
sej+ lukD	4	3,67
spa +sej + lukD	2	1,83

(sea, seb, sec4, seh, sei, sej).

with 70 strains (64%). The gene coding for the leucotoxin sub-unit D (lukD) was present in 68 strains whereas the gene coding for the leucotoxin sub-unit S-PV (lukS-PV) was found in only one strain (1%), and all strains tested negative with the PCR for the genes coding for the leucotoxin sub-units F-PV and M (lukF-PV, lukM) and for the toxic shock syndrome toxin (tst-1). Thirty-six (33%) of the 109 S. aureus strains were positive with one of the PCR specific of the genes coding for entérotoxines: 3 for the seb gene, 4 for the sei gene and 29 for the sej gene.

Associations of toxin-encoding genes was present in some strains: 40 (37%) strains possessed both spa and lukD genes; 23 (21%) both sej and spa genes; 4 (4%) both sej and lukD genes; 2 (2%) the SPA, sej and lukD genes.

The results of the antibiotic sensitivity assay gave high levels of sensitivity of the 109 strains of S. aureus to erythromycin (83%), oxacillin (90%), and to cefuroxim, neomycin, gentamicin, tetracycline and enrofloxacin (94%) A lower level of sensitivity to penicillin was obtained (54%) (Fig.1).

Discussion

S. aureus represents ca. 45% of all *staphylococci* isolated from cases of subclinical mastitis in six farms of the peri-urban region of Dakar. They were identified to S. aureus on the basis of the results of the *Pastorex Staph-Plus* and rabbit blood coagulation tests. On the other hand the PCR assay targeting the nuc gene was negative with 2 strains and 39 strains were non-haemolytic. The results of the former test corroborate two other published studies in which 98% to 100% of the strains were positive (Kuzma *et al*., 2003; Kalorey *et al*., 2007). The reasons for the few strains testing negative may resides in some difference in the nuc gene. PCR targeting the nuc gene can was prevalent in strains from caprine (68.5%) and ovine (75.8%) sources.

Studies reported variability between production of capsular antigen 5h and 8h by strains of *S. aureus* isolated from cases of bovine mastitis from different geographic regions. This information is important for the rational design of a capsule-based vaccine to prevent *S. aureus* bovine mastitis (Tollersrud *et al.,* 2000b). The high prevalence of nonreactive (NR) strains with CP serotype 5 (CP5) - or CP8-specific antibodies, reported by authors (Sordelli *et al.,* 2000), cast doubt on the roles of CP5 and CP8 in the pathogenesis and immunoprophylaxis of bovine mastitis. Concerning the profile of the virulence factors of these 109 *S. aureus* strains (or *virulotyping*), no actual consensus has been found by testing them with PCRs for various virulence factor-encoding genes; only the genes

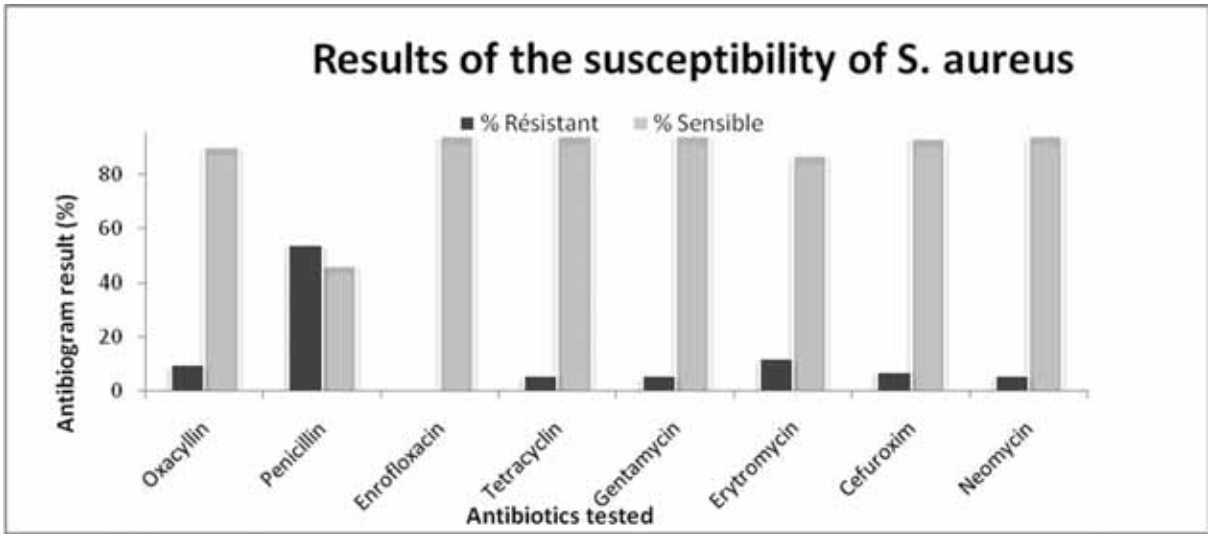

Fig. 1: Result of the susceptibility of S. aureus strains

coding for the Protein A (spa) and for the leucotoxin D (lukD) were detected in more than 50% of the strains. Conversely, the genes coding for the Panton Valentine leukocidin (lukS-PV and lukF- PV) and for the leucotoxin M (lukM) were absent in virtually all strains, though they are found in more than 50% of the strains investigated by others (Fueyo *et al*., 2005; Taminiau *et al*., 2007; Zecconi *et al*., 2006). Studies have shown the important role played by *leukotoxins* (Panton Valentine leukocidin: lukF-PV and luk M) in the induction of inflammation of the udder (Younis *et al*., 2005). Indeed, the results suggest that LukM/LukF' induce inflammation into the udder by a mechanism similar to that of LPS or by a unique mechanism(s) which requires further nvestigation. Rainard *et al*., (2003) showed also, the importance of lukM/lukF-PV to the *pathogenesis* of *mastitis* in ruminants and the protective ef ect of antibodies to this *leukotoxin* therefore be considered as a reliable assay during large scale studies. On the other hand the latter test is not a reliable marker of *S. aureus* species. Two different major capsula antigens have been associated with virulent S. aureus: antigen 5 and antigen 8 (O'rioran *et al*., 2004). The genes coding for these two capsular antigens are also present in the mammary strains of this study but most of the strains tested positive for the type 5 capsule (94%). This prevalence is above 51.4% obtained by Poutrel *et al*. (1988) in strains from bovine sources. These authors noted that, type 5 was predominant in strains from bovine sources (51.4%), whereas type 8 was prevalent in strains from caprine (68.5%) and ovine (75.8%) sources.

Studies reported variability between production of capsular antigen 5h and 8h by strains of *S. aureus* isolated from cases of bovine mastitis from different geographic regions. This information is important for the rational design of a capsule-based vaccine to prevent *S. aureus bovine mastitis* (Tollersrud *et al*., 2000b). The high prevalence of nonreactive (NR) strains with CP serotype 5 (CP5) - or CP8-specific antibodies, reported by authors (Sordelli *et al*., 2000), cast doubt on the roles of CP5 and CP8 in the *pathogenesis* and *immunoprophylaxis* of *bovine mastitis*.

Concerning the profile of the virulence factors of these 109 *S. aureus* strains (or *virulotyping*), no actual consensus has been found by testing them with PCRs for various virulence factor-encoding genes; only the genes coding for the Protein A (spa) and for the leucotoxin D (lukD) were detected in more than 50% of the strains. Conversely, the genes coding for the Panton Valentine leukocidin (lukS-PV and lukF- PV) and for the leucotoxin M (lukM) were absent in virtually all strains, though they arefound in more than 50% of the strains investigated by others (Fueyo *et al*., 2005; Taminiau *et al*., 2007; Zecconi *et al*., 2006). Studies have shown the important role played by *leukotoxins* (Panton Valentine leukocidin: lukF-PV and luk M) in the induction of inflammation of the udder (Younis *et al*., 2005). Indeed, the results suggest that LukM/LukF' induce inflammation into the udder by a mechanism similar to that of LPS or by a unique mechanism(s) which requires further investigation. Rainard *et al*., (2003) showed also, the importance of lukM/lukF-PV to the *pathogenesis* of mastitis in ruminants and the protective ef ect of antibodies to this *leukotoxin* Their results establish that lukM/lukF-PV is very active on PMN of ruminants and suggest that this *leukotoxin* could be the most active *leukotoxin* produced by *mastitis* isolates. According to some authors (Barrio *et al*., 2006), among *leukotoxins*, the association luk M / F'Pv may constitute a particular virulence attribute of mastitis-causing S. aureus strains. LukM/F0- PV was by far the most *cytotoxic leukotoxin*, but it was closely followed by ∼-hemolysin for PMN activation.

About lukD gene, in our study, the high prevalence (62.8%) obtained is in agreement with other authors results. Indeed, Yamada *et al*. (2005) noted high frequencies of lukE and lukD genes in almost all (96.0%) of the bovine isolates of S. aureus that were collected from mastitic cow's milk and farm bulk milk. Von Eiff *et al*. (2004) reported that the lukE and lukD genes were found at high prevalence in S. aureus isolates from humans, significantly more so in blood (82%) than in nasal isolates (60.5%). In domestic animal isolates of *S. aureus*, these genes were detected in all the *S. aureus* isolates from ruminants with *mastitis* by PCR amplification.

LukE+LukD is a bicomponent toxin which was as effective as the Panton-Valentine leucocidin for inducing dermonecrosis when injected in the rabbit skin, but not *hemolytic* and poorly *leucotoxic* compared to other *leucotoxins* expressed by *Staphylococcus aureus* (Gravet *et al*., 1998).

Similarly, none of the strains of *S. aureus* of our study possessed the genes coding for the toxic shock syndrome toxin, though found in some strains isolated from mastitis by others: 15% (Akinden *et al*., 2001), 13% (Fueyo *et al*., 2005) and 68% (Tollersrud *et al*., 2000a).

With regard to the frequency of the genes coding for the *enterotoxins*, only one third of the *S. aureus* strains were positive, with the sej gene detected in 29 strains. On the other hand the sei and seb genes were rare (present in only 7 strains) and the sea, sec and seh genes were not detected, confirming, in part, other published results (Zschöck *et al*., 2005) . The presence of some *enterotoxin*-encoding genes may raise the question of the role of these mammary strains of *S. aureus* to cause food poisoning in humans. Nevertheless before confirming any rela-

tionship between these isolates and food poisoning, the production of *enterotoxin(s)* at levels that are sufficient to cause diseases should be demonstrated.

Associations of virulence factor-encoding genes have been found in many published studies, like in ours in which 64% of the strains possess the spa, lukD and/or sej genes, though no consensus profile of mammary strains of *S. aureus* has ever been identified. Presence of both seg and sei genes or sed and sej genes are frequently found in *S. aureus* isolates from bovine mastitis or raw milk (Lammler *et al*., 2000; Omoe *et al.*, 2002). The correlation between prevalence of novel *S. aureus enterotoxin* types seg through seo-encoding genes (seg to seo) (Rhem *et al*., 2000; Zhang *et al*., 1998; Orwin *et al*., 2001) and clinical types of *bovine mastitis* as well as their public health significance remains to be elucidated in future *epidemiological* studies (Omoe *et al.,* 2002).

Antibiotic resistance testing revealed a good sensitivity of *S. aureus* to most antibiotics tested. The high resistance (54%) of *S. aureus* strains against *penicillin* is most probably related to the massive use of this drug during treatment (general or local) in most farms.

Conclusion

These PCR assays, especial y the nuc gene PCR, 212 are useful to validate the rapid identification and characterization of mammary isolates of *S. aureus* previously identified by *phenotypic* assays. Though the prevalence of genes coding for various properties including toxins by strains of *S. aureus* vary depending on the dairy farms their expression determines the *pathogenicity* of the strains responsible for subclinical *mastitis* and the importance of economic losses. However it should not be forgotten that, besides *S. aureus,* the importance of coagulase- negative *staphylococci* (SCN) in subclinical mastitis is increasing. F.i. in this study, the SCN represent more than half of the *staphylococci* isolates (135/244). Although their virulence factors are not fully identified, this increasing importance of SCN may partly due to the production of a slime *polysaccharide*, considered as responsible for adherence to host cells.

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ORGANS WEIGHT AND PERFORMANCE CHARACTERISTICS OF BROILER CHICKENS EXPOSED TO CRUDE PETROLEUM FLAME AND FUMES

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POIDS DES ORGANES ET PERFORMANCES DES POULETS DE CHAIR EXPOSÉ À UNE FLAMME DE PÉTROLE BRUT ET DES FUMÉES

Résumé

La performance des poulets de chair exposé aux flammes et aux fumées de la combustion du pétrole brut à des distances variables au cours d'une période quotidienne de 16 heures a été évaluée pendant 56 jours dans un poulail er. La combustion du pétrole brut a été simulée dans un brûleur métal ique de 22,86 cm de haut avec un diamètre de 17,8 cm et une épaisseur de 1,17cm spécialement inventé pour cet objectif. Le concept expérimental était de facteur 2. dans un concept complètement aléatoire (CRD) avec le facteur A comme étant les distances à partir des flammes de la combustion du pétrole brut et le facteur B comme étant l'âge des volail es en semaines. Cent quatre-vingt (180) poussins de chair de race Anak âgés d'un jour ont été divisé par groupes de 4 à 45 oiseaux chacun en trois répartitions de 15 oiseaux chacun. Les oiseaux témoins ont été maintenus dans un autre poulail er situé loin du lieu des flammes. Les distances mesurées étaient de 4m, 8m et 12m à partir des flammes. Les oiseaux ont été nourris à ad-libitum avec des aliments ayant des propriétés de la pâtée de démarrage pour une période de 5 semaines puis avec de la moulée de finition pour poulet à gril er pendant 3 semaines. L'eau a également été fourni ad-libitum. Lorsque cela était néces-saire, des vaccinations de routine ainsi que l'administration d'autres médicaments ont été ef ectués. Le microenviron-nement météorologique expérimental (température ambiante, humidité relative et intensité lumineuse) du poulail er a été enregistré. Les émissions gazeuses issues de la combustion du pétrole brut (qualité de l'air) étaient examinées. L'alimentation de la volail e et le pétrole brut ont aussi été analysé. Les résultats ont indiqué que l'augmentation du poids corporels, le gain hebdomadaire de poids corporels, le gain quotidien, la consommation hebdomadaire de nourriture, le taux de conversion alimentaire hebdomadaire, le poids des organes et (alimentation/gain) la mortalité hebdomadaire n'étaient pas af ectés par le traitement de manière significative (P>0,05). L'âge des oiseaux (semaines) a eu une influence très importante (P>0,05) sur tous les aspects de performances mesurées. Les tendances normales de croissance ont été observées dans toutes les analyses inclues lors du contrôle sur une période de 8 semaines. Un faible taux de mortalité a généralement été constaté dans toutes les études.

Mots clés: Poulets de chair, Combustion, Flammes et fumées, Poids des organes et performances, Simulation de pétrole brut.

Summary

Performance of broiler chickens exposed to crude petroleum flame and fumes at varying distances over a period of 16 hrs daily were evaluated for 56 days in a poultry house. The crude petroleum burning was simulated in a metal burner, 22.86cm high with a diameter of 17.8cm and a thickness of 1.17cm designed for the purpose. The experimental design was a 2.factor factorial in a completely randomized design (CRD) with factor A as distances from the crude petroleum flame and factor B as the age of the birds in weeks. One hundred and eighty (180) Anak day old broiler chicks were divided into 4 groups of 45 birds each, replicated thrice at 15 birds per replicate. The control birds were located in another poultry house outside the flame area. The measured distances were 4m, 8m and 12m from the flame point. The birds were fed ad-libitum on a proprietory starter mash for 5 weeks, and a broiler finisher mash for 3 weeks. Water was also provided ad-libitum. Routine inoculations and other medications were administered when due The micro meteorological experimental environment (ambient temperature, relative humidity and light intensity) of the poultry house were recorded. Gaseous emissions from the burning crude oil (air quality) were monitored. Poultry feed and crude oil were also analyzed. Result indicated that weekly body weights, weekly body weight gains, daily gains, weekly feed consumption, weekly feed conversion ratio, organ weights and (feed/gain) weekly mortality were not significantly (P>0.05)

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affected by the treatment. Age of birds (weeks) had highly significant (P< 0.05) influence on al the performance traits measured. Normal growth pattern was observed in al treatments including the control over the 8 weeks period. Low mortality was general y observed in al treatments.

Key Words: Broiler chickens, burning, flames and fumes, organ weights and performance, simulated crude petroleum.

Introduction

When crude oil is burnt and natural gas is flared, various gaseous byproducts are emitted into the atmosphere causing air pol ution. Flames and fumes from crude oil wel and flared natural gas release approximately 82% of al pol utants discharged into the atmosphere by the oil industry (Uchegbu, 1998, Vil asenor *et al*, 2003).

The effects of crude oil spil s and gas flaring on animals are many and varied. Ingestion of crude oil contaminated feed by rabbits (Berepubo et al., 1994), goats (Ngodigha *et al*., 1999), and poultry (Nwokolo *et al*., 1984; Monsi *et al*., 1991) have been known to produce adverse effects. Inhalation of noxious gases by livestock has been known to produce respiratory disorders (Ukaegbu and Okeke, 1987). Natural oil and gas flaring by products are stressors with extensive adverse effects on the environment with harmful consequences on man, soil, aquatic life, animal and plant agriculture (Odu 1972; Kuhnhold, 1978; Baker, 1983; Ekweozor and Snowden, 1987; Isirimah *et al*., 1989). Limited data have been generated on the impact of crude oil on some domestic animals in Nigeria. Livestock may be exposed to crude oil contamination through feed ingredients and water intake in areas with oil spil age and natural gas flaring. In the Niger Delta of Nigeria some poultry farms are located in communities where natural gas is flared or in areas where crude oil spil s often occur. Inhalation of the noxious gases produced by these flares could be detrimental to the chickens, causing toxicological health hazards. The objective of this study is to determine the ef ect of burning crude petroleum on broiler chicken performance.

Materials and Methods

One hundred and eighty (180) unsexed day old broiler chickens were divided into 4 groups of 45 birds each, replicated thrice with 15 birds per replicate in a completely randomized design trial. The distances from the flame point were 4m 8m, 12m and control, representing the treatments as I, I , III and IV respectively (Fig. 1). Treatment IV (control) was located in a separate building without flame. The birds were distributed randomly into 12 pens and brooded in an open sided brooding pen on deep litter. Brooding temperatures ranged from 33-35^{0}C. The crude was ignited in a designed metal burner, 22.86cm high, 17.8cm diameter and a thickness of 1.17cm (Fig. 2). This study was done at Rivers State University of Science and Technology Teaching and Research Farm in Port Harcourt.

The birds were fed ad libitum on a proprietory broiler starter mash containing 2435.6 kcalME/kg and 25.5% crude protein) for 5 weeks, and a broiler finisher mash (2660.6kcalME/kg and 20.6% crude protein for 3 weeks (Table 1). Water was provided ad-libitum. Feed intake and mortality values were recorded daily. Body weights were taken on a weekly basis. Body weight gains and feed conversion ratio were calculated on a weekly basis. At the end of the 8th week, three birds per treatment were slaughtered by severing the jugular vein and liver, lungs, heart and kidney removed and weighed. Statistical analysis was carried out using analysis of variance (ANOVA) procedure and the means were separated using Duncan's Multiple Range Test (1955).

Results of growth performance of the treated broilers are presented in Tables 2 and 3. The results showed that there were no significant treatment effects (P > 0.05) on the birds. Age of birds had significant (P<0.05) influence on all the performance traits measured. Organ weights also showed no significant differences (P>0.05) between the liver, heart and kidney of the birds. However, the lungs of the birds at 12m were significantly heavier, than those of the birds at 4m and 8m respectively, but similar to the control (Table 4). Even though a normal growth pattern was observed in this study for all the treatment groups, indicating that their performance was not negatively impacted, being a simulation study. However, it is well known that in the field where gas flaring is carried out, man, animals, vegetation, soil and indeed the entire environment are affected adversely through high environmental temperatures, high thermal radiation, production of toxic gases during combustion, high noise levels and continuous light intensity, this was confirmed by Egbuna (1987). It was observed that body weight gains of birds nearest to the flame tended to decline as compared to those in the outermost group, though not significantly. This may be due to stress effects from the increased temperature and inhaled fumes.

Feed intake was lower towards the flame location, but did not give an improved feed conversion ratio. This must be due possibly to metabolic adjustment, which was not however specifically evaluated in the study. Birds have been

Table 1: Proximate Analysis of the Experimental Diets

Nutrients	Broiler starter	Boiler finisher
Moisture (%)	10.8	11.47
Carbohydrate (%)	28.82	29.03
Lipid (%)	2.92	3.06
Protein (%)	25.5	20.6
Ash (%)	4.56	5.72
Fibre (%)	5.48	6.02
Trace Elements		
Calcium (%)		
Phosphorus (%)	0.67	0.69
Iron (%)	0.86	0.82
Potassium (%)	0.35	0.41
Vitamin A (I.U)	0.45	0.56
Metabolisable Energy (Kcal ME/kg)	110	98.5
	2435.6	2660.6

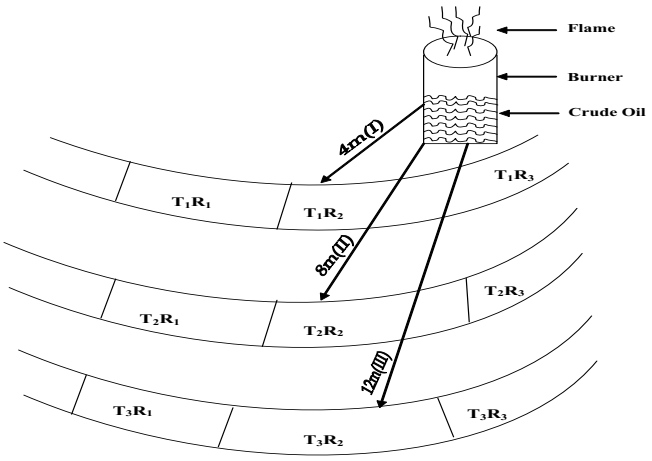

Fig. 1: Poultry pen experimental design, showing the distances from the flame point

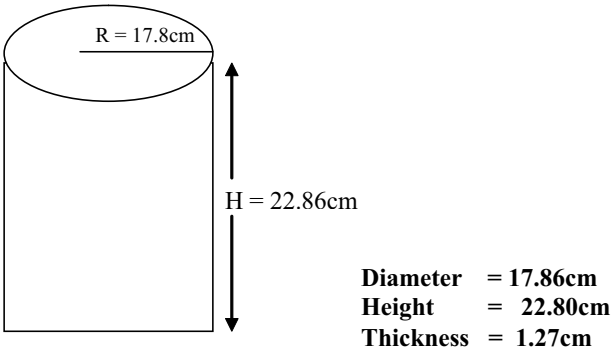

Fig. 2: Design metal burner that was used to burn the crude oil to produce the flame

Table 2: Effects of Crude Petroleum Flame Placed at Various Distances on Growth Performance of Broilers Chickens.

Distances to crude oil flame (m)	Weekly weight gains (kg) (Mean ± SEM)	Body weight (kg) (Mean ± SEM)	Average daily gains (kg) (Mean ± SEM)	Feed consumption (kg) (Mean ± SEM)	Feed conversion (Mean ± SEM)	Mortality (%) (Mean ± SEM)
4m	3.81 ± 0.02	13.81 ± 2.10	0.54 ± 0.05	9.97 ± 1.20	2.62 ± 0.14	0.13 ± 0.06
8m	3.89 ± 0.43	13.99 ± 2.10	0.56 ± 0.06	9.98 ± 1.19	2.56 ± 0.12	0.04 ± 0.04
12m	3.95 ± 0.45	14.38 ± 2.16	0.56 ± 0.06	10.30 ± 1.26	2.61 ± 0.15	0.21 ± 0.08
Control	3.96 ± 0.45	14.39 ± 2.16	0.57 ± 0.06	10.02 ± 1.18	2.53 ± 0.18	0.04 ± 0.04

Table 3: Weekly Values of Various Performance Characteristics of Broiler Chickens Exposed to Crude Petroleum Flame at Various Placement Distances

Weeks	Distances	NWT (kg) (Mean ± SEM)	BWTG (kg) (Mean ± SEM	ADG (kg) (Mean ± SEM)	Feed cons (kg) (Mean ± SEM)	For (Mean ± SEM)	Mortality (Mean ± SEM)
	4m	1.60 ± 0.08a	0.90 ± 0.05a	0.13 ± 0.03	2.03 ± 0.02a	2.25 ± 0.13a	0.67 ± 0.33a
1	8m	1.68 ± 0.09a	0.95 ±0.08a	0.13 ± 0.03	2.03 ± 0.02a	2.14 ± 0.12a	0.00 ± 0.00b
	12m	1.52 ± 0.06a	0.78 ± 0.03b	0.11 ± 0.02	1.97 ± 0.01b	2.53 ± 0.31a	0.67 ± 0.33a
	Control	1.52 ± 0.08a	0.88 ± 0.04a	0.13 ± 0.03	2.27 ± 0.03a	2.58 ± 0.02a	0.00 ± 0.00a
	4m	3.62 ± 0.22b	2.02 ± 0.13b	0.30 ± 0.02	3.03 ± 0.03a	1.50 ± 0.02a	0.00 ± 0.00a
2	8m	3.72 ± 0.33b	2.04 ± 0.13b	0.29 ± 0.02	3.15 ± 0.05a	1.54 ± 0.03a	0.00 ± 0.00a
	12m	3.42 ± 0.20b	1.90 ±0.12b	0.27 ± 0.02	3.20 ± 0.06b	1.68 ± 0.04b	0.33 ± 0.33b
	Control	3.27 ± 0.18b	1.65 ± 0.10b	0.24 ± 0.02	3.22 ± 0.02a	1.95 ± 0.05c	0.00 ± 0.00a
	4m	6.58 ± 0.20c	2.97 ± 0.17a	0.42 ± 0.03	6.73 ± 0.12a	2.27 ± 0.13a	0.00 ± 0.00a
3	8m	6.62 ± 0.23c	2.90 ± 0.14c	0.41 ± 0.03	6.77 ± 0.12a	2.33 ±0.15a	0.00 ± 0.00a
	12m	6.42 ± 0.15c	3.00 ± 0.18b	0.43 ± 0.03	6.77 ± 0.12a	2.26 ± 0.13a	0.00 ± 0.00a
	Control	6.75 ± 0.35c	3.48 ± 0.21b	0.50 ± 0.04	6.80 ±0.13a	1.95 ± 0.11b	0.00 ± 0.00a
	4m	9.72 ± 0.44d	3.13 ± 0.27a	0.45 ± 0.02	7.00 ± 0.00a	2.24 ± 0.12a	0.00 ± 0.0a
4	8m	10.10 ± 0.46d	3.48 ± 0.30a	0.50 ± 0.03	6.97 ± 0.15a	2.00 ± 0.11a	0.00 ± 0.00a
	12m	8.98 ± 0.42d	2.57 ± 0.24b	0.37 ± 0.01	6.90 ±0.14a	2.68 ± 0.24b	0.00 ± 0.00a
	Control	10.13 ± 0.46d	3.38 ± 0.29a	0.48 ± 0.03	6.97 ± 0.15a	2.06 ± 0.11a	0.00 ± 0.00a
	4m	14.15 ± 0.53e	4.43 ± 0.33a	0.63 ± 0.03	12.00 ± 0.00a	2.71 ± 0.20a	0.33 ±0.33a
5	8m	14.45 0.60e	4.35 ± 0.30a	0.62 ± 0.03	11.93 ±0.48a	2.74 ±0.20a	0.00± 0.00b
	12m	14.23 ± 0.55e	5.25 ± 0.60b	0.75 ± 0.05	14.63 ± 0.97b	2.79 ± 0.22a	0.33 ±0.00b
	Control	15.10 ± 0.74a	4.97 ±0.43b	0.71 ± 0.04	11.90 ± 0.48a	2.39 ± 0.16b	0.00 ± 0.00b
	4m	19.34 ± 1.33f	5.24 ± 0.99a	0.75 ± 0.12	14.00 ± 0.00a	2.67 ± 0.16a	0.00 ± 0.00a
6	8m	19.60 ± 1.40f	5.15 ± 0.90a	0.73 ± 0.12	14.00 ± 0.00a	2.72 ± 0.20a	0.33 ± 0.33b
	12m	18.92 ± 0.98f	4.68 ± 0.85b	0.67 ± 0.11	14.00 ± 0.00a	2.99 ±0.26a	0.00 ± 0.00a
	Control	19.28 ±1.32f	4.18 ± 0.56b	0.60 ±0.10	14.00 ± 0.00a	3.35 ± 1.10b	0.33 ± 0.33b
	4m	25.05 ±1.57g	5.66 ± 0.50a	0.81 ± 0.14	17.50 ± 0.00a	3.09 ± 0.29a	0.00 ± 0.00a
7	8m	26.19 ± 1.58g	6.24 ± 0.52b	0.89 ± 0.15	17.50 ± 0.00a	2.80 ± 0.21b	0.00 ± 0.00a
	12m	25.84 ±1.59g	6.92 ± 0.70b	0.99 ±0.21	17.50 ± 0.00a	2.53 ± 0.17b	0.00 ± 0.00a
	Control	26.42 ± 1.60g	7.14 ± 0.90c	1.02 ± 0.40	17.50 ± 0.00a	2.45 ± 0.15b	0.00 ± 0.00a
	4m	31.84 ± 1.48h	6.78 ±.92a	0.97 ± 0.12	17.50 ± 0.00a	2.58 ±0.15a	0.00 ± 0.00a
8	8m	32.71 ±1.50h	6.53 ± 0.76a	0.93 ± 0.12	17.50 ± 0.00a	2.68 ± 0.16a	0.00 ± 0.00a
	12m	31.71 ± 1.28h	5.37 ± 0.76a	0.77 ± 0.10	17.50 ± 0.00a	3.26 ± 0.61b	0.00 ± 0.00a
	Control	32.39 ± 1.49h	5.98 ± 0.72b	0.85 ± 0.11	17.50 ± 0.00a	2.93 ± 0.25b	0.00 ± 0.00a

Within columns, mean ± SEM with different superscripts are significantly different ($P < 0.05$). FOR = feed conversion ratio (feed/gain)

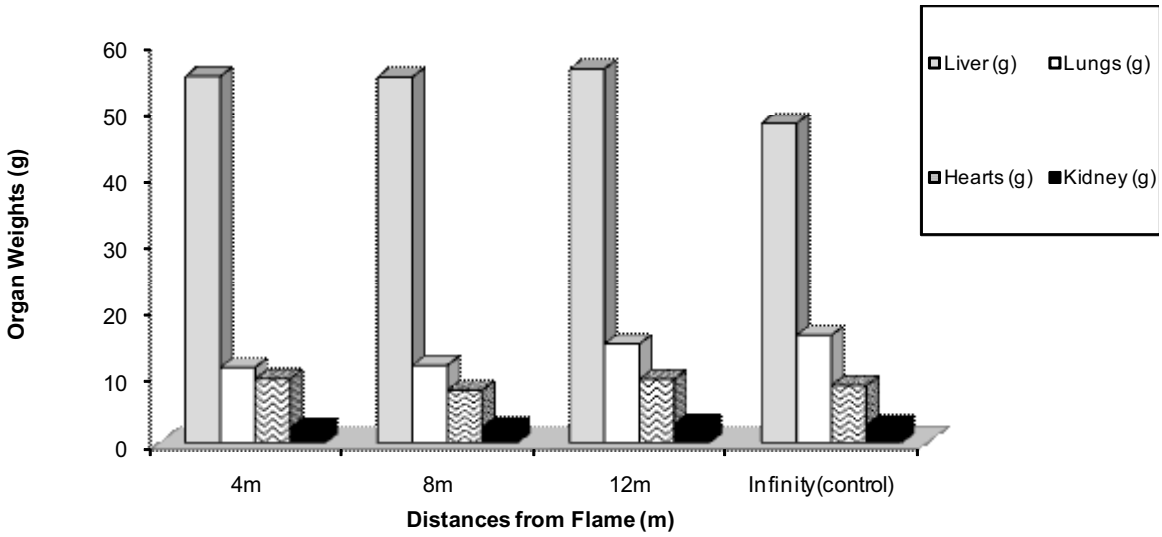

Fig. 3: Effects of Distances from Crude Petroleum Flame on Organ Weights (g)

credited with an innate capability of adjusting their feed intake under different lighting regimes, through a genetically controlled mechanism in the hypothalamus (ventral medial nucleus) (Anand and Brobeck, 1952). Mortality was generally low among the birds (0.04 ? 0.04% to 0.21 ? 0.8%). Mortalities in 4m and 12m groups were due to heat prostration leading to panting, asphyxiation and disease (coccidiosis). There were no mortalities in weeks 3, 4, 7 and 8 in al treatments. Mortality patterns and modalities in birds exposed to crude oil effects through ingestion and inhalation procedures may not be similar probably because of the toxicity levels. Ingested crude oil has been observed to cause mortality from alterations in the general morphology of tissues and organs in rabbits (Berepubo et al., 1994); goats (Ngodigha *et al*., 1999) and poultry (Nwokolo *et al*., 1984 and Monsi *et al*., 1991). Inhaled crude oil fumes are known to cause respiratory disorders with adverse ef ects on the lungs. Increased levels of inhalation of toxic gaseous emissions such as Nitrogen dioxide (NO_2¬¬¬), Sulphur dioxide (SO2), Hydrogen sulphide (H_2S), Carbon Monoxide (CO) and Suspended particulate matter (SPM) from crude oil may eventually predispose various mortality levels.

Conclusion

Inhalation of gaseous fumes produced by burning crude oil did not result in very severe toxicological effects, as compared to ingestion

The significant observations obtained from this study showed that performance traits (body weight, body weight gains, average daily gains, feed intake, feed conversion ratio, mortality and organ weights were not adversely affected. This might be due to length of exposure period as well as indoor/outdoor temperature dif erences, suggesting that the route or method of exposure is of important relevance in such investigations.

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FLUOROQUINOLONE RESISTANT SALMONELLA ENTERICA OF POULTRY ORIGIN FROM SOUTH WESTERN STATES OF NIGERIA

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ENTERICA SALMONELLES RÉSISTANTES AUX FLUOROQUINOLONES D'ORIGINE VOLAILLE PROVENANT DES ÉTATS DU SUD-OUEST DU NIGERIA

Résumé

On a déterminé la susceptibilité de 41 isolats de Salmonella enteric isolés des maladies des épidémies de la volail e entre avril 2005 et août 2007 dans les Etats d'Oyo et d'Ogun du Nigéria à la ciprofloxacine, à l'acide nalédixique, au chloramphénicol, au streptomycine, au kanamycine, à l'ampicil ine, au néomycine et au tétracycline. En utilisant la méthode de microtitration pour la détermination de la CMI (concentration minimale inhibitrice), 22 profils de résistance ont été obtenus. Il s'agit notamment du modèle résistant octuple manifesté par six isolats; trois différents profils de résistance septuple exposés par quatre isolats, huit isolats qui manifestent trois différents profils de résistance aux sextuples, six isolats présentaient cinq différents modes de résistance quintuple, trois différents modes de quadruple résistants ont été présentés par six isolats, cinq différents profils de résistance aux triples ont été montrées par huit isolats alors que deux isolats ont montré un double modèle résistant et une résistance à l'ampicil ine uniquement a été observée dans un isolat. Un total de 30/41 (73,2%) résistait aux médicaments multiples, présentant une résistance à quatre ou huit antibiotiques. Le type R cip.amp.str.chl.kan.neo.tet.nal était plus fréquemment vu dans six isolats. De tous les isolats de 26/41 (63,4%) étaient résistant à la ciprofloxacine. Cette enquête révèle la présence généralisée de la Salmonel a de type sérologique multirésistant dans les épidémies de maladies de la volail e au Nigeria.

Summary

The susceptibility of 41 *Salmonella enterica* isolates isolated from poultry disease outbreaks between April 2005 and August 2007 in Oyo and Ogun States Nigeria to *ciprofloxacin, nalidixic* acid, *chloramphenicol*, *streptomycin, kanamycin*, *ampicil* in, *neomycin*, and tetracycline was determined. Using microtitre method for MIC determination, 22 resistance patterns were obtained. These include an octuple resistant pattern manifested by six isolates; three different septuple resistance patterns exhibited by four isolates, eight isolates manifested three dif erent sextuple resistance patterns, six isolates exhibited five different quintuple resistant patterns, three dif erent quadruple resistant patterns were presented by six isolates, five dif erent triple resistance patterns were shown by eight isolates, whereas two isolates showed double resistant pattern and a single resistance to ampicillin was seen in one isolate. A total of 30/41 (73.2%) were multi-drug resistant, showing resistance to four or eight antibiotics. R-type cip.amp.str.chl.kan.neo.tet.nal was the commonest seen in six isolates. Of al the isolates 26/ 41(63.4%) were *ciprofloxacin* resistant. This investigation reveals the widespread occurrence of multidrug resistant Salmonel a serovars from poultry disease outbreaks in Nigeria.

Key words: *Fluoroquinolone* resistant Salmonel a enterica, Poultry, Multidrug-resistant.

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Introduction

Salmonel a species are prominent among major pathogens responsible for infections in human and animals and salmonel osis currently constitute a global major public health problem (Harish *et al*., 2006; Torpdahl, *et al*., 2007). In most cases, Salmonel a infections in humans are usual y associated with food-borne transmission through ingestion of meat, dairy products and food contaminated with animal feces (Holmberg *et al*., 1984; Winokur, 2003). There are increasing reports of various profiles of Salmonel a resistance to *ciprofloxacin*. Salmonel a isolates with reduced *ciprofloxacin* susceptibility but uniformly resistant to nalidixic acid has been reported (Hakanen, *et al*, 1999). While in other investigation quinolone resistance pattern in which *S. enterica* isolates which were susceptible to nalidixic acid but had reduced susceptibilityto ciprofloxacin was reported (Hakanen, *et al,* 2005). *Ciprofloxacin* (fluoroquinolone) have been used as ef ective and life saving treatment for extra cel ular infections in severe *S. typhimurium* infections (Holmann, 2001). It has been observed that fatality rates are usual y higher for patients infected with drug resistant Salmonel a species than for those infected with drug susceptible strains (Helms *et al*., 2002; Verma *et al*., 2005). The fol owing profiles of resistance among the Salmonella isolates in slaughtered pigs in Ethiopia: ciprofloxacin, nalidixic acid and nitrofurantoin (R type CipNalNit, 10%), ciprofloxacin, nalidixic acid, spectinomycin, streptomycin, sulfisoxazole and tetracycline (R type CipNalSptStrSulTet, 14.3%) and to *ciprofloxacin, knamycin, nalidixic* acid, neomycin, nitrofurantoin, *streptomycin* and *tetracycline* (R type Cip-KanNalNeoNitStrTet, 10%) have been reported (Mol a *et al*., 2006). This work reports the emergence of high *fluoroquinolone/quinolone* and multidrug resistant Salmonel a enterica subspecies enterica isolated from poultry disease outbreaks in two South Western States: Oyo and Ogun States, of Nigeria.

Materials and methods

Salmonella isolates

Forty one, *S. enterica* used for this study were isolated from samples of livers, kidneys, spleen, heart blood, and bone marrow col ected from commercial layers and broilers that died of septicemic diseases in Oyo State and Ogun State, South Western, Nigeria between April 2005 and August 2007. The samples were inoculated into Selenite- F- broth, incubated aerobically at 37^{0}C overnight. Subsequently, samples were sub- cultured from the Selenite- F- broth into sterile plates of MacConkey agar and *deoxycholate* citrate agar, incubated at 37^{0}C overnight. Al the non lactose fermenters on MacConkey agar were sub cultured into DCA, incubated at 37^{0}C overnight. They were further identified morphologically, biochemically and serologically according to Kauffman White Scheme with Polyvalent and monovalent (O), (H), and (V1) antisera (Wellcome, Research Laboratories, UK) as S. enterica (Edwards and Ewing, 1989; Barrow and Feltham, 1993). The isolates were subsequently studied for antimicrobial susceptibility to, *ciprofloxacin, kanamycin, chloramphenicol, streptomycin, neomycin, nalidicic* acid, ampicillin and tetracycline.

Determination of antibiotic resistance

Minimum inhibitory concentration The minimum inhibitory concentration (MIC) of *ciprofloxacin, nalidixic* acid, ampicil in, *streptomycin, chloramphenicol, kanamycin, neomycin, tetracycline* for each of the forty one, S. enterica isolates was determined respectively by microtitre method (Adetosoye and Rotilu, 1987).

Known weight of antibiotic powder of ampicil in, streptomycin, neomycin, kanamycin, tetracycline and ciprofloxacin (Sigma-Aldrich inc, 3050 spruce Street, St Louis MO63103, USA), were respectively dissolved in sterile distil ed water to a final concentration of 70 µg/ µl, 64 µg/µl, 64 µg/µl, 64 µg/µl, 70 µg/µl, 60 µg/ µl, respectively. Nalidixic acid was dissolved with 2 drops of 0.2M NaOH and made up to a final concentration of 64 µg/µl, with sterile distil ed water while chloramphenicol powder was dissolved in 1ml of 96% alcohol and made up to a final concentration of 200 µg/µl with sterile distil ed water. A 0.1% concentration of 2.3.5 triphenylteterazolium chloride (Sigma Chemical Company, USA) prepared with sterile distil ed water was used as indicator.

Fifty microlitre of sterile TSB was delivered into each of ten wel s in a row of a microtitre plate (WHO plate). Fifty microlitre of the antibiotic under test was delivered to the first wel The mixture was thoroughly mixed and fifty microlitre was transferred to the next wel .The same procedure was carried out up to the tenth wel , thus the mixture was serial y diluted down to the tenth wel where fifty microlitre of the mixture was discarded. Then twenty five microlitre of the overnight broth culture of the *S. enterica* isolate under investigation was delivered to each wel , fol owed by the addition of twenty five microlitre of 0.1% indicator. A negative control was delivered into 11th row containing a mixture of twenty five microlitre of 7/26 (26.9%) were nalidixic acid sensitive at MIC <8 µg/ µl. Of the fifteen isolates with decreased ciprofloxacin susceptibility, 9/15 (60%) were sensitive to nalidixic acid at MIC of <8 µg/ µl, 5/15 (33.33%) were resistant to nalidixic acid at MIC of =32 µg/µl, and 1/15 (6.67%) showed intermediate resistance to nalidixic acid at MIC range of 8-16 µg/µl. Twenty two resistant patterns were

Table1: Antibiotic sensitivity patterns of drug resistance *Salmonella enterica* of poultry origin in two south Western States of Nigeria

S/N	Antibiotic resistant patterns	Frequency	
1	Amp	1	2.4
2	amp.neo	2	4.9
3	amp.neo.tet	3	7.3
4	amp.neo.nal	1	2.4
5	amp.kan.neo	1	2.4
6	cip.amp.neo	2	4.9
7	Str.neo.nal	1	2.4
8	cip.amp.neo.nal	3	7.3
9	amp.kan.neo.tetra	1	2.4
10	cip.amp.kan.neo	2	4.9
11	cip.amp.str.neo.tet.	1	2.4
12	amp.kan.neo.tet.nal	1	2.4
13	amp.str.neo.tet.nal	2	4.9
14	amp.chl.kan.neo.t et.	1	2.4
15	cip.amp.str.neo.nal	1	2.4
16	cip.amp.str.neo.tet.nal	5	12.2
17	cip.amp.str.chl.neo.nal	1	2.4
18	cip.amp.str.kan.neo.tet.	2	4.9
19	cip.amp.str.chl.kan.neo.tet.	1	2.4
20	cip.amp.str.kan.neo.tet.nal	2	4.9
21	amp.str.chl.kan.neo.tet.nal	1	2.4
22	cip.amp.str.chl.kan.neo.tet.nal	6	14.6

exhibited as shown in table1. An octuple sterile TSB and twenty five microlitre of 0.1% indicator. The positive control on the other hand contained twenty-five microlitre of the overnight broth culture of *S. enterica* under test, twenty- five microlitre of 0.1% indicator, and fifty microlittres of sterile TSB delivered into the twelfth row. The microtitre plate was subsequently covered and incubated at 37^{0}C for 18hours. The procedure was repeated for each of the antibiotics under test and for each of the forty one *S. enterica* isolate studied. The MIC of the respective antibiotic was taken as the lowest concentration of the antibiotic that inhibits the growth of the S. enterica isolate. The change in colour (a red formesan) indicated growth of the resistant isolate as seen in positive control, and no colour change was seen in sensitive strains. The wel nearest to where there was colour change (red formesan) was taken as the minimum inhibitory concentration of the antibiotic tested.

Results

A total number of 26/41 (63.4%), of the forty one isolates were ciprofloxacin resistant at MIC >1 µg/µl, 15/41 (36.6%) had low *ciprofloxacin* susceptibility at MIC >0.06 µg/µl, 14/26 (58.8%) of the high fluoroquinolone resistant isolates were also nalidixic acid (quinolone) resistant at MIC =32 µg/µl, another 2/26 (7.7%) of the high ciprofloxacin resistant isolates had MIC of 25 µg/µl to nalidixic acid, 3/ 26 (11.5%) had intermediate resistance to nalidixic acid at MIC range of 8-16 µg/µl, while 7/26 (26.9%) were nalidixic acid sensitive at MIC <8 µg/µl. Of the fifteen isolates with decreased ciprofloxacin susceptibility, 9/15 (60%) were sensitive to nalidixic acid at MIC of <8 µg/ µl, 5/15 (33.33%) were resistant to nalidixic acid at MIC of =32 µg/µl, and 1/15 (6.67%) showed intermediate resistance to nalidixic acid at MIC range of 8-16 µg/µl. Twenty two resistant patterns were exhibited as shown in table1. An octuple resistant pattern (R-type-cip.amp.str. chl.kan.neo.tet.nal) was manifested by six isolates, three dif erent septuple resistance patterns (R-types-cip.amp. str.kan.neo.tet.nal; amp.str.chl.kan.neo.tet.nal; and R-type-cip.amp.str.chl.kan.neo.tet) were manifested by four isolates. Eight isolates manifested three differerent sextuple resistance patterns as (R-types- cip.amp.str.neo.tet.nal; cip. amp.str.chl.neo.nal and R-type-cip.amp.str.kan.neo.tet). Six isolates showed five different types of quintuple resistant patterns as (R-types-cip.amp.str.neo.tet; amp.kan.neo.tet. nal; amp.str.neo.tet.nal; amp.chl.kan.neo.tet and R-type-cip.amp.str.neo.nal). Three different quadruple resistant patterns (R-types-cip.amp.neo.nal; cip.amp.kan.neo and R-type-amp.kan.neo.tet) were presented by six isolates. Five dif erent triple resistance patterns (R-types-cip.amp. neo; amp.kan.neo; amp.neo.nal; amp.neo.tet; and R- type-str.neo.nal) were exhibited by eight isolates. Two isolates showed double resistant pattern as R-type-amp.neo and a single resistance (R- type- amp.) was seen in just one isolate. Thirty of the forty one isolates, 30/41 (73.2%) were resistant to between four and eight of the *antimicrobial* tested.

Discussion

The MIC value of a Salmonel a species to a given antibiotic is used to determine the resistance status of the strains. A strain with an MIC>1 µg/µl for ciprofloxacin for example wasconsidered high ciprofloxacin resistant, an MIC>0.06 µg/µl as decreased ciprofloxacin susceptible, MIC of 8-16 µg/µl for intermediate resistance to nalidixic acid, but MIC <8 µg/µl as being sensitive to Nalidicic acid (Cavaco *et al*., 2007; Cavaco ., 2007). However for nalidixic acid resistant strains the MIC was =32 µg / µl (Cui

et al., 2008) . Using these MIC values of *ciprofloxacin* and nalidixic acid, it was observed that 26/41 (63.4%) isolates had high *ciprofloxacin* resistance in the current work and this finding is higher than 10/45 (22.2%) of high ciprofloxacinresistant strains of *S. enterica* reported in the United Kingdom (Katie *et al*., 2008). It has been earlier reported that reduced susceptibility to *fluoroquinolone* causes poor response to treatment by Salmonel osis patient as wel as encouraging prolonged bacteria shedding (Wain *et al*., 1997). In the current work, 15/41 (36.6%) showed decreased ciprofloxacin susceptibility, and just like previous workers observation (Wain *et al*., 1997), there were reports of treatment failures and high mortalities associated with the diseases outbreaks where the *S. enterica* isolates studied were isolated (personal communication). This observation is thought to be due to similar reason earlier reported by previous worker that patient infected with quinolone resistant strain of Salmonel a species are more likely to die than those infected with *quinolone* susceptible strains (Helms *et al*., 2002; Helms *et al*.,2004).

The concurrent nalidixic acid resistance among the ciprofloxacin resistant isolates 53.8% agrees with the earlier reports (Hakanen, *et al*, 1999; Hakanen, *et al*, 2005). Consequent upon the use of *Enrofloxacin* and *Sarafloxacine* in general veterinary and poultry industries there were increase in fluoroquinolone resistant strains of Salmonel a species in UK and USA (Anonymous *et al*., 1996; Threlfal *et al*., 1996). The observation of ciprofloxacin resistance in the current investigation may be attributed to the consequences of unguarded use of fluoroquinolone based drug and other antibiotics by some poultry farmers in the area studied.

In 1990S a strain of *S. typhimurium* resistant to ampicil in, chloramphenicol, streptomycin, sulphonamide and tetracycline (R-typeACSSUT) emerged in the United States and Europe (Glynn *et al*.,1993). The isolates studied in the current work however showed resistance to higher number of antimicrobials with R-type cip.amp.strep.chl. kana.neo.tet.nal being the commonest type as seen in six isolates. Five other isolates had R-type cip.amp.strep.neo. tet.nal, while two isolates had R-type cip.amp.strep. kana. neo.tet.nal. In al , 73.1% were multidrug resistant, being resistant to between four and eight antibiotics. *S. enterica* have been identified as the cause of food borne disease and it is of public health importance worldwide (Weil *et al*., 2004).

The occurrence of antibiotic resistance in *pathogenic* organism is not desirable especially in Salmonel a species being a very important zoonotic disease (Anderson *et al* 1999; Lo Fo Wong *et al*., 2002). Nevertheless, there has been reports of increase in occurrence of antimicrobial drug resistance (including nalidixic acid), among Salmonel a species in Thailand (Hoge *et al*.,1998), Netherland (Van Duijkeren *et al*., 2003), Asia (Wang *et al*., 2006). France (Caicol et al.,2006). These isolates could be a potential source of human multidrug resistant Salmonel a infections. Hence, there is a need for public health enlightenment campaign on antibiotics misuse in Nigeria. Consequently, a nation-wide antibiotic resistance surveil ance among food animals in Nigeria is advocated to evaluate the possible risk of drug resistance transfer from animal to man. From this work, the need for reviewing and enforcement of legislation regarding prudent/ judicious use of antibiotic in the livestock industry is suggested, so that no antibiotic especial y those that are used for human treatment should be used for treatment in food animals as was recommended in Great Britain during an outbreak of diarrhea in calves infected by *S. typhimurium* that was resistant to chloramphenicol (the then drug of choice) for treatment of typhoid fever among other antibiotic whereby there were death of six human infected due to treatment failure (Swann, *et al*.1969). Also the use of any antibiotics in food animals must be based on Veterinary prescription and strict supervision to ensure its judicious usage when need be.

Conclusions

Salmonella species constitutes a very important zoonotic disease with sporadic outbreaks in livestock farms with resultant economics losses and profitability in terms cost of treatment and mortalities around the globe. However, the occurrence of emerging of multidrug-resistant pathogenic strains to antimicrobials recorded in this investigation cal s for concern against the backdrop of the fact that the pathogens constitute a potential health hazard to the farm workers as wel as the consumers of such poultry products with the risk of untreatable food poisoning that the pathogens may produce in exposed man and animals. The need to educate the populace and the livestock farmers on the danger of indiscriminate use of unprescribed antimicrobials for treatment cannot be overemphasized.

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PRELIMINARY STUDY ON THE IMPACT OF BOVINE TUBERCULOSIS ON THE REPRODUCTIVE EFFICIENCY AND PRODUCTIVITY OF HOLSTEIN DAIRY COWS IN CENTRAL ETHIOPIA

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ETUDE PRÉLIMINAIRE SUR L'IMPACT DE LA TUBERCULOSE BOVINE SUR L'EFFICACITÉ DE LA REPRODUCTION ET LA PRODUCTIVITÉ DES VACHES LAITIÈRES HOLSTEIN DANS LE CENTRE DE L'ÉTHIOPIE

Résumé

Une étude rétrospective a été menée sur 100 vaches laitières (50 bovins ayant un test positif pour la TB et 50 bovins ayant un test négatif) pour déterminer l'effet de la tuberculose bovine (TB) sur l'ef icacité de la reproductivité et de la productivité des vaches laitières Holstein. Pour atteindre cet objectif, les enregistrements de cinq années de la reproduction / des éléments variables de la production notamment l'âge à la puberté, l'âge au premier vêlage, le nombre de services par conception, l'interval e de vêlage, la production laitière et les jours de traite ont été évalués. L'âge moyen (moyenne au cours du mois± erreur-type de la moyenne) au moment de la puberté pour les vaches positives (26,36 ± 0,93) et pour les vaches négatives (28,26 ± 0,93) ne présentaient pas de différence notable. De même, l'âge lors du 1er vêlage ne présentait pas de différence notable entre les vaches positives (36,09 ± 0,94) et négatives (36,23 ± 1,37). D'un autre côté, la moyenne du nombre de services par conception était nettement (P<0,05) plus élevée chez les vaches positives (2,02 ± 0,18) que chez cel es qui sont négatives (1,25 ± 0,16) pour le 3ème vêlage. En outre, le nombre de jours de traite au premier vêlage (294,43 ± 5,96 contre 327,66± 9,53; P=0,004) et au second (286.58±7.21 vs. 329.16±7.35; P=0.0001) était clairement plus faible chez les vaches positives que chez cel es qui sont négatives. La production de lait a respectivement diminuée de 10%, 13% et 5% au cours du 1er, du 2ème et du 3ème vêlage chez les vaches positives pour la TB par rapport à la production des vaches qui sont négatives. Cette étude préliminaire a montré l'ef et négatif de la tuberculose sur l'ef icacité de la reproduction et de la productivité des vaches laitières Holstein en Ethiopie.

Mots-clés: Impact de la tuberculose bovine, Ef icacité en matière de reproduction, Productivité, Vaches laitières Holstein

Summary

A retrospective study was conducted on 100 (50 bovine TB positive and 50 negative) dairy cows to determine the effect of *bovine tuberculosis* (TB) on reproductive efficiency and productivity of *Holstein* dairy cows. In order to achieve this, five year records of reproductive/ productive variables including age at puberty, age at first calving, number of service per conception, calving interval, milk yield, and milking days were assessed. The mean age (Mean in month± SEM) at puberty for positive cows (26.36±0.93) and negative cows (28.26±0.93) did not dif er significantly. Similarly, age at 1st calving did not dif er between the positive (36.09±0.94) and negative (36.23±1.37) cows. On the other hand, the mean of the number of service per conception was significantly (P<0.05) higher in positive (2.02±0.18) cows than in negative (1.25±0.16) cows for the 3rd calving. Moreover, the average number of milking days at first (294.43±5.96 vs. 327.66±9.53; P=0.004) and second calving (286.58±7.21 vs. 329.16±7.35; P=0.0001) was significantly lower in positive cows than in negative cows. Milk production was reduced by 10%, 13%, and 5% during 1st, 2nd, and 3rd calving, respectively in TB positive cows as compared to that of the negative cows. This preliminary study showed the negative impact of bovine TB on the reproductive efficiency and productivity of Holstein dairy cows in Ethiopia.

Keywords: Impact of *bovine tuberculosis*, reproductive efficiency, productivity, *Holstein* dairy cows

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Introduction

Bovine tuberculosis (TB) is an infectious disease of cattle characterized by the formation of tubercle in any tissue or organ of the animal. According to Cosivi *et al*., (1998), 60% of the African 47% of Asian, and 38% of the American and Caribbean countries have reported the occurrence of bovine TB from sporadic to enzootic level. Thus, bovine TB is one of the devastating diseases of cattle in developing countries throughout the world (Berrada and Barajas-Rgas, 1995). Bovine TB has severe implication for animal welfare and animal health since it causes reduced productivity and premature death in cattle and affected farms suffer severe economic losses (Zinsstag *et al*., 2006). Studies have reported that the prevalence of bovine TB ranging from 3.4% in smal holder production system that keeps zebu cattle to 50% in per-urban (intensive) dairy production system in Ethiopia (Ameni and Roger, 1998; Ameni *et al*., 2001; Laval and Ameni, 2004; Ameni *et al*., 2007; Regassa *et al*., 2007; Ameni *et al*., 2008; Demelash *et al*., 2008). However, the effects of bovine TB on reproduction and productivity have not yet been studied in Ethiopia so far. Clinical tuberculosis in cattle is typically debilitating illness characterized by progressive emaciation and development of tubercle in any tissue (Radostits *et al*., 1994). Such excessive loss of body condition could lead to reduction in milk production and reduced reproductive efficiency, which can be estimated by measuring different reproductive and productivity variables including age at puberty, age at 1st calving, number of service per conception, calving interval, milk yield, milking days etc.

Thus, the objective of this study is to investigate the effects of bovine TB on the reproductive efficiency and productivity of Holstein dairy cows

Material and Methods

Study farm

The Farm which served as a source of data was first established in 1955 and started with 120 in-calf Holstein heifers, which had been imported from the United States. In addition, some pure Holstein heifers were introduced from Kenya in 1959. After about 20 years, an additional 120 in-calf Holstein heifers were donated by the Government of Cuba in 1980 and formed the nucleus of the present Farm. The Farm is located 43km west ofAddisAbaba at Holetta Town on the main road to western Ethiopia. Because of the occurrence of clinical signs suggestive of bovine TB (e.g. respiratory distress, coughing, weight loss, emaciation) and post mortem lesions in animals that had died, the herd was tuberculin skin tested in 2002. On the basis of the result of this test the Farm was divided into positive and negative herds, which were physical y separated but kept under Holstein dairy cows. a similar cattle husbandry and management.

Study animals and data collection

A total of 100 pure Holstein cows, 50 bovine TB positive and 50 negative, were used for this study. The reproductive records including age at puberty, age at first calving, number of service per conception and calving interval were analyzed. In addition, the number milking days and milk yield were analyzed. Both positive and negative cows belong to a single farm. But they were kept physically separate while they were managed uniformly under similar management practices. All records of each of the cow including date of birth, calving date and the first date of service were kept and thus used to calculate the age at puberty the age of 1st calving, and calving interval. The 1st service date for the first lactation was considered as the age at puberty of the animal.

Data analysis

Means (Mean±SEM) were calculated for al reproductive parameters. Student t test was used to compare the means of the measured variables of negative and positive cows. Statistical significance was set at a P-value of <0 .05.

Result

The analyzed comparative reproductive variables for positive and negative cows were presented in Table 1. The mean age at puberty (26.36± 0.93 vs. 28.26±0.93) and mean age at 1st calving (36.09±0.94 vs. 36.23±1.37) did not differ between negative and positive cows. Similarly, the means of number of service per conception for the first calving did not differ between positive (1.62±0.14) and negative (1.56±0.15) cows. Nevertheless, although not statistically significant, the number of service per conception for the first calving was slightly higher in positive cows as compared to negative cows. Further as the number of calving increases, the difference in means of number of services per conception between the positive (2.02±0.18) and negative (1.25±0.16) cows was evident (P<0.05).

Although the mean milk yield did not differ between the positive and negative cows, the mean milking days did show difference across the dif erent calving (Table 1). From Table 1, it can be extracted that milk production was reduced by 10%, 13%, and 5% during 1st, 2nd, and 3rd calving, respectively in TB positive cows when compared with that of the negative cows.

Table 1: Effect of bovine tuberculosis on the reproductive/productivity variables of dairy cows in central Ethiopia

Reproduc tive/productivit y variable	Bovine TB positive (Mean + SEM)	Bovine TB negative (Mean + SEM)	t- test	P-value
1st calving				
Number of service	1.62±0.14	1.56± 0.15	0.04	0.85
Milking d ays	294.43±5.96	327.66±9.53	8.64	0.004
Milk yield (liter)	3106 ± 129.40	3216.66±138.98	0.33	0.565
2nd calving				
Number of service	1.92 ± 0.19	1.92 ± 0 .18	-	-
Calving interval (days)	465.22± 017.85	461.06±17.60	0.03	0.868
Milking d ays	286.58±7.21	329.16±7.35	16.97	0.0001
Milk yield (liter)	3937.4±197.15	4017.52±150.41	0.10	0.748
3rd calving				
Number of service	2.02±0.18	1.25± 0.16	0.937	0.01
Calving interval (days)	437.32±16.27	457.2±18.56	0.64	0.424
Milking d ays	267.84± 8.33	282.81± 8 .96	2.55	0.113
Milk yield (liter)	3862.65± 6 0.94	3691.53±58.98	0.50	0.479
4th calving				
Age at puberty	26.36±0 .93	28.259±1.086	1.76	0.187
Age at 1st calving	36.09±0 .94	36.229±1.373	0.01	0.933

Discussion

A comparative assessment of the variables of reproduction and productivity was made in bovine TB positive and negative Holstein cows to estimate the effects of bovine TB on the reproductive efficiency of dairy cows. The result showed that there was no difference between positive and negative cow in reaching age at puberty and age at 1st calving. The reason could be the fact that bovine TB is affected by age of the animals i.e. young animals are less likely to be infected and the risk of infection increases as the age of the animal increases. Furthermore, as bovine TB is a chronic disease, even if younger animals are skin test positive (infected), as the disease is at its earliest stage its effects could not be so much serious and hence the consequences could be then be milder.

On the other hand, the mean of number of service per conception was greater in bovine TB positive cows than in negative cows particularly as the number of calving increases. As explained above, the effects of bovine TB increase as the age of the animal increases because of its chronic nature. Its effects could be direct on the reproductive organs like uterine and ovaries thereby hindering their physiological functions (Andrews, 1992), or indirect including loss appetite, loss of condition and emaciation, respiratory distress, which could affect cow's reproductive efficiency and productivity. Infection of the uterine with M. bovis causes tuberculous metritis with characteristic feature of chronic purulent discharge that affect the conception rate of infected cows.

At all the three (1st, 2nd, and 3rd) calving the milk yield was slightly greater in bovine TB negative cows than in positive cows although not statistically significant. The absence of statistical significance could be due to the low sample size. Otherwise, obvious difference in milk yield was observed between positive and negative cows. The mean number milking days in each calving was significantly greater in negative cows than in positive cows. Thus, as the total number of milking days has a direct effect on the total volume of milk production i.e. the lower number milking days in bovine TB positive cow causes reduction in the total volume of milk production. Similarly, previous studies in Germany (Meisinger 1970) reported 10%±2.5% loss of milk production and 4%±2% loss of meat production attributed to bovine TB. In Hungary, milk losses were assumed to 12% of the total yield, and loss caused by sterility at 5% in infected animals (Danes, 1986). Further in Spain, Bernues *et al.* (1997) assumed losses of meat production at 10% in calves born from infected cows.

On top of its effect on the reproductive efficiency and productivity, its control has huge cost implications. Efforts to control bovine TB were initiated in 19th century (Bang,

1884), and have occupied substantial part of human resources and institutional capacity of the veterinary sector in the 20th century (Zinsstag *et al*., 2006). Many of the developed countries have put enormous efforts into the control of bovine TB, and finally declared free of the disease, as they could accomplish the important aspect of the control of bovine TB, which is the compensation of farmers for the culled animals. For example, the cost/benefit analysis of M. bovis eradication in the United States showed an actual cost of $538 million between 1917 and 1992 (Frye, 1994). By reducing the number of cattle lost from 100,000 to less than 30 per year, the program saves $150 million per year in replacement costs alone, and as a consequence of such efforts, farmers have benefited by eliminating the indirect cost of losses in milk and meat production, stock replacement, decontamination procedures. Similarly, in the Republic of Ireland, the present value of the benefit of control exceeded the present value of the costs by 85% and the rate of return of the scheme was 15.5% (Sheehy and Christiansen, 1991). Nonetheless, in the United Kingdom, negative results were obtained in the economic evaluation of the control campaigns because of the wildlife reservoir of *M. bovis* (Power and Watts, 1987).

However, most developing countries could not undertake such control scheme and hence bovine TB could be considered as a disease of poverty since the rural communities of most developing countries are not only at higher risk of its zoonosis but also vulnerable to poverty resulting from the loss of livestock productivity.

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EFFICACY OF CHLOROQUINE, IVERMECTIN AND ARTEMETHER ON ONCHOCERCA GUTTUROSA IN ZEBU CALVES IN SUDAN

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EFFICACITÉ DE LA CHLOROQUINE, L'IVERMECTINE ET L'ARTÉMÉTHER SUR ONCHOCERCA GUTTUROSA CHEZ LES VEAUX DE RACE ZÉBU AU SOUDAN

Résumé

Les densités de microfilaires (mff) de la peau, la récupération du ver parasite adulte et l'embryogenèse des vers femel es gravides ont été utilisé pour évaluer l'ef icacité de la Chloroquine,de l'Ivermectine et de l'Artemether contre l'Onchocerca gutturosa dans trois groupes de chacun cinq veaux de race zébus âgés de 2-3 ans et étant naturel ement infectés. La Chloroquine et l'Artemether se sont avérés très ef icaces à la fois contre la microfilaire dermique que contre les vers parasites adultes. Les densités de microfilaires ont rapidement diminuées deux à trois jours après l'injection et la disparution complète est survenue au cours de la période de suivie (4 mois). Quatre mois après la dernière dose de chaque médicament, les animaux ont été abattus et les vers adultes examinés. Les trois médicaments ont montré des taux de mortalité élevé de 27% à 32% parmi les vers adultes femel es ($P<0,05$) par rapport au groupe témoin. Les embryogrammes ont montrés des proportions nettement élevées ($P<0,05$) de stades embryonnaires et microfilaires intra-utérines morphologiquement touchés en plus des signes de dégénération qui ont eu pour conséquence un arrêt partiel ou total du développement embryonnaire.

Mots clés: Onchocerca gutturosa, Ef icacité médicamentaire, Macrofilaricide, Embryogenèse

Summary

Skin *microfilarial* (mff) densities, adult worm recovery and embryogenesis in gravid female worms were used to evaluate the efficacy of *Chloroquine, Ivermectin* and *Artemether* against *Onchocerca gutturosa* in three groups of five natural y infected zebu calves 2-3 years old. *Chloroquine* and Artemether were highly effective against both dermal mff and adult worms. Skin mff densities was declined rapidly within two- to three- days of injection and complete clearance occurred thereafter for the fol ow up period (4 months). Four months after the last dose of each drug, the animals were slaughtered and the adult worms examined. Al three drugs showed significant mortality rates i.e. 27%- 32% among adult female worms ($P<0.05$) as compared to the control group. *Embryogrammes* showed significantly increased proportions ($P<0.05$) of morphologically affected embryonic stages and intrauterine mff in addition to signs of degeneration that resulted in partial or complete inhibition of *embryonigenesis*.

Key words: *Onchocerca gutturosa*, Drug Efficacy, Macrofilaricide, *Embryogenesis*

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INTRODUCTION

The target objective of control of human *Onchocerciasis* is to find a drug with *intrinsic filaricidal* effects that is potent, safe and cheap for long-term treatment of the disease with the ultimate goal of eradication and elimination of *Onchocerca volvulus*. Repeated 6 or 12 monthly standard doses of *Ivermectin* affected the proportion of recovery of living adult *Onchocerca volvulus* (Duke *et al*., 1990) arrested microfilarial production and resulted in only mild to moderate *macrofilarial* effects (Awadzi *et al*.,1999)The more frequent administration of *Ivermectin* from annually to a 3- or 6 month regime was recommended by (Gardon *et al*., 2002) to increase the rate of elimination of the parasite in the long-term control, and to reduce the *microfilarial* load in infected individuals, thereby reducing the transmission of the parasite. However, in a recent conference on onchocerciasis it was concluded that its eradication is not feasible with the present tools alone (Dadzie *et al*., 2002; Borsboom *et al*., 2003) In addition, sub-optimal efficacy of *Ivermectin* and/or Ivermectin resistance in humans has been reported in from Ghana; where despite multiple treatments with *Ivermectin*, *microfilaridermia* persisted in some patients (Awadzi et al., 2004a; Awadzi *et al*., 2004b) It is important therefore, to continue the development of alternative drugs which have *macrofilaricidal* efficacy or which show total and long-lasting suppression of embryogenesis, in order to complement the *microfilaricidal* effects of Ivermectin. *Chloroquine* and other *quinoline*- containing drugs showed *filaricidal* effects in some animal species and in vitro trials with *Onchocerca* species and other *filarial* worms (Guderian *et al*., 1987; 1988; Thompson *et al*., 1968; Trees *et al*., 2000; Van de Waa *et al*., 1989) However, (Guderian et al., 1991; 1997) observed reduction in dermal mff load after treatment of acute malaria in Equadour, suggesting an interaction between *Chloroquine* and O. volvulus.
Since *Onchocerca volvulus* only infects humans, this study was intended to use *Onchocerca gutturosa* in zebu calves as an animal model for testing the micro- and *macrofilaricidal* effects of *Chloroquine* and *Artemether* compared to repeated doses of Ivermectin.

Materials and Methods

Experimental animals:

Twenty zebu male calves, 2-3 years old, and naturally infected with *Onchocerca gutturosa* were used. The animals were housed at the premises of CVRL, Khartoum and had free access to water and sorghum straw. They were divided into four equal groups, each of five, weighed and subjected to the following treatment regimens:
Group I (*Chloroquine*) received daily I/M injection of Chloroquine (*Chloroquine - Phosphate Base*, France Lab.) at a dose of 200 mg/day for 7 consecutive days then repeated weekly for 14 weeks.
Group II (Arthemedine) were injected I/M with 160 mg Artemether (Artemedine, Kunming Pharmaceutical Corp, China) for three successive days.
Group III (Ivermectin) received weekly S/C injection of Ivermectin (Ivomec®, Merck Sharp and Dohme, New Jersy, USA) at a dose rate of 150 µg/kg body weight for 14 weeks;
Group IV (Control) was kept as an infected untreated control.

Assessment of skin mff densities:
Skin snips were taken from the mid-dorsal line of the animals around the hump before treatment and at frequent intervals after treatment. A designated area of about five cm2 was shaved and cleaned. The skin was held apart by a thicker and an area of about 1-2 cm2 was cut to the full thickness of skin, weighed, washed with tap water and cut into small pieces and incubated in clean polystyrene tube with one ml of *Phosphate Buffer Saline* (PBS, with PH 7.0-7.2) supplemented with penicillin, (100 iµ/ml) and *streptomycin* (100 µl /ml).
After 3–4 hours of incubation at 37oC, the active mff emerged in the PBS. The PBS was then aspirated by a pipette into a watch glass and the number of the mf was microscopically counted using magnification of X4 and X10. The mf densities were expressed as mf per gm of skin and their count percentages were calculated out of their initial densities.

Recovery of adult worms:
Calves were sacrificed four months after the last dose of

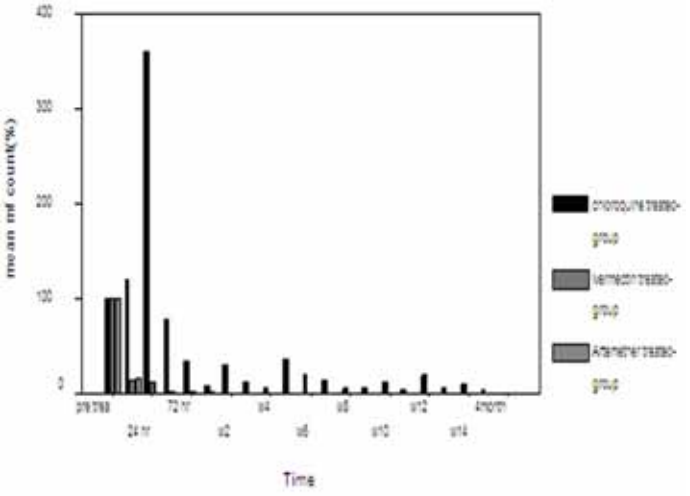


Figure 1: The mean skin mff count (%) in the treated groups

each treatment. The ligamentum nuchae of each calf was removed carefully with its connective tissue intact. After trimming, the tissue containing adult worms was washed in PBS (PH 7.0 - 7.2) and incubated at 37oC in 0.3% collagenase in PBS supplemented with 100iμ/ml penicillin and 100μl/ml streptomycin. After two to three hours, the digested tissue was transferred to a petri dish with fresh PBS. The partially released worms were gently extracted from the digested tissue by a fine forceps, and then washed three times with PBS. Each worm was kept individually in a *polystyrene* tube containing one ml of PBS.

Evaluation of drug efficacy on adult worms:
Each worm was examined morphologically and identified as a male or a female, and further categorized as alive or dead according to {Chavasse et al., 1992}. Live worms were recorded on the basis of their motility, viability of intra-uterine mff and embryos or uterine musculature and intact internal morphology in case of immotile worms. Worms that were collapsed degenerated with disintegration of some or all internal organs, transparent cuticle with irregular interspaces between cuticle and hypodermis which may be filled with inclusions and vacuoles were classified as dead worms (Chavasse *et al*., 1992) Dead worms were further classified as fragmented or partially to completely calcified. Live females were grouped further according to their size, colour and reproductive status as young, mature gravid, or mature non-gravid worms. Small transparent female worms without fully developed ovaries and uteri were classified as immature and their number excluded from all analysis concerning reproductivity. Worms that showed developing oocytes and other uterine forms were classified as mature worms according to (Schultz-key 1988).

Evaluation of drug efficacy on embryogenesis:
The preparation and evaluation of embryogrammes was done as described by (Chavasse *et al.,* 1992). Gravid females obtained from each calf were pooled together in a watch glass, cut into small pieces and crushed gently to allow uterine stages to emerge in PBS. The homogenate was transferred to a sterile clean and well-stoppered eppendorf tube, and completed to a known volume. Fifty μL from the homogenate were taken by a micropipette, diluted into one ml PBS and transferred to a MCMaster counting chamber for embryogramme analysis according to (Renz *et al.*, 1995). Differential counts of the various developing uterine stages were made; viz the stretched mf, coiled mf, oocytes and the developing morulae of different sizes. Degenerating and damaged stages were distinguished morphologically from the normal ones and were also recorded. The deformed (damaged) stages showed an irregular appearance, dark in color and undefined segmentations. The degenerated mff showed disappearance of internal nuclei, irregular cuticle with fragmentation and vascularization. The approximate number of each embryonic stage per female worm was recorded and the percentages of affected forms were calculated for each treated- and control groups.

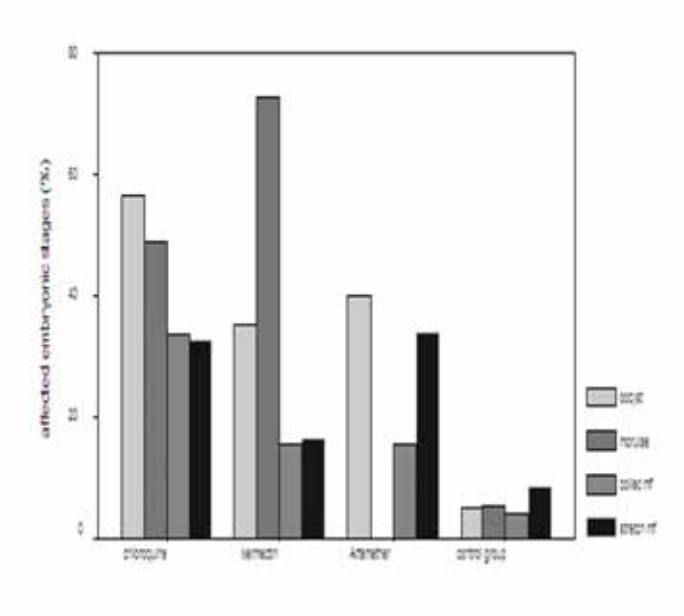


Figure 2. Affected embryonic stages (%) in the treated and the control groups

Statistical Analysis:
Statistical Packages for Social Science (SPSS programme) was used to evaluate the significance of the results. Data were subject to either ANOVA-test or Chi square test and the significance was considered at P-level <0.05.

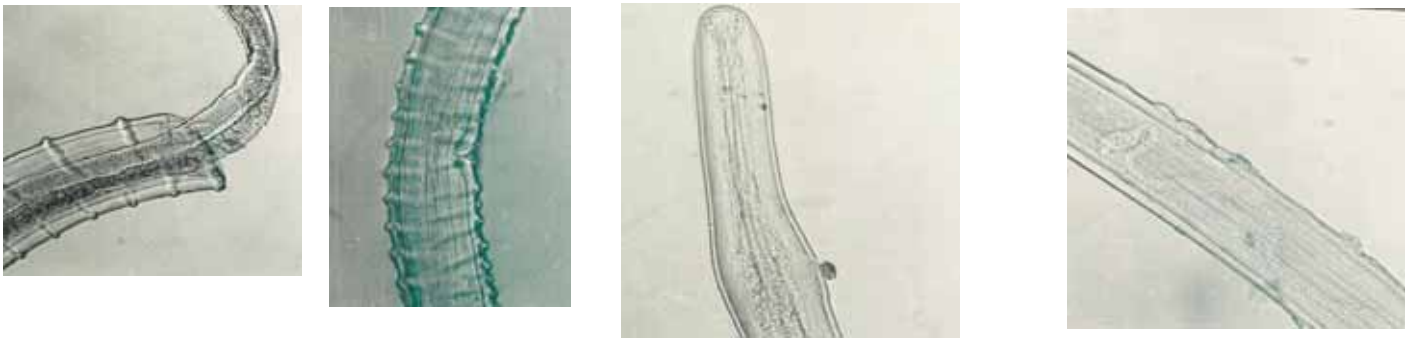
Results

Effects on skin mff counts:
Although great variations were observed in the initial mff count of each calf in each of the four study group, yet the group means were almost similar (P>0.05) ranging as 5.630, 1.935 and 1.409 mff/mg skin for Chloroquine-, Ivermectin-, and Artemether-treated group, respectively. Fig. 1 shows the mean mf counts at different time points after treatments, as percentages out of their initial counts.
In the *Chloroquine*- treated group the mean mff count started to increase significantly within 6 hours of the first injection to 118% (6.658 mff/ mg) of their initial count and reached its highest count at 24 hours, 359% (20.227 mff /mg skin) then it decreased sharply to 78% and 33% at 48 and 72 hours, respectively. During the course of treatment, the mff density decreased gradually until below 20%

Table 1. Total numbers and percentages of adult females of O. gutturosa recovered, from control and treated groups, 4 months post last dose of treatment

Group	No of recovered males	Status of female *O. gutturosa*				
		Dead	Living	Gravid	Non-gravid	Immature
Control	14	0.0(0.0%)	24 (100%)	21 (100%)	0.0(0.0%)	3
Chloroquine	0.0	7 (31.8%)	15 (68.2%)	9 (75.0%)	3 (25.0%)	3
Ivermectin	7	11(32.4%)	23 (67.6%)	19 (91.5%)	2(8.5%)	2
Artemether	0.0	3(27.3%)	8 (72.7%)	5 (83.3%)	1(16.7%)	3
P- value	----	0.034*	0.034*	0.275	0.275	---

Significant at P <0.05: Chi2 test



A B C D

Plate 1. Unaffected female and male worms of O. gutturosa from the control group of calves:
(A) Portion of a fully gravid female with million oocysts and developing mf (X25).
(B) Portion of a female showing the vulva(X25)..
(C) Anterior end of a male (X25).
(D) Portion of a male showing normal gonads(X25).

at week two and at the end of the 14 weeks of treatment, the mff clearance was almost 90% -100%. This group remained almost mff- free during the four months post last dose of *Chloroquine*.

The mean percentages of dermal mff count in the Ivermectin- and Artemether- treated groups were similar and fell sharply to about 15% of its counts within the first 48-72 hours of medication. Skin *microfilariae* were completely absent thereafter (Fig.1). All treated calves remained mff-free throughout the follow -up period of four months after the last treatments.

Effects on adult worm recovery:

All living adult worms of both sexes from the control group were found to be highly active, motile and morphologically normal (Table 1 and plates 1). In contrast, the recovery of living worms was much lower in all three treated groups. The proportion of dead female worms ranged between 27.3%- 32.6% compared to (0.0%) in the control group (P< 0.05, Table1). The proportion of gravid worms among the living ones ranged between 75%–91.5% in the treated groups compared to 100% in the control group (Table 1). The dead females were found transparent with degenerated internal organs and irregular cuticle structures some of them were found fragmented and/ or partially calcified (plate 2, 3, and 4). In *Chloroquine*- and Artemether-treated calves, adult males were not seen in the digested nuchal ligamnets whereas, seven adult males were extracted from one calf, which was highly parasitized with *O. gutturosa* adult worms, in the *Ivermectin* - treated group.

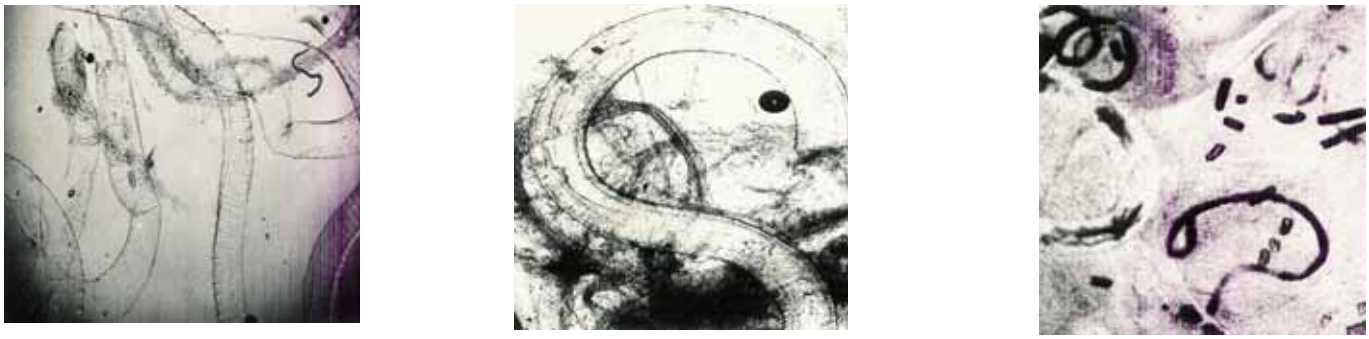
Effects on embryogenesis:

Repeated doses of both *Chloroquine* and *Ivermectin* as well as a single dose of Artemether resulted in clear effects on the *embryonic* development of *Onchocerca gutturosa*. The analysis of the embryogrammes of treated worms showed significant proportions of all *morphologically* affected developing stages (P< 0.05- chi2 test, Table 2, Figure2, Plates 5,6 and 7). The morula stage was highly affected by Ivermectin (72%) followed by Chloroquine (48.85%) whereas in Artemether treated group this stage was almost completely absent (Fig. 2). In Chloroquine- and Artemether- treated calves the number of affected stretched mff within the female uteri was significantly higher than in Ivermectin-treated group (P< 0.021) Table 2.

Table 2: The mean total numbers and percentages of affected embryonic stages in the control and treated groups

Group	Oocyts	Morulae forms	Coiled mff	Stretched mff
Control	20962 (5.2)[a]	17274 (5.5)[a]	5336 (4.04)[a]	3458 (8.5)[a]
Chloroquine	19932 (56.4)[b]	8613.5 (48.5)[b]	6642 (33.6)[b]	4188 (32.8)[b]
Ivermectin	17747 (40.6)[b]	1741 (72.7)[b]	285 (15.8)[ab]	3672 (16.4)[c]
Artemether	24230 (40.4)[b]	0.00	14735 (15.8)[a]	7570 (34.1)[b]
P- value	0.001	0.028	0.031	0.021

Mean values with different superscript letters within same column are significant at P <0.05: Chi2 test



A B C

Plate 2. Portions of affected of O. gutturosa females in Chloroquine-treated calves:
(A) Degenerated dead worm with completely absorbed internal organs and loss of cuticle structure(X10).
(B) Dead fragmented and transparent female worm embedded within the host tissue(X10).
(C) Fragmented and calcified female(X10).

Discussion

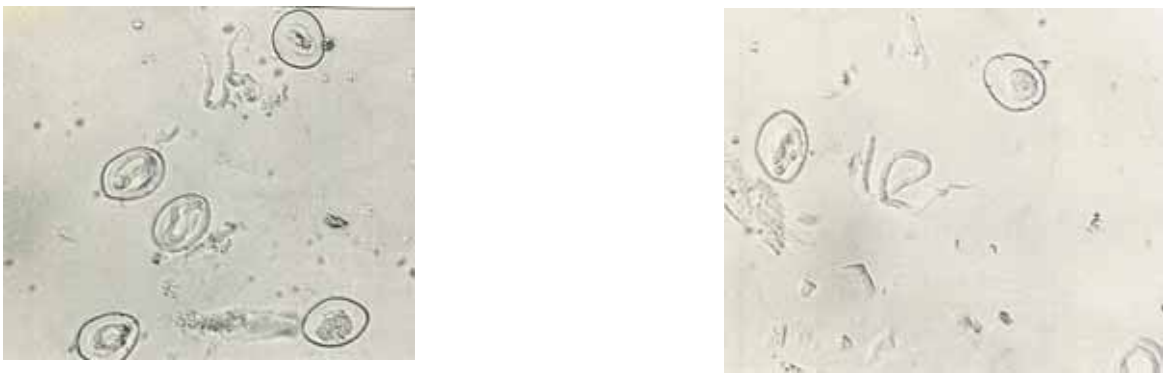
Since *O. volvulus* only infects man, the use an alternative animal filaria for testing drugs is imperative. Onchocerca species in cattle have successfully been used in a number of drug trials e.g. *O. gibsoni* in Australia, *O. gutturosa* and *O. lienalis* in Europe and *O. ochengi i*n Cameroon, (Trees *et al*., 2000; Renz *et al*., 1995). From a number of observations in human treatment, some antifilarial activity of antimalarial compounds including quinoline and chloroquine became evident (Van de Waa *et al*., 1989; Guderian *et al*., 1991). In this study we compared two antimalarial drugs, chloroquine and arthemether in a bovine model using natural infections with *O. gutturosa* as a drug screen. The density of skin mff increased immediately after administration of *Chloroquine* for 24 hours and then, the reduction became consistent and by the end of the 14-weeks the mf count almost reached zero with complete clearance up to the end of the experimental period (four months post last dose of treatment). This shows that the efficacy of Chloroquine is repeated dose- and time dependent as shown in our previous in vitro studies (Husna *et al*., 1998) This is in line with those of (Guderian *et al*., 1991) in that there is a clear association between reduction of dermal mff densities of *O. volvulus* and treatment with *Chloroquine* in Ecuadorian patients suffering from acute malaria and kept on prophylaxis. On the other hand, reappearance of skin-mff in patients treated with *Chloroquine* over 3 days (Guderian *et al*., 1991) supports our observation of elevated mff counts at the first 24 hours in this study. Treatment with *Chloroquine* and Fansidar (*Pyrimethamine/sulfadoxine*) showed 100% clearance of experimentally inoculated mf of *O. gutturosa* into albino mice on day six (Husna *et al*., 2008a) Histological sections of ear-skin on day five-post treatments showed dead mf with signs of degeneration and cellular reaction around them.

Anti-filarial activities of other quinoline- containing antimalarial compounds have been reported against other filarial nematodes in animal models and in vitro studies (Thompson *et al*., 1968;Van de Waa *et al*., 1989) According to (Mahmoud *et al*., 1991) *Chloroquine* has a high affinity towards melanine-containing cells (melanocytes) in the skin which may explain its potential microfilaricidal efficacy. Although its antifilarial activity is not fully understood, it had been suggested that *Chloroquine* may inhibit the aerobic energy metabolism in filarial worms {Van de Waa et al., 1989}. Weekly repeated doses of Ivermectin, (150 µg/kg) for 14 weeks resulted in a significant increase of dead female worms in the ligamentum nuchae. In contrast, other workers showed that even a higher dose (800 µg/kg body weight) or repeated high doses of Ivermectin could not kill adult O. volvulus in humans {Awadzi et al., 1995}and O. ochengi in cattle (Renz *et al*., 1995) but rather led to a permanent sterilization of the females worms in

A B

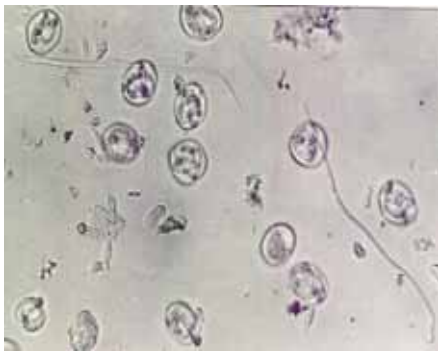
Plate 4. Portions of affected O. gutturosa females in Artemether- treated calves:
(A) Dead female worm with partially calcified anterior end, affected cuticle and degenerated internal organs (X10).
(B) Posterior end of an unaffected female (X10).

cattle (Bronsvoort *et al*., 2005). On the other hand, the present results are in partial agreement with (Duke *et al*., 1990), who stated that 12 monthly doses of Ivermectin killed 12% of male worms and 22% of female worms of *O. volvulus* in man. Adult male worms were only seen in one calf out of five, this agrees with the work of (Renz *et al*., 1995)who reported that fewer males were recovered from *O. ochengi* nodules after Ivermectin treatment and this was considered a useful, early indicator of *macrofilaricidal* activity. However, in other studies on *O. ochengi* in cattle the number of recovered adult male worms varied over time of treatment (Bronsvoort *et al* 2005). Similar to Ivermectin, Artemether showed profound and rapid effects in reducing mff densities of *O. gutturosa* soon after the first dose. Although this group of calves had pretreatment mean count of skin mf about 1.400 mf/mg skin, it sharply dropped to 15% six hours after the initial dose. Treatment continued to be highly effective against dermal mf for four months post last dose of treatment. In all treated calves no allergic reaction was observed and no side effects reported in this treated group (Husna *et al*., 2008b). Although this group showed the lowest worm recovery, yet 27.3% were found dead and no male worms were observed. The macrofilaricidal effect of Artemether on the reproductive potential of living female worms was assessed four months after treatment. Only one female (16.6%) had empty uteri indicating complete inhibition of *embryogenesis*. Other gravid females showed generating *oocytes*, coiled mff and stretched mff whereas, the morula stages were even completely absent. This may be due to high percentage of affected *oocytes*, which failed to develop further on. This study also indicates that male worms are possibly more sensitive to *Chloroquine* and Artemether as indicated by their low recovery rates.



A B

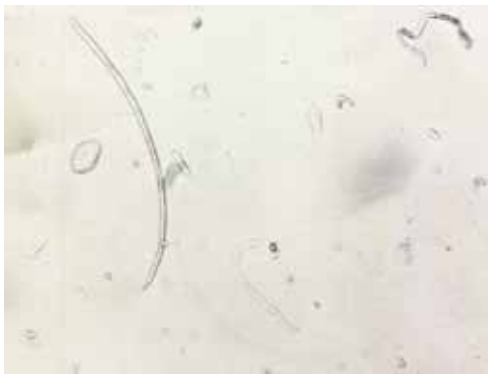
Blate 5. *Embryogenesis* of *O. gutturosa* females in *Chloroquine*- treated calves:
(A) *Embryonic* suspension with affected and degenerating stretched and coiled mff (X40)
(B) Affected mff, morulae stage and oocyst (X40).



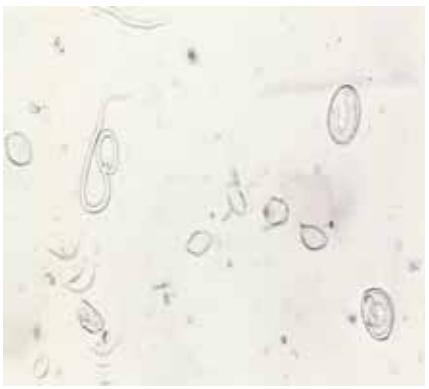
A

B

Plate 6. *Embryogenesis* of *O. gutturosa* females in *Ivermectin*- treated calves:
(A) *Embryonic* suspension with normal mf and affected oocyst and coiled mff (X14).
(B) Affected oocyst, morulae stages and degenerated mff (X40) .



A



B

Plate 7. *Embryogenesis* of *O. gutturosa* females in *Artemether*- treated calves:
(A) *Embryonic* suspension with affected and degenerating mff (X40).
(B) Unaffected mff and degenerating morulae, and affected *oocyst* (X40).

Conclusion:

From this study it can be concluded that the use of *Chloroquine* and *Artemether* may reduce the level of transmission of *Onchocerciasis* through their action on reducing the dermal *microfilarial* load and their inhibitory effects on *emberyogenesis*. Although the use of both drugs in the treatment of helminths is limited, this trial may provide a tool for further studies on the control of both animal and human onchocerciasis.

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Impact:

The global objective in *onchocerciasis* endemic areas is the control and eradication of the disease through both patients treatment and vector control measures. The WHO on-going programs in this field have achieved high progress. Our recent study may be a valuable contribution in searching for potent, safe and cheap treatment of the disease with ultimate goal of eradication and elimination of *Onchocerciasis*.

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STUDY ON SEROPREVALENCE OF BOVINE BRUCELLOSIS AND ABORTION AND ASSOCIATED RISK FACTOR

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ÉTUDE SUR LA SÉROPRÉVALENCE DE LA BRUCELLOSE BOVINE ET L'AVORTEMENT COMME FACTEUR DE RISQUE ASSOCIÉ

Resumé

De novembre 2008 à mars 2009 une étude transversale sur la *brucellose bovine* a été menée pour déterminer la prévalence sérologique et la répartition de la brucellose bovine dans des sites sélectionnés de la région de Jimma au sud-ouest de l'Ethiopie. Un total de 950 animaux (541 femel es et 409 mâles) a été soumis au test sérologique par l'utilisation de RBPT (épreuve d'agglutination sur plaque à l'antigène tamponné) comme test de dépistage et CFT (réaction de fixation du complément) comme test de confirmation. Parmi les animaux testés, un total de 26 (2,27%) animaux a donné des résultats positifs pour les réacteurs de l'anticorps de la brucel ose par la RBPT et cela a également été testé par la CFT dans lequel 10 animaux (1,1%) ont réagit positivement aux anticorps de la *brucellose*. D'importantes différences ont été observées dans les données sur la *séropositivité* des animaux ($p<0,05$). La *séropositivité* était associée de manière importante aux antécédents d'avortement ($p<0,05$). Aucune différence significative n'a été observée entre le sexe, l'âge, la tail e du troupeau et la catégorie des animaux ($p>0,05$). En outre, aucune dif érence significative n'a été également constatée entre la gestation, la lactation et la parité avec la *séropositivité* de la maladie ($p>0,05$).

Mots clés: *Brucellose bovine*, CFT, RBPT, Facteurs de risque, *Séroprévalence*

Summary

A cross sectional study was carried on *bovine brucellosis* from November 2008 to March 2009 to determine the sero-prevalence and distribution of bovine brucel osis in selected sites of Jimma zone, Southwestern Ethiopia. A total of 950 animals (541 female and 409 male) were examined *serologically* by using RBPT as screening test and CFT as confirmatory test. Out of the total animals tested 26 (2.74 %) were found positives reactors for brucella antibody by RBPT and this was further tested by CFT in which 10 (1.1%) animals were found positive reactors for *brucella* antibodies. Significant variation were observed among woredas with *seropositivity* of the animals ($p<0.05$). Seropisitivity was significantly associated with history of abortion ($p<0.05$). No significance differences was found between sex, age, herd size and categories ($p>0.05$). In addition, no significance dif erence observed among pregnancy, lactation and parity status ($p>0.05$) with *seropositivity* to the disease.

Key Words: *Bovine brucel osis*, CFT, RBPT, Risk Factors, *Seroprevalence*

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Introduction

Bovine brucellosis has an impact on animals and human health, as well as wide socioeconomic impacts, especially in countries which the rural income relies largely on livestock breeding and dairy products. Brucellosis is a zoonotic disease and has public health and economic importance in most countries of the world (Nicoletti, 1984).

In Ethiopia *bovine brucellosis* is one of the infectious diseases of animals and humans which cause loss of milk production, infertility and sterility in bovine and undulant fever in human as reported by earlier studies (Asfaw *et al.*, 1998; Bekele, 1999; Tolosa, 2004; Berhe *et al*., 2007; Tolosa *et al*, 2007; Kebede *et al*., 2008; Tolosa *et al*., 2008).

Chora Botor and Limu kosa are found in Jimma zone which are almost located in Gibe valley of southwestern part of Ethiopia where a large numbers of indigenous breed are concentrated. The status of bovine brucellosis in extensive management system of the zone was reported (Tolosa *et al*., 2008), but in the Chora botor district there is no data available on the prevalence of bovine brucellosis and its epidemiological risk association. Therefore, the objectives of this study are:

- To determine the sero-prevalence of bovine brucellosis
- To assess the effect of risk factors upon the occurrence of the disease

Materials and Methods

Study area

The study was carried out in the Southwestern part of the Oromia regional state in Jimma zone, Chora Botor and Limu kosa districts which are located around Gibe valley at 120 and 75 km North from Jimma town. Chora Botor and Limu kosa are under sedentary cattle husbandry system where livestock owners and their livestock remain permanently settled in one area without practicing seasonal migration to other areas in search of feed and water.

Study Animals

The study animals are indigenous cattle in the district which were under individual ownership, or management system. Animals above 6 months of age with no history of vaccination were included in the study. The animals were categorized as cow, calve, heifer and bull.

Study design

A cross sectional epidemiological study was carried out between November 2008 up to March 2009 on indigenous cattle by using serological tests: Rose Bengal plate Test (RBPT) and complement Fixation Test (CFT).

Sample size and sampling technique

Sample size was done with an expected prevalence of 0.77 % (Tolosa *et al*., 2008), with a 95% confidence interval and 5% desired absolute precision (Thrusfield, 1995). Accordingly, a total of 950 animals were sampled and tested using RBPT and confirmed further by CFT. Simple random sampling technique was used to select the study animals. Accordingly two PAs from Limu Kosa and two from Chora Botor were selected. From each PAs one village was randomly selected and from each village blood samples were collected from all animals above 6 months of age.

Study Methodology

Blood sample collection and handling

About 10 ml of blood was collected from jugular vein of each selected animals by using plain vacutainer tubes and serum was separated and transported in icebox to Jimma University, college of Agriculture and veterinary medicine at microbiology laboratory and stored at -20^{0}C until testing.

Serological tests

Rose Bengal plate test (RBPT)

Rose Bengal plate test was performed according the standard procedures. RBPT used for screening test of the serum for the presence of Brucella agglutinin which was obtained from Veterinary Laboratory Agency UK. The serum samples stored at -20°Cwas first removed and left to melt and then 30µl of serum was taken from each sample by using micropipette and put in one of each microscopic slide wells. Again 30µl of antigen commercially prepared was taken and put also on the other sides of each slide and mixed thoroughly and left for 4 minutes. Finally positive results were categorized based on their degree of agglutination. The test was performed, at Jimma University, College of Agriculture and Veterinary Medicine, at Microbiology laboratory

Table 1. Seroprevalence of RBT and CFT for brucel osis by study districts

District*	N	RBT Number(%) positive	CFT* Number(%) positive
Chora Botor	456	26(5.7)	10 (2.2)
Limu Kosa	494	0 (0)	0 (0)
Total	950	26 (2.74	10 (1.1

Complement fixation test (CFT)

Those positive serum samples by Rose Bengal plate test (RBPT) were further tested by CFT for confirmation. In the Complement fixation test (CFT), all reagents were evaluated by titration. The CFT was done at National Veterinary Institute, Debre Ziet, Ethiopia according to the protocol recommended by OIE (2004). Sera with strong, more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and at dilution of 1:20 were classified as positive (OIE, 2004).

Data analysis

Data obtained from the laboratory results were stored in Microsoft Excel spread sheet program. Analysis for brucella sero prevalence was carried out by computer software SPSS version 15.1. The total prevalence was calculated by dividing the number of Rose Bengal Plate Test (RBPT) and Compliment Fixation Test (CFT) positive animal by the total numbers of animals tested (Thrusfield, 1995). The association between different risk factors and the prevalence were assessed using Chi-square. Herd prevalence was calculated by dividing the number of herds with at least one reactor in Rose Bengal Plate Test (RBPT) and Compliment Fixation Test (CFT) by the number of all herds tested.

Results

Overall seroprevalence of bovine brucellosis

Out of the total sera tested using RBPT, 26 (2.74 %) reacted positively to brucellosis. These were further tested using CFT and 10 (1.1 %) animals were confirmed to be seropositive for brucellosis (Table 1). The prevalence was recorded in Chora Botor (2.2 %) district, but none in Limu Kosa district (Table 1).

Seroprevalence versus risk factors (sex, age and herd size)

Although higher seroprevalence was observed in females (1.29%) than males (0.73 %) there was no statistically significance difference between sex groups ($p > 0.05$). Older animals were more reacted to brucellosis than the others. Animals above five years of ages has higher seroprevalence (1.5%) than animals less than five years (1.3 %) (Table 2). Herd size has no significant effect with prevalence of brucellosis in individual animals.

Seroprevalence versus reproductive status

Significant association was found between cow with serpositivity and history of previous abortion (Table 3). However, there was no significant association with factors like parity number, lactation status, and pregnancy status with prevalence of brucellosis.

Discussion

In this study an overall 1.1 % seroprevalence at animal level was established in the study area. This is in line with that of Abay (1999) with 1.5% seroprevalence in southeastern Ethiopia; and Mulugeta (2006) with seroprevalence of 1.13% in Addis Ababa and Sululta abattoirs. The present finding is higher than that of Tadesse (2003) who reported 0.14% prevalence in north Gondar and Lidiya (2008) with

Table 2. *Seroprevalence* of *bovine brucel osis* in cattle according to sex, age and herd size

Description	No of tested animals	CFT Number(%) positive	CI (95%)	c2	p-value
Sex					
Female	541	7 (1.29)	0.34-2.24	0.73	0.70
Male	409	3 (0.73)	0.10-1.56		
Total	950	10 (1.9)			
Age					
≥0.5-2year					
≥2-5year					
≥ 5year					
Total	950	10(1.1)			
Herd size					
<10	291	1 (0.34)	0.30-1.02	5.37	0.68
≥10-20	392	3 (0.8)	0.10-1.63		
≥ ≥20	267	6 (2.2)	0.40-4.04		
Total	950	10 (1.1)			

seroprevalence of 0.045% in four zones of Shoa, but much lower than Kibru (1985) who reported 18.4% prevalence in and around Addis Ababa, and Bayleyegn (1989) who reported 7.6%, in Arsi region. This difference might be due to the difference in husbandry system, management, the breed of animals and other related factors. According to FAO-WHO (1986), the level of brucellosis infection tends to be relatively high in intensive farms than where there has been indigenous cattle or introduced breed, it has been also reported that the risk of infection increase with change from the pure extensive to more intensive cattle management.

Occurrence of the disease was different from district to district. The result showed that in Chora botor the prevalence was very high and none in Limu Kosa. The spread of disease from one herd to other and from one area to other area is almost always due to the movement of infected animal from an infected herd into a non-infected susceptible herd (Radostits *et al*., 2000).

In the present study, higher prevalence was observed in female animals than males. Tolosa *et al*. (2008) and Yayeh, (2004) reported that males were non reactor for brucellosis tests. Susceptibility of cattle to brucella abortus infection is influenced by the age, sex, and reproductive status of the individual animals (Radostits *et al*., 2000).

In this study, higher prevalence was observed in older age group than younger age group. This observation is in agreement with previous findings reported by other authors (Asfaw *et al*., 1998; Taye, 2005; Tolosa *et al*., 2008). Sexually mature pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex (Radostits *et al*., 2000; Kebede *et al*., 2008; Tolosa *et al*., 2008).

Table 3. *Seroprevalence* of *bovine brucellosis* in cattle according to reproductive status

Description	No of tested animals	CFT Number (%) positive	$\chi 2$	p-value
Abortion history				
Aborted	22	2 (9.1)	6.42	0.01
Non aborted	289	4 (1.4)		
Total	311	6 (1.9)		
Pregnancy status				
Pregnant	61	2 (3.3)	0.73	0.39
Non Pregnant	250	4 (1.6)		
Total	311	6 (1.9)		
Lactation status				
Lactation	171	3 (1.8)	0.06	0.80
Non Lactation	140	3 (2.1)		
Total	311	6 (1.9)		
Parity				
One parturition	59	0 (0)	5.56	0.62
Two parturition	81	4 (4.9)		
Three parturition	171	2 (1.2)		
Total	311	6 (1.9)		

In the present study, higher prevalence in larger herd size was recorded. This findings also in line with that of other authors in different parts of the country (Asfaw et al., 1998; Tolosa et al., 2008). Herd located close to other infected herds whose owners made frequent purchases of cattle had an increased risk of acquiring brucellosis (PAHO/WHO, 2001).

In this study, it is observed that there was significant association between abortion and the prevalence of brucellosis. This is in agreement with the previous findings with other authors (Tolosa *et al*., 2008). Higher prevalences were observed in pregnant cows than non pregnant cows; in non lactating cows than lactating cows, and in cows with two parturition than less than two or above.

Conclusion

In conclusion, the present *seroprevalence* study on brucellosis showed the presence of brucellosis in the study area even though the recorded prevalence of the disease is low. However, it is observed that there was significant association between abortion and the prevalence of brucellosis which needs further investigation to study

the loss incurred due to the disease, and its public health importance in the area.

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CAMEL MASTITIS, ASSOCIATED BACTERIAL PATHOGENS AND ITS IMPACT ON MILK QUALITY IN GEWANE DISTRICT, AFAR REGIONAL STATE, NORTHEASTERN ETHIOPIA

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MAMMITE CHEZ LA CHAMELLE, ASSOCIES AUX AGENTS BACTÉRIENS PATHOGÈNES ET IMPACT SUR LA QUALITÉ DU LAIT DANS LE DISTRICT GEWANE, ETAT RÉGIONAL D'AFAR, NORD DE ETHIOPIE

Résumé

La présente étude a été menée de septembre 2006 à avril 2007, dans le but d'évaluer la présence de mammites chez les chamelles, de bactéries qui lui sont associées ainsi que les quantités de matière grasse et de protéines dans le lait de chamel e dans le district de Gewane de l'Etat Régional d'Afar, dans le nord-est de l'Ethiopie. Des chamelles al aitantes issues de différents troupeaux et gérées de manière traditionnelle ont été sélectionnées de manière aléatoire, des échantillons de lait par quartier ont été prélevés . Des échantillons de lait (n=404) émanant des chamelles allaitantes (Camelus dromedarius) du district de Gewane ont été analysés en vue de dépister des mammites. Vingt et un (20,8%) quartiers ont été diagnostiqués comme étant des cas de mammite clinique. Des pis examinés, trente et un (30,7%) étaient infestés de tiques et avaient des lésions, tandis que 45,2% avaient des mammites. Il existe un lien fort ($p< 0,05$) entre l'infestation par les tiques et la mammite. Il a été découvert que les chamel es al aitantes, au début ou au cours de la période de lactation, étaient plus atteintes par les mammites ($p<0,05$) comparés a ceux des périodes plus avancées de la lactation. Cent quatre-vingt six échantillons de lait par quartier (48,5%) ont donné des résultats positifs pour le test californien de dépistage des mammites (CMT) alors que 164 (83,7%) ont produit des bactéries pathogènes. Une correlation forte et positive ($r = 0,76$) a été a été constatée entre les résultats positifs au CMT et la présence d'importants agents pathogènes dans les échantillons de lait. Les principaux agents pathogènes de la mammite qui ont été isolés étaient la *Staphylocoque* doré, la *Staphylocoque* négatif (quant à la coagulasse), le *Streptococcus agalactiae,* le *Streptococcus dysagalactiae* et *Ecoli*. Les pourcentages moyens de matière grasse et de protéines dans le lait testé négatif pour le CMT étaient respectivement de 3,83% ± 0,081 et de 2,85 ± 0,024. Toutefois, dans le lait testé positif pour le CMT, ce pourcentage était respectivement de 1,97 ± 0,071 et de 2,91 ± 0,13. Les résultats de l'analyse suggèrent que la mammite de chameau est répandue dans le district de Gewane et qu'el e est liée à une quantité relativement faible de matière grasse et de protéines dans le lait de chamel e. Des recherches plus approfondies sur le lait de chamelle sont essentiel es pour promouvoir et rétablir la bonne santé et pour aider les associations de fermiers qui sont spécialisées dans l'élevage de chameaux.

Mots clés : Prévalence, étiologie, mammites, chamelles, test californien,

Summary

The present study was conducted between September 2006 and April 2007 with the aim of assessing the occurrence of camel mastitis and bacterial causes associated with it and evaluating Fat and Protein content of camel milk in Gewane district, Afar Regional State, Northeastern Ethiopia. Lactating camels which are traditional y managed were randomly selected and quarter milk samples were col ected from camels in different herds. Quarter milk samples (n = 404) from lactating camels (Camelus dromedarius) in Gewane district were examined for mastitis. Twenty one (20.8%) was diagnosed as clinical mastitis cases. Thirty one (30.7%) udders examined were infested with ticks, had lesion and

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45.2% had mastitis. There were significant association ($p<0.05$) between tick infestation and mastitis. Lactating camels at early and mid lactation were found most affected by mastitis ($p<0.05$) than those at late lactation. One hundred ninety six quarter milk samples (48.5%) were positive for California mastitis test (CMT) and 164 (83.7%) yielded pathogenic bacteria. A positive correlation ($r = 0.76$) was observed between CMT positive results and presence of major pathogens in camel milk samples. The main mastitis pathogens isolated were Staphylococcus aureus, Coagulase-negative staphylococci, Streptococcus agalactiae, Streptococcus dysgalactiae and E.coli. The average mean fat and protein percentage in CMT-negative milk were 3.83%± 0.081 and 2.85 ± 0.024 respectively. However, in CMT-positive milk it was 1.97 ± 0.071 and 2.91± 0.130 respectively. The study results suggest that mastitis in camels was prevalent in Gewane district and was associated with a relatively low protein and fat content of camel milk. Detailed research on camel milk is essential to promote and restore health and to help farming communities who have perfected camel- keeping.

Key words: Camelus dromedarius, *Mastitis*, CMT, *Etiology*, Prevalenc

Introduction

Camels are mainly kept for milk production, which is a valuable human food source in the arid environments of camel keeping countries (Schwartz and Dioli, 1992). Food Agriculture Organization has reported that more than 18 million camels around the world support the survival of millions of people (FAO, 2003). World wide production of camel milk merely contributes to 0.23% of the total milk production (Stefan, 1998). From a global perspective the economic significance of camel production is minimal as the comparison of livestock numbers. Even for Africa or Eastern Africa alone the economic potential of the camel, judged by numbers only, remains limited in comparison to other livestock species. Certainly the importance of camel production becomes more evident if one considers the arid lowlands of Eastern Africa alone where camels represent more than half of the regional herds (Schwartz and Dioli, 1992).

Camel milk is one of the main components of the diet of the nomads in Ethiopia. It is very difficult to imagine life in the arid and semi-arid areas without camels. It represents every thing concerning the survival of the pastoralists. It is a symbol of life, pride, wealth, hope and continuity in afar, which also holds true in other camel rearing areas of the country (Teka, 1991; Gebremariam, 1991). Camel milk is one of the underutilized resources and it is a valuable source of energy, water and vitamins for the camel herders and the calves (Mohamed, 2007). It is rich in fat and protein and remarkably in vitamin C (Dostolova et al., 1993). Camel milk not only contains more nutrients compared to cow milk (Arrowal *et al*., 2005), but also it has therapeutic and antimicrobial agents (Elagamy *et al*., 1992). How ever a quantitative picture has not been drawn on the important subsistence value of this species. No plausible estimates of milk in pastoral diets and income are quite unreliable.

Nowadays, public health concern associated with microbial food safety has arisen. Numerous epidemiological reports have implicated non-heat treated milk and raw-milk products as the major factors responsible for illnesses caused by food-borne pathogens (De Buyser et al., 2001; Harrington et al., 2002). Cross-contamination with pathogenic microorganisms can gain access to milk either by fecal contamination or by direct excretion from the udder into milk. Most of camel milk is consumed in the raw state with out any heat treatments or acid fermentation and kept at high ambient temperature coupled with lack of refrigeration facilities during milking and transporting. These conditions turn the milk to be unsafe, capable of causing food-borne diseases and it even spoils fast (El-Ziney and AL-Turki, 2007). Furthermore, any infection of the mammary gland of the camel affects seriously the milk yield and consequently the calf and the family (Almaw and Molla, 2000). A number of pathogens have been isolated from camel's milk (Barbour *et al*., 1985; Mostafa *et al*., 1987; Abdurahman *et al*., 1995; Obied ., 1996; Bekele and Molla, 2001). Very little work is done concerning camel mastitis as the disease was thought to be uncommon in the camel. However, cases of mastitis in camel have recently been reported from several camel keeping countries including Egypt, Saudi Arabia, Somalia, Sudan, Ethiopia and the United Arab Emirates (Quadil and Quadar, 1984; Hafez et al., 1987; Karmy et al., 1990; Obied et al., 1996; Almaw and Molla, 2000; Abdel Gadir, 2001).

The aim of the present study were to assess the occurrence and cause of camel mastitis and bacterial causes associated with it and evaluate Fat and Protein content of camel milk in Gewane district, Afar Regional State, Ethiopia.

Material and Methods

The study was conducted in Gewane district of Afar National Regional State between September 2006 and April 2007. A total of 101 lactating camels which are traditionally managed were randomly selected and sampled from different herds.

Physical examination of the udder and milk

The udders were examined for any gross signs of inflam-

mation such as swelling, heat and pain or indurations and hardening of the udder on digital palpation and visible alterations of color and consistency of the milk.

Milk samples

Quarter milk samples were collected after wiping the teats with cotton wool moistened with 70 % alcohol, paying particular attention to the teat orifice, the first few squirts of milk were discarded and about 10-20 ml of milk was collected in a sterile universal bottle. The quarter milk samples were transported on ice to the microbiology laboratory and kept at 4 °C for a maximum of 24 hours until inoculation onto a standard bacteriological medium.

California Mastitis Test (CMT)

A squirt of milk from each quarter of the udder was placed in each of the four shallow caps in the CMT paddle. An equal amount of commercial CMT reagent was added to each cap and mixed well. Reactions were graded as 0, T, +1, +2 and +3. The California Mastitis Test was carried out using the method described by Quinn *et al*. (1994).

Bacterial isolation and identification

Frozen quarter milk samples were thawed at room temperature. The bacteriological cultures were performed following standard microbiological techniques (NMC, 1990; Sears *et al*., 1993; Quinn *et a*l., 1994). One loop full of milk was streaked on 5 % sheep blood agar and MacConkey agar in parallel to detect any Gram- negative bacteria that are able to grow on the medium. The plates were incubated aerobically at 37°C for 24 to 48 hours. Presumptive identification of bacteria on primary culture was done on the basis of colony size, morphology, heamolytic characteristics, Gram stain reactions and catalase test. Staphylococci were identified based on catalase test, their growth characteristics on mannitol salt agar, and coagulase production. Edwards's medium is used for the growth of streptococci. Streptococcus species and Enterococcus feacalis were determined according to CAMP reaction, type of haemolysis, growth characteristics and esculin hydrolysis on Edwards's medium. Corynebacterium species, Actinomyces species and Bacillus cereus are identified based on heamolytic characteristics, catalase test, and growth on 9% NaCl, CAMP reaction and sugar fermentation test. Gram- negative isolate were cultured on MacConkey agar and further identification was made by catalase test, oxidase reaction, triple sugar iron agar (TSI), 'IMViC' (indole, methyl red, Vogues- Proskauer and citrate) test, urease and sugar fermentation test.

Fat and Protein determination

CMT-positive quarter milk samples from individual camel were pooled together. Similarly, CMT-negative quarters were pooled and each was evaluated for fat and protein content. The Fat and Protein content were determined by Gerber method (Europe) and Kjeldhal method respectively (Richardson, 1985; ILRI, 1995).

Table 1: Relationship between CMT score and *bacteriological* culture

CMT score	Examined	Culture positive	Culture negative
0	164	45(27.4)	119(72.6)
Trace(T)	44	13(29.5)	31(70.5)
1+	51	17(33.3)	34(66.7)
2+	89	41(46.1)	48(53.9)
3+	56	48(85.7)	8(14.3)
Total	404	164(40.6)	240(59.4)

Data analysis

All the collected data were stored in Microsoft Excel spread sheet and transferred to SPSS version 11.0 for analysis. P-values < 0.05 were considered significant.

Results

Prevalence of clinical and sub clinical mastitis

Out of 101 camel udders examined, 21 (20.8 %) had clinical mastitis. Subclinical mastitis was detected by CMT in 196 (48.5 %) of the quarter milk samples of these164 (83.7 %) were culture positive (Table 1).

Bacteriological pathogens isolated from subclinical and clinical mastitis

The major bacterial pathogens isolated from clinical mastitis were Staphylococcus aureus, Streptococcus dysgalactiae and, E. coli and those from subclinical mastitis were Staphylococcus aureus, coagulase-negative staphylococci, E. coli, Corynebacterium species and micrococcus species (Table 2).

Fat and Protein percentage in CMT-negative and CMT-positive milk

The average mean of fat percentage in CMT-negative milk was 3.83%± 0.081 whereas in CMT- positive milk it was 1.97 ± 0.071. The average mean of protein percentage in CMT-negative milk was 2.85 ± 0.024 whereas in CMT-positive milk it was 2.91± 0.13 (Table 3). There was significant difference in fat percentage between CMT-negative and CMT- positive milk samples ($r = 0.746$, $p = 0.01$).

Table 2. *Pathogens* isolated from mastitis milk samples of camels

Bacteria isolated	Clinical mastitis	Subclinical mastitis	Total number
Staphylococcus aureus	23(54.8)	15(16)	38(28)
Streptococcus agalactiae	1(2.4	-	1(0.7)
Streptococcus dysgalactiae	4(9.5)	3(3.2)	7(5.1)
Coagulase-negative staphylococci	3(7.1)	-	41(30.1)
Escherichia coli	7(16.7)	9(9.6)	16(11.8)
Streptococcus uberis	1(2.4)	-	1(0.7)
Enterococcus faecalis	-	2(2.1)	2(1.5)
Corynebacterium spp.	1(2.4)	8(8.5)	9(6.6)
Micrococcus spp.	-	6(6.4)	6(4.4)
Bacillus spp.	-	4(4.3)	4(2.9)
Actinomyces pyogens	-	4(4.3)	4(2.9)
Pasteurella haemolytica	-	3(3.2)	3(2.2)
Klebsiella pneumonia	294.8)	2(2.1)	4(2.9)
Total	42	94	136

Table 3. Mean Fat and Protein % in CMT-negative and CMT-positive milk samples

Pooled milk samples	No. of samples examined	Mean Fat %	Mean Protein %
CMT-positive	20	1.97	2.91
CMT-negative	20	3.83	2.85

Table 4. Multivariate analysis of association between clinical mastitis and potential risk factors

Variable level	Level	Odds ratio	p-value	95%CI
Parity number	one	0.757	0.772	0.116-4.959
	two	0.999	0.999	0.149-6.704
	three	0.354	0.359	0.038-3.258
	four	-	-	-
Stage of lactation	early	0.022	0.001*	0.002-0.221
	mid	0.118	0.050*	0.014-0.996
	late	-	-	-
Tick infestation	no	-	-	-
	yes	0.261	0.019*	0.085-0.803
Teat lesion	no	-	-	-
	yes	11.19	0.001*	2.71-46.26

*Significant ($p<0.05$)

Risk factors for mastitis

Lactating camels with udder or teat lesion, tick infestation and at early and mid lactation were more affected by clinical mastitis. There were significant associations ($p< 0.05$) between these factors and clinical mastitis (Table 4). Subclinical mastitis was prevalent in she-camel with three or more parity, udder infested with tick and those at early lactation. There were significant associations ($p< 0.05$) between these factors and subclinical mastitis (Table 5).

Discusion

The camels with clinical mastitis showed gross signs of inflammation such as swelling, heat and pain or indurations and hardening of the udder on digital palpation and visible alterations of color and consistency of milk. Abdurahman *et al*. (1997) reported that clinical mastitis in lactating camels is well recognized and feared by the camel owners because of its striking effect on milk yield and quality and consequently on the family and on the calf. The result is in agreement with that of Bekele and Molla (2001), Barbour *et al.* (1985), and Obied *et al.* (1996) who reported 12.5%, 15% and 19.5% in north eastern part of Ethiopia, Saudi Arabia and Sudan respectively. The presence of clinical mastitis was higher than that of Abdurahman *et al.* (1995) who reported 5.9% clinical mastitis in the Sudan and Almaw and Molla (2000) who reported 2.1% in north eastern part of Ethiopia.

The teat lesions were mostly chronic, non penetrating superficial wounds. This could be attributed to the practice of camel herders cauterizing the udder so as to treat mastitis. It is also observed that they put sticks into the nostrils of calves to prevent suckling. Tick bites on the udder can cause skin irritation and localized inflammatory response, which can lead to secondary bacterial infections. Almaw and Molla (2000) reported that camel herders in the afar tie the teats with soft bark to prevent the calf from suckling when calves began to herd together with their dams. Obied *et al*. (1996) indicated that heavy

tick infestation of the udder, harmful treatment of affected quarters by cauterization and use of anti- suckling devices could be some of the factors, which predispose camel udders to bacterial infections.

California Mastitis Test revealed a prevalence of 48.5% sub clinical cases among the quarter milk samples examined. 164 (83.7%) were culture positive. A positive correlation was found between CMT scores and bacteriological results (Table 1). It is higher than the result found by Almaw and Molla, (2000), 8.6% were CMT positive from 753 quarter milk samples examined of which 57 (87.7%) revealed pathogenic bacteria. The result was in agreement

Table 5. Multivariate analysis of association between subclinical mastitis and potential risk factors

Variable	level	Odds ratio	p-value	95%CI
Parity number			0.119	
			0.230	
	three		0.032*	
	four	-	-	-
Stage of lactation	early	96.8	0.00*	0.24-374.5
	mid	-	-	-
	late	-	-	-
Tick infestation	no	41.05	0.002*	3.97-42.9
	yes	-	-	-

with Bekele and Molla, (2001) who reported a prevalence of 47.3% of subclinical mastitis from 543 quarter milk samples examined by CMT, where 162 (63.0%) revealed *pathogenic* bacteria. Barbour *et al.* (1985) examined 140 milk samples and score by CMT technique. He found a highly significant correlation ($r = 0.803$, $p < 0.01$) between positive CMT results and mastitis.

Thirty two (16.3%) of CMT positive samples were culture negative. Almaw and Molla (2000) reported 10.8% of CMT positive samples resulted culture negative. Whereas Abdurahman et al. (1995) and Bekele and Molla (2001) reported 37% and 43% of CMT positive quarter milk samples of camels did not show any bacterial growth respectively. This could be possibly because the samples were taken during the convalescent phase of infection but with high leukocyte counts giving a CMT positive result. It has been reported that 57% coliforms infections are less than 10 days duration and are rapidly destroyed by inflammatory reactions (Rodostits et al., 1996). The organisms may no longer be present, and the positive CMT could be due to some byproducts such as endotoxins. Further more, storage and transportations and the antimicrobial effect of camel milk might reduce the number of viable organisms (Sears et al., 1993; Quinn *et al.*, 1994).

The Gram positive cocci were the main bacteria isolated in mastitis cases in camel milk. Staphylococcus aureus was identified as the most common bacteria. The organism constitutes 38 (28%) of the total isolates. Similarly 20.8%, 34.4% and 31.5% was reported by Bekele and Molla (2001), Karmy (1990) and Obied *et al.* (1996). Coagulase-negative *staphylococci* were the major isolates; 41 (30.1%) from subclinical cases of camel mastitis. *Streptococci* constituted 8% of the isolates of which *Streptococcus dysgalactiae* was 5% of the isolates. The Gram negative bacteria constituted 16.9% of the isolates of which E.coli was 11.8% of the isolates. The organisms isolated (Table 2) are also regarded as important mastitis causing agents in Ethiopia and other countries (Barbour *et al.*, 1985; Mostafa *et al.*, 1987; Karmy, 1990; Abdurahman *et al.*, 1995; Obied *et al.*, 1996; Abdel Gadir, 2001).

The average mean of fat and protein percentage in healthy milk is in agreement with Hashi (1989) where he reported 4.6% and 3.15% respectively. Similarly Khaskheli *et al*, 2005 reported fat content of camel milk ranged between1.8 to 5.0 g and the total protein content of camel milk within the range of 1.8 and 3.20 g per 100 g with an average percentage of 2.54±0.19. The protein content was similar to those found by Sawaya et al. (1984) where they reported 2.95 ± 0.09 and was lower than Mukasa- Mugarawa (1981) where he reported 4.02. The average mean of fat and protein percentage is 1.97 ± 0.071 and 2.91 ± 0.13 in CMT-positive milk samples respectively. Variation in fat content was observed to be directly or indirectly related to the total solids content of camel milk, i.e. as the total solids increased, the fat content also increased and vice versa and may depend more on diet type and end products of digestion or mobilized body reserves used for milk secretion. Furthermore fat percentage of camel milk varies with season, stage of lactation and pregnancy (Khaskheli et al, 2005; Knoess, 1986, Rodriguez et al., 1985). There was a decrease in fat content from 4.3 to1.1% with increase in water content of milk produced by thirsty camels (Yagil and Etzion, 1980). The average casein and whey protein content in camel milk varies between 2.3% and1.9% and 0.7% and 1.0% respectively (Dostolova et al., 1993). The slight increase in protein in CMT-positive milk samples may attribute to the animal immune response those immunoglobulins which are proteins, present in milk.

The results found from the present study suggest that mastitis in camels was prevalent in the study area. California mastitis test has shown to be valuable indicators of udder infection of the camel. Further investigations using larger number of camels are necessary to understand the dynamics of mastitis in camels before such indicators could be routinely used in predicting udder infections in the camel. Gram positive cocci were the dominant species among mastitis pathogens isolated. Tick infestations

together with teat or udder lesions were found predisposing factors to the occurrence of mastitis. Although camels in the Afar pastoralist are managed traditionally on poor quality feed and scarce water source, the protein and fat content of the milk were in the normal range. A decrease in fat and increase in protein contents were observed in CMT-positive milk samples. In order to improve the udder health of Afar camels, an attempt should be made to increase awareness among pastoralist on the significance of udder health problems associated with reduced milk production and quality. Furthermore mastitis control programs such as hygiene during milking of the udder and proper treatment of mastitis cases are essential and need to be practiced. Further detail research covering large areas and representative samples on camel milk is essential in order to understand the epidemiology of mastitis in camel, associated bacterial pathogens, risk factors and constituents and properties of camel milk in pastoral camel- keeping communities in the region.

Impact

From the present study it is revealed that pastoralists' camels are affected by udder infection and caused by various pathogenic microorganisms and the condition is exacerbated by tick infestation and teat lesion. The disease affects the nutritional quality of milk as a result first and most consumable milk can be reduced; secondly, it reduces the income gained from sell and even there will be total loss in chronic infection because the udder can be blocked totally. Avoiding the practice of using anti suckling sticks on calves and treating for clinical and sub clinical cases is important. Mastitis control program based on the present study findings will benefit the pastoralists to use more from their camels.

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THE CLINICAL AND PATHOLOGICAL FEATURES OF EXPERIMENTAL MANNHEIMIA HEMOLYTICA A2 INFECTION IN WEST AFRICAN DWARF GOATS

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LES SIGNES CLINIQUES ET PATHOLOGIQUES DE L'INFECTION EXPÉRIMENTALE DE MANNHEIMIA HAEMOLYTICA A2 SUR LES CHÈVRES NAINES EN AFRIQUE DE L'OUEST

Résumé

Mannheimiosis causée par Mannheimia hemolytica a été provoqué expérimentalement chez 15 chèvres naines d'Afrique de l'ouest âgées de six mois par inoculation intra trachéale de 1 x 10^9 d'unités formant colonies de phase de croissance logarithmique de quatre heures de *Mannheimia hemolytica* de type sérologique A:2 avec cinq chèvres servant de témoins. Les signes cliniques ont été observés et deux chèvres ont été euthanasiées à des interval es prédéterminées pour faire des analyses *bactériologique*s et *pathologique*s sur les tissus prélevés à l'aide des techniques usuel es. Les signes cliniques par ordre d'apparition étaient l'anorexie, la pyrexie, la dyspnée, l'écoulement nasal, le décubitus et la mort. Les observations importantes faites à l'autopsie étaient la congestion pulmonaire, un œdème avec une hépatisation et des adhérences pleurales des lobes apicaux, la pleurésie fibrineuse ainsi que des pétéchies et ecchymoses hémorragiques des muscles squelettiques. Le pourcentage moyen de l'hépatisation des poumons pour l'infection était de 10,0% sans variations remarquables entre les sexes (P>0,05) et le poumon droit était le plus touché (p<0,05) alors que le taux de mortalité global était de 20,0% au 12ème jour après inoculation. Sur l'aspect histologique, les lésions pulmonaires étaient caractéristiques de la bronchopneumonie fibrineuse avec le gonflement des cloisons alvéolaires, l'œdème et l'infiltration des neutrophiles de l'interstitium. *Mannheimia hemolytica* A : 2 a de nouveau été isolé de manière bactériologique des poumons, du foie, des reins, de la rate et du sang du cœur lors de l'autopsie. D'un point de vue de diagnosticien, un diagnostic fondé sur les critères cliniques et *morphologique*s décrits dans cette étude peut être utile dans les cas où l'examen microbiologique est relativement dif icile.

Mots clés: Chèvres, pneumonie, Mannheimia hemolytica A2, infection expérimentale

Summary

Mannheimiosis due to *Mannheimia hemolytica* was experimental y induced in 15 six -month-old West african dwarf goats by intratracheal inoculation of 1 x 10^9 colony-forming units of four hour log-phase *Mannheimia hemolytica serotype* A:2 with five goats serving as controls. The clinical signs were observed and two goats were euthanized at predetermined intervals for bacteriological and pathological investigations on tissues col ected using standard techniques. The clinical signs in order of manifestation were anorexia, pyrexia, dyspnea, nasal discharge, recumbency and death. The prominent lesions observed at necropsy were pulmonary congestion and edema with consolidation and pleural adhesions of the apical lobes, fibrinous pleurisy as wel as, petechial and ecchymotic hemorrhages of skeletal muscles. The average percentage lung consolidation for the infection was 10.0% with no significant sex variation (P>0.05) and the right lung was more affected (p<0.05) while the overall mortality was 20.0% at 12 days post-inoculation. Histological y, the lung lesions were typical of fibrinous bronchopneumonia with thickened alveolar septa, edema and neutrophilic infiltrations of the interstitium. *Mannheimia hemolytica* A:2 was re-isolated bacteriological y from the lungs, liver, kidneys, spleen and heart blood at necropsy. From a diagnostician point of view, a diagnosis based on clinical and morphologic criteria as described in this study might be helpful in cases where microbiological examination is relatively difficult

Keywords: Goat – *Pneumonia* – *Mannheimia hemolytica*, Experimental infection – Nigeria.

Introduction

Sheep and goats represent an important aspect of the livestock economy in developing countries in the humid tropics (Boyazoglu *et al*., 2005) with goats and sheep from West and Central Africa constituting about 37% and 21% of the livestock population in Africa, respectively. These animals require a low capital outlay for their production. Over 80 % of rural families keep ruminants especially sheep and goats with women and children being more involved in their upkeep (Kumar *et al*., 2003)

Over the past 15 years however, there has been a steady growth in goat production. Goats therefore have a higher population than sheep in most countries (Ademosun, 1985). Goats are considered to be one of the most important protein producing animals in Nigeria and they provide 30-36% of the total meat consumption of the Nigerian populace annually (Ademosun, 1985).

The major impediment to their production in most developing countries including Nigeria is the high incidence of infectious diseases with Peste des Petit Ruminant (PPR), bacterial *pneumonia* and parasitic *gastroenteritis* being the most important (Ojo and Obi 1996). Previous investigations in Nigeria showed that *Mycoplasma* spp. and Mannheimia *haemolytica* are the most important non viral agent associated with *caprine pneumonia* (Odugbo *et al*., 2004). The *pneumonia* is associated with pleuritis and *septicaemia* in ruminants (Odugbo *et al*., 2003). In most developed countries of the world, investigations on Mannheimia hemolytica have been carried out on beef cattle and sheep with very few reports in goats. A review of literatures indicates that very little attention has been paid to the pathogenesis of the disease in goats. In this report, the observations on the pathology including sequential clinical features associated with experimental *Mannheimia haemolytica* infection in West African Dwarf (WAD) goats are presented.

Materials and Methods

Study Location

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

Experimental Animals

Twenty apparently normal West Africa Dwarf goats (WAD) six months of age (10 females and 10 males) were used for the experiment. The animals were divided into two groups and housed in well partitioned pens. Group A had 15 goats (7 females and 8 males) while group B with 5 goats (2 females and 3 males) served as control. They were conditioned for 14 days before the intervention and vital signs (rectal temperature and respiratory rates) were monitored daily to observe whether they remained afebrile and free of any clinical signs of diseases. Wheat bran and water were provided ad libitum daily. The nasal swabs of the animals were negative for *Mannheimia haemolytica* by cultural isolation prior to inoculation. The animals were also confirmed seronegative by Agar gel precipitation technique for antibody to Peste des petit ruminants virus (PPRV) prior to inoculation.

Preparation of M. haemolytica inoculum

M. haemolytica A2, which had been isolated earlier from the pneumonic lungs of a goat, was selected from a stock culture, which was supplied by Professor A.I. Adetosoye of the Department of Veterinary *Microbiology* and *Parasitology* of University of Ibadan was subcultured onto blood agar and incubated at 37^0C overnight. Thirty colonies of the same size were then inoculated into 50 mL brain heart infusion broth (Oxoid) and incubated at 37^0C for 18 h before the number of colony-forming units (CFU) was estimated using the total plate count method (Quinn *et al*., 1994). The live inoculum was prepared by diluting the prepared broth to give an end concentration of 1×10^9 CFU/mL.

Challenge Infection.

Group A with fifteen goats were infected intratracheally according to the method described by Ames *et al*; (1985) while five uninfected control goats were inoculated intratracheally with 1 ml of sterile brain infusion broth.. The infection was done with 1ml of pure culture (10^9 CFU) of a 4 hour log phase culture of *Mannheimia haemolytica* A2 in brain in fusion broth. The goats were then closely monitored for clinical signs.

Pathology.

The animals that died and two that were euthanized at predetermined periods were necropsied. The study has been independently reviewed and approved by an ethical board of the faculty. Adequate measures were taken to minimize pain or discomfort.

Necropsy was carried out on 8, 12, 19, 28, 45 and 48 day post inoculation (dpi). Samples from most body organs (oral mucosa, lungs, liver, heart, spleen, messenteric lymph nodes and intestine) were collected in 10% buffered formalin, routinely processed and stained with haematoxylin and eosin for histological examination using the light microscope.

For the lung pathology, the degree of consolidation or pneumonia as a percentage of the total lung volume, was

Table 1: Clinical Feature in Experimental MH infection in West African Dwarf

Clinical features	No of animals affected	Time of occurrence in days (dpi)
Dull and withdrawn	10	7-11
Rough coat	10	3-7
Serous nasal discharge	6	6-10
Mucoid nasal discharge	10	8-15
Pyrexia	10	5-6
Cough (Occasional)	7	8
Cough (Paroxysmal)	14	15
Serous ocular discharge	6	22-25
Death	3	8 and 12

estimated as described by Odugbo *et a*l., (2004). The extent of pneumonia was determined by visual observation, palpation and measurement of the lesion which was manually plotted onto a lung diagram and then estimated as a percentage of each lobe.

Statistical Analysis

Statistical analysis was carried out with ANOVA and Duncan multiple range test of significance for means of the parameters recorded (Petrie and Watson, 1999).

Results

Clinical Features

Tables1 shows the animals involved, severity and timing of the clinical features in the course of the experiment. Ten of the animals were dull and withdrawn with rough hair coats. There was arching of back 2 – 3 days post inoculation (dpi) which was marked between 5 and 8 dpi. The nasal discharge was initially serous 6 dpi and later mucopurulent from 8 dpi. Sneezing and cough were first noticed 5 dpi. The cough was initially occasional but paroxysmal later. The severity of the cough increased on 6 dpi till day 29 pi. Pyrexia and emaciation were also observed from 5 dpi with fever of 40°C lasting for 2 – 3 days. Pneumonic stance, staggering and weaknesses were noticed from 9 dpi. Serous ocular discharges were noticed later in the course of the experiment between 22 to 25 dpi. The first death was recorded 8 dpi while the second and third deaths were recorded 12 dpi. The overall mortality was 20%.

As shown in Fig. 1, the weight of the animals in the control group was relatively stable throughout the course of the

TABLE 2: Lung lesion score (% consolidation) in experimental Mannheimia hemolytica infection in West African Dwarf goats.

Serial numbers	Tag numbers	Post inoculation days	sex	Left cranial	Left Posterior cranial	Left caudal	Accessory	Right cranial	Right posterior cranial	Right middle	Right caudal	Total	sum Total
				5%	6%	32%	4%	6%	5%	7%	35%		
1.	AB	8	M	H	H	H	H	0.6				0.6	6.6
2.	AM	8	F	-	-	-	-	4.2(F)	3.5(F)	4.9		12.6	
3.	AC	9	M	0.25	0.3	-	-	1.2	1.25	-		3.0	3.0
4.	AJ	9	F	0.2	0.35			1.3	1.15			3.0	
5.	AK	12	M	0.25	4.2(F)	9.6		0.3	0.25	3.5(F)	10.5	28.60	
6.	AF	12	F	0.25	2.4(F)	3.2(F)		2.1	1.75	4.2(F)	7.0(F)	20.9	24.75
7.	AL	19	M	2.0	2.4	3.2		2.4	-	6.7	3.5	19.8	
8.	AD	19	M	H	H	H		H	H	H+	H	-	
9.	AE	28	M	0.25	0.12	0.94(H)		0.3	0.1	-	-	1.71	9.9
10	AH	28	F	0.1	0.3	-	-	0.12	0.25	-	-	0.77	
11	AA	41	M	-	-	-	-	4.2	3.75	1.4	3.5	13.15	
12	AO	41	F	-	-	-		4.0	3.85	1.7	3.46	13.31	1.24
13	AN	45	M	4.0(F)	4.8(F)	10.56		-	-	-	-	19.36	
14	AI	45	F	-	-	-		3.6	3.0	3.5	-	10.1	13.15
15	AG	48	F	-	-	-		-	-	-	-	0	
	total			7.3	14.87	27.5		24.32	18.85	25.9	27.96	146.8	
	Ave			left	3.3			Right	6.47			9.8	14.73
				femal	8.67			Male	10.8		Ave.	10.0	

H - hemorrhage (F) - Fibrin deposition

experiment while that of MH infected group had a drop in weight between 14 dpi and 21 dpi. During the recovery phase a weight increase occurred between day 28 and 35 post inoculation (pi) with a drop between day 35 and 42 pi.

Fig. 2 shows that there was pyrexia on the 5th and 6th dpi and subsequently there was slight drop in the temperature than those of the control group till the end of the experiment.

Fig. 3 also shows that the MH infected goats had lowered respiratory rates when compared to the control goats especially on 3, 7, 20 to 35 dpi.

Pathology

Gross pathology:

The animals 8 day pi were moderately emaciated. The bony protuberances especially those of the ribs and the pelvis were very prominent. The mucosa of the nasal turbinate of the animal sacrificed as at day 8 were hyperemic. The respiratory airways contained moderate amount of froth. In the animal, there was consolidation of the anterio-ventral portion of the cranial lobe of the right lung while the left lung had petechiation on the cranial, middle and proximal aspect of the caudal lobe.

There were severe petechiations on the subcutaneous tissue in the submandibular, axilar, subscapular regions, thoracic muscles, myocardium, coronary fat and spleen. There were fibrin deposits on the cranial and middle lobes of the right lung, and involving the pericardium (Fig 1); but there were no lesion on left lung.

On the day 9 pi, the dead animal showed hyperemic turbinates with moderate froth in the airways. There were patchy areas of consolidation on the anterior right lung,

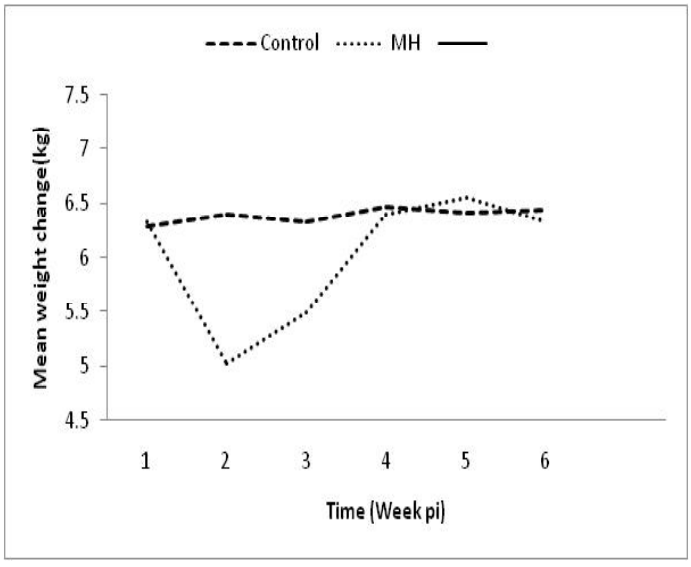

Figure 1: Weight changes in experimental MH infection in West African Dwarf goats

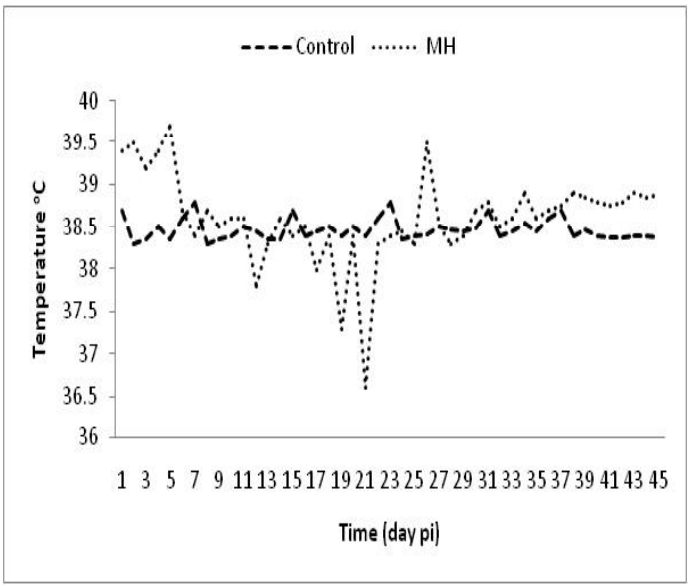

Figure 2: Temperature changes in experimental MH infection in West African Dwarf goats

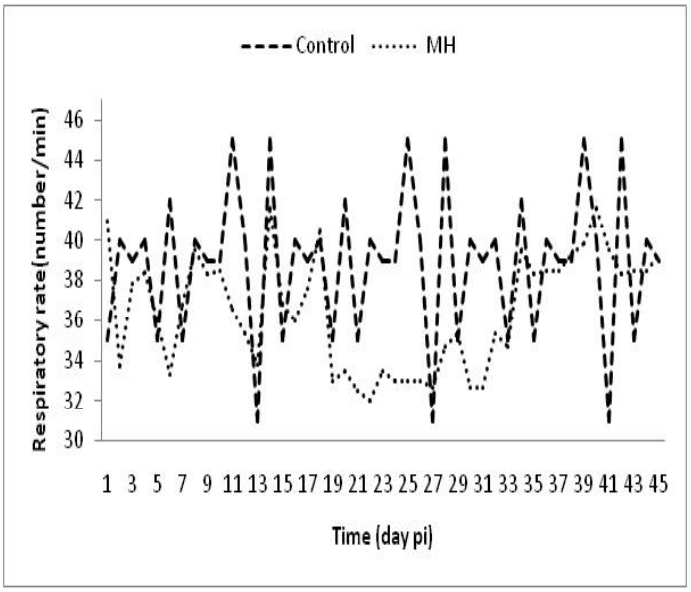

Figure 3: Respiratory rates in experimental MH infection in West African Dwarf Goats

and ventral posterior aspect of the middle lobe. The consolidation was lobular and dorsal in distribution with fibrin deposition on the cranial lobes of the left lung. The gall bladder was markedly distended with bile.

On day 12 pi, the upper respiratory lesions were more severe and diffuse. The consolidation affected all the lobes. In addition, there was fibrous adhesion of the pleura to the rib cage. There was also a focus of necrosis of about 0.5cm in diameter on the liver. The lesions in the second animal which was euthanised were similar to the one that died on day 12 pi in distribution and severity.

At day 19 pi, the lung and upper respiratory tract lesions

were similar to those of day 12 pi but less severe. Petechial hemorrhages were present in the anterior, middle and proximal aspects of the caudal lobe of both lungs.

On day 28 pi, although the lesions followed similar distributions as those seen on day 9 pi, consolidation was patchy affecting between 2-3% of the lung tissue and there was no fibrin deposition but the liver had diffuse necrosis.

The upper respiratory tract lesions on 41 dpi, were characterized by moderate amount of froth in the tracheobronchial airway, hyperemia of the entire cranial lobe of the right lung and consolidation of the anterior aspect of the middle lobe. The mediastinal lymph nodes were oedematous and enlarged while the liver was slightly congested with distended gall bladder. The spleen in one of the animal was mildly enlarged and pulpy in consistency.

At 45 dpi, one of the animals, showed consolidation of the anterio-ventral portion with fibrous attachment of the right cranial lobe of the lung to the thoracic wall and between the middle lobe and the *pericardium*.

The entire left cranial, middle and proximal 1/3rd of the caudal lobe of the left lung were severely consolidated with deposition of fibrin and thickening of the interlobular septae. The pericardium was moderately thickened by fibrin deposits while the pericardial cavity contained about 4 mls of yellowish turbid fluid. The spleen was slightly enlarged and the kidney had mild cortical congestion. Another animal that was euthanized but with good body condition had moderate consolidation of the right cranial and proximal half of the middle lobes of the lung while the gall bladder was markedly distended.

On 48 dpi, there was marked emaciation and dehydration of the carcass. The lungs were moderately congested and oedematous with mild amount of froth in the respiratory airways.

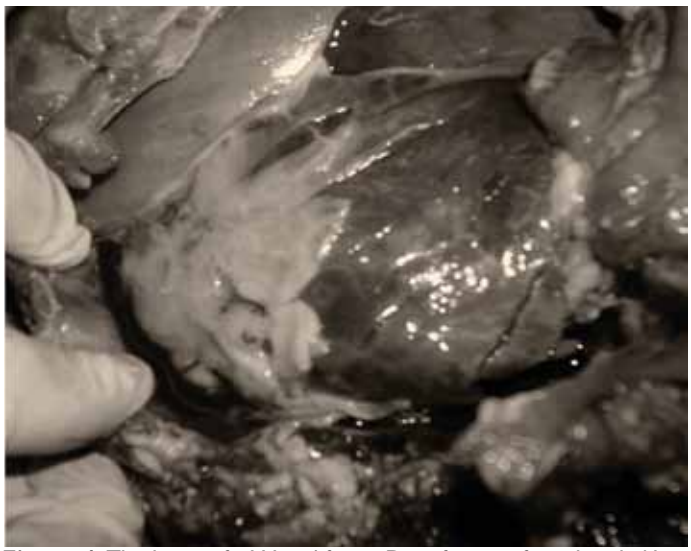

Figure 4: The lungs of a West African Dwarf goat infected with *Mannheimia haemolytica* showing fibrinous deposits on the pleura and pericardium

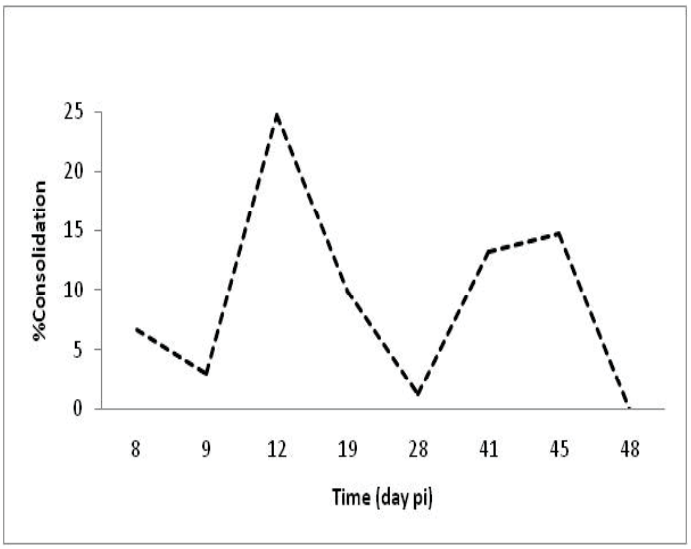

Figure 5: Percentage *pulmonary* consolidation in Experimental MH infection in West African Dwarf goats.

Lungs of control goats were normal.

Pulmonary Consolidation Score

The average percentage pulmonary consolidation score for the infection was 10.0%. The average pulmonary consolidation score in female (7 goats) was 8.7% while that of male (8 goats) was 10.8%. The weight loss in the infected group was marked especially during the period that corresponded with increasing lung consolidation (in the first three post inoculation) as shown in figure 1.

On Table 2 and figure 5 was shown the percentage pulmonary consolidation in experimental MH infection with an initial peak at day 12pi (25%) and a later lower peak at day 45pi. The lowest consolidation percentage was observed on day 28pi. There was no pulmonary consolidation in the control group.

Histopathology

Histopathological changes associated with goats inoculated with M. haemolytica are acute, subacute, and chronic bronchopneumonia. Lungs of goats that died at 8 day p.i. had extensive areas of necrosis, purulent exudates, oedema, hemorrhage and fibrin deposits (Fig. 3). There was purulent exudate in the alveoli and bronchioles (Fig. 4). The subcutaneous and muscular hemorrhages were seen at histology. Lungs of goats euthanized at 12 days p.i. were characterized by mild pulmonary oedema and congestion. There was massive fibrin deposition in the alveoli and interlobular septa and pleura with marked proliferation of type II alveolar cells and numerous alveolar macrophages within the alveoli. Lungs of goats euthanized at 45 days p.i. had epithelial cell proliferation in alveoli and bronchioles, and marked pleural and interlobular fibrosis. In addition, the airway lumens were partially obliterated by fibrous connective tissue. Lungs of control goats were normal.

Discussion

The results of this study describes the clinical and pathological features of experimental Mannheimia hemolytica infection in West African dwarf goats using the serotype A2, the most predominant serotype in Nigeria Odugbo *et al*., 2003 and one of the cause of ovine and bovine pneumonia (Rowe *et al* 2001). The results suggest that the bacterium was moderately virulent as it caused 20% mortality between day 8 and 12 pi. The rapid onset and severity of lesions observed may be attributed to the effect of the cytotoxin produced by MH as the challenge bacterium was in the log phase before being used (Shewen and Wilkie 1985). This log phase helps the bacterium to circumvent the host defences contrary to the reproduction of experimental pneumonic pasteurellosis by combined viral and Pasteurella infection. It also contradicted the use of dexamethasone and transport stress to induce the experimental disease has described by (Zamri-Saad *et al*., 1991).

The clinical features observed were comparable with those reported previously by Odugbo *et al*. (2003) in sheep but the course of the disease was longer in the experimentally infected goats. The incubation period was within hours with animals withdrawn, dull and with rough hair coats. Death occurred 8 days pi and 6 days after the first clinical sign of illness. This may be associated with virulence and dose as death was reported to have occurred 12 hours after the first sign of illness in a natural outbreak. This timing of death is simialr to the report of Zamri-Saad et al., (1991) where death was reported on day 3 and 7pi in dexamethasone treated and transport stressed goats.

The sudden death of the animal with fibrinous pneumonia and septicaemia noticed in this experiment depicts the acute phase of the disease which has also been reported in sheep (Odugbo *et al*., 2004). The affected goat in this study had a good body score as reported in previous studies in sheep (Odugbo et al., 2004). The staggering, pneumonic stance and marked emaciation noticed 12 dpi were not reported by other workers. These signs may be associated with the severe pneumonia observed in this study. The respiratory rates were lower at some points where affected animals were small and at days where terminally affected animals were more commonly observed however dypnoea where commonly observed with grunts (Odugbo *et al*., 2004).

Although MH is normally found inhabiting the *nasopharynx* and tonsils of healthy goats (Ngatia *et al*., 1985), MH was not isolated from goats examined prior to challenge. The reason for this observation may be related to the fact that the rate at which MH is isolated from apparently healthy goats which is low and known to be between 7-10% (Emikpe *et al*., 2009). Also that the carrier state of the organism is animals has been shown to fluctuate over time in sheep and calves (Magwood *et al* ., 1969).

However, the challenged dose of the organism produced disease and the virulence factors such as leukotoxins, capsule and lipolysaccharide produced aid the bacteria to escape the host defensive mechanisms like the antimicrobial barriers consisting of beta defensin, anionic peptides found in the by the organism epithelial cells, resident and inflammatory cells. This ability enhances the colonization and invasion of the lungs (Brogden *et al*., 1998).

The most prominent pathological changes were found in the lung with initial hemorrhages, oedema and congestion which later progressed to consolidation with fibrin exudation. This series of events are associated with leukotoxin produced by the organism which enhances neutrophil me-

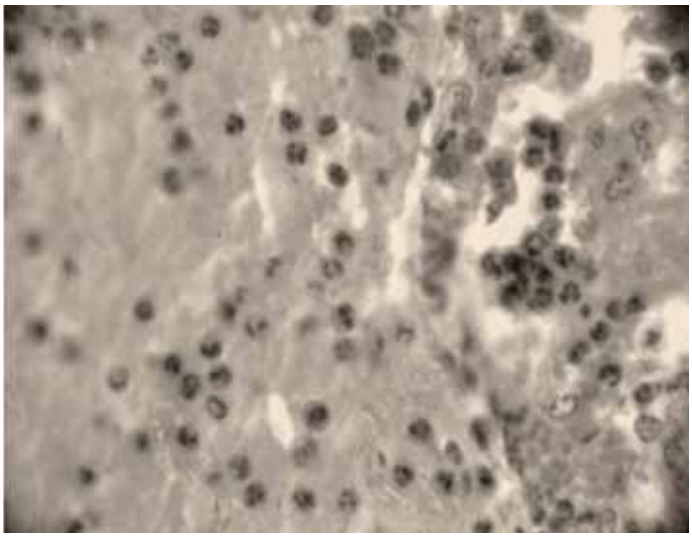

Figure 6 Photomicrograph of the lung section of a West African Dwarf goat infected with MH showing marked fibrin deposits and numerous neutrophilic infilterates in the pleura (H &E., X 400).

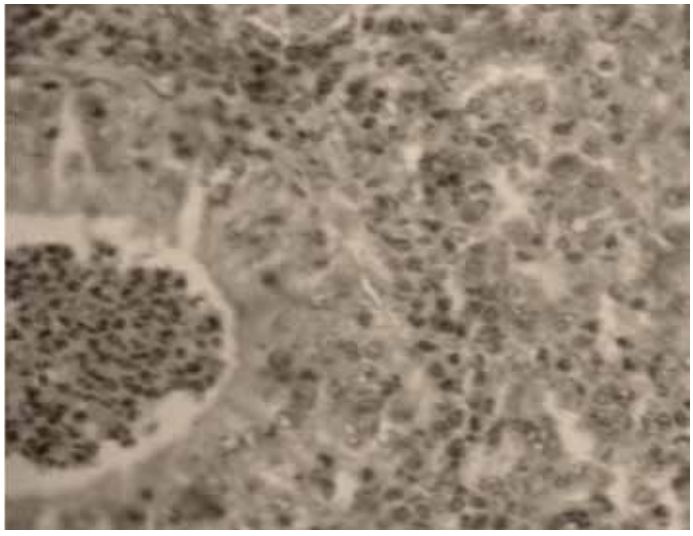

Figure 7: Photomicrograph of the lung section of West African Dwarf goat infected with MH showing acute pneumonia with purulent exudate in a bronchiole (arrow) (H & E., X 400).

diated damage of endothelial cells, platelet lysis associated with pulmonary vascular thrombosis and fibrin exudation (Maheswaran *et al*., 1993).

The pattern of the lung consolidation or pneumonia revealed an initial peak 12 dpi which corresponded to the time of the occurrence of nearly all the lesions associated with MH infection in cattle as most lesions associated with pasteurellosis occur in two weeks. The percentage consolidation of 10.0% is similar to the 12% recorded in cattle by Thompson *et al*., 1998 but not the 66.3% reported for sheep in Nigeria Odugbo *et al*., 2004 and 19% for goat in dexamethasone treated and transport stressed (Zamri-Saad *et al*., 1991). This higher lesion score reported for sheep in Nigeria by Odugbo *et al*., 2004 may be associated with the challenge dose used which was about seven times that used in this experiment while that reported by Zamri-Saad *et al*., (1991) may be due to the stressors the animals were subjected to. The study also revealed a significant relationship between pulmonary consolidation and weight loss ($P<0.05$). This observation is in agreement with the findings of some workers in finished pigs that the degrees of *pneumonia* and carcass quality are interrelated (Ostanello *et al*., 2007).

There is no sex predisposition in the spread and pattern of the lung lesions in this study. The possible reason for this observation is not clear while the fact that the right lung is more affected had been earlier described in some experimental viral and bacterial pneumonia in the same breed (Emikpe and Akpavie, 2009).

The pattern of pneumonia is usually bronchopneumonia in nature and more of the lesions are found peribronchiolar in nature with fibrinopurulent exudate in the alveoli, bronchioles and bronchi (Gilmour and Gilmour 1989). The presence of large numbers of spindle-shaped cells with intensely basophilic nuclei (oat cells) which is considered the pathognomonic feature of the lesion was observed in this study which showed that West African Dwarf goats in this study were very susceptible to MH infection. This observation has not been previously described in this breed. The peak lung consolidations recorded at days 12 and 45 (sub acute and chronic phases) corresponded with the time of isolation of pure cultures of MH. The peak consolidations also suggested subacute and chronic infection as the acute phase occurred in first week pi with death and marked subcutaneous hemorrhages and fibrin deposits on the pleura. These observations have been reported for cattle by Grubor *et al.,* (2004). From a diagnostician point of view, although a definitive diagnosis of MH is by bacterial isolation, a diagnosis based on clinical and morphologic criteria as described in this study might be helpful. The study on the susceptibility of various serotype and different isolates of MH to commonly available antibiotics is on going.

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EPIDEMIOLOGICAL STUDIES ON LISTERIOSIS IN SHEEP

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ETUDES ÉPIDÉMIOLOGIQUES DE LA LISTÉRIOSE CHEZ LES OVINS

Résumé

Cette étude a été réalisée dans le gouvernorat d'El-Dakahilia sur 2448 moutons appartenant à six troupeaux, d'âges variés et avec un historique de manifestation nerveuse. La prévalence des manifestations nerveuses a été de 4,9% (105/2448). Les taux de cas d'issue fatale et de mortalité ont été 77,14% et 3,3% respectivement. Le pourcentage de Listeria monocytogene était de 26,66% (30/08). L'examen du Liquide Cérébro-spinale (LCR) chez les malades et chez les moutons témoins sains a révélé une élévation importante du nombre total de cellules, des protéines totales et de la Créatinine cytokinase chez les malades par rapport aux moutons témoins.

Mots clés: *signes cliniques* de la *listériose*, moutons, *Listeria monocytogenes*, Examen du LCR

Summary

This study carried out at El-Dakahilia governorate on 2448 sheep located in six flocks, varied ages and with history of nervous manifestation. The Prevalence of nervous manifestations was 4.9% (105 /2448). The case fatality rate and mortality rate were 77.14 % and 3.3 % respectively. The percent of Listeria monocytogenes was 26.66% (8/30). Examination of CSF of diseased and control healthy sheep revealed significance elevation of total cell count, total protein and Creatinine cytokinase of diseased than control sheep.

Key words: *Listeriosis clinical signs,* Sheep, *Listeria monocytogenes*, CSF examination

Introduction

Listeria monocytogenes is a Gram positive non sporulating rod which produce encephalitis, septicemia and abortion in both human and livestock, further more Listeria monocytogenes is a major human bacterial food born *pathogen* that annually accounts for an estimated 2500 cases of meningitis, encephalitis, sepsis, fetal death, premature births and 504 deaths in USA at an estimated loss of $ 200 million.(Wesley; *et al* .,2002):

Clinical *Listeria monocytogenes* isolates from humans and ruminants present many similarities and often belong to the same genetic lineages thus, ruminants and their environment may represent an important source of food contamination and infections for humans (Wagner *et al*., 2000)

The incidence of listeriosis in ruminants has markedly increased over the last few decades and feeding of poorquality silage seems to be a key factor in disease transmission. (Boerlin *et al*., 1996). Among farm animals, sheep appear to be particularly susceptible to listeriosis. The organism is spread world-wide in nature (decaying herbage, soil, feces, and sewage) and occurs usually in low numbers(Fenlon *et al*., 1988).

The current methods for the isolation and identification of *L. monocytogenes* are laborious and time consuming. Therefore, molecular techniques are increasing being used as new alternative faster diagnostic methods with enhanced sensitivity and reproducibility. The diagnosis of listeriosis in ruminants, based on characteristic clinical signs, may be virtually confirmed by detection of an elevated white blood cell count, comprised principally of mononuclear cells, in the CSF(Woolford *et al*., 1990). The first positive step in elimination of human listeriosis is the elimination of animal listeriosis so; this study designed to throw some lights on clinical, epidemiological and laboratory diagnostic studies on sheep listeriosis in Egypt.

Materials and Methods

Animals:

Sheep

Throughout one year, (November 2006 to October 2007) six sheep flocks, with history of nervous man festations, at El- Dakhlia, Governorate were noticed for nervous signs as circling; head tilt drooping of the ear; blindness and recumbence ,the flock number ranged from 100 -800 sheep varied in their ages and Localities.

Rabbits and Swiss white mice

Ten groups (N =3) Rabbits 0f 800-900 gm B.W. and Ten groups (N =5) Swiss white mice of 16–18 gm B.W. used for studying the Anton's test and *pathogenisity* test of *Listeria monocytogens* isolates, 8 group from each species for the 8 isolates and other 2 groups are control.

Samples

Blood samples

Blood samples with EDETA were collected from 70 varied age sheep, 50 of them from clinically suspected and 20 from apparently normal contact sheep for hematological examination. While Blood samples were collected without anticoagulant for serum separation were collected from 110 sheep, 50 of them from clinically suspected and 60 randomly collected samples from apparently normal contact sheep for biochemical examination and antibodies detection by ELISA. Serum samples were stored at - 20°C until used.

Cerebrospinal fluid (CSF)

Cerebrospinal fluid (CSF) samples were collected from 24 sheep showing nervous signs (12 samples from diseased, and 12 from in contact healthy sheep) for isolation of Listeria species and for *biochemical* and cellular examination of CSF. The samples were put in screw sterilized caped bottle, transported to Laboratory of infectious Diseases Faculty of Veterinary Medicine Mansoura University, in cold chamber container within few hours for immediately *bacteriological* and cellular examination. Postmortem examination and tissue samples:

Brain, liver, spleen, and kidney tissues collected from each one of recent dead or emergency slaughtered sheep (30 samples for each organ). The selected samples divided into two parts, one was *bacteriological* processed for isolation of *Listeria* species and the other part preserved in formalin 10% for *histopathological* examination.

Isolation and identification of *Listeria monocytogenes* was done according to (Cruickshank *et al*., 1975). Typing of isolated *Listeria* monocytogenes using molecular technique (PCR) (Borucki *et al*., 2003).

Manual erythrocytic and leukocytic count:

Erythrocytes and *leukocytes* performed by manual method using improved neubauer hemocytometer and diluting fluids of erythrocytes and leukocytes. (Feldman *et al*., 2000).

Hemoglobin concentration (Hb) (gm/ dl) estimated *spectrophotometrically* using the cyanomethaemoglobin method (Drabkin, *et al*., 1949). Determination of packed cell volume (PCV) was determined using *microhaematocrite* capillary tubes. (Drabkin, *et al*., 1949). Red cell indices, MCV (fl), MCH (pg) and MCHC (%) were calculated from measured PCV%, Hb concentratrion and RBCs count, (Feldman, *et al*., 2000). For the differential *leukocytic* count, The blood film was made as soon as possible after collection of blood sample by manual method, two blood films were made from each blood sample, stained by Giemsa stain (Coles

et al., 1986).

The total cell count were done using the methods described by (Coles *et al.,* 1986). *Biochemical* analysis of CSF for total protein, albumin, glucose, creatinin kinase (CK) and AST using the available commercial test kits (Coles *et al*., (1986).

Total serum protein, glucose, creatinine cytokinase and AST were determined spectrophotometrically using the commercial test kits (Henry *et al*., 1974).

The Indirect Enzyme-linked immunosorbent assay (ELISA) test was applied in diagnosis of *L. monocytogenes* in diseased and apparently healthy sheep. (Engvall et al. 1971).

The obtained data in ELISA test were statistically analyzed. (Snedecor *et al.* 1973).

Results and Discussion

Clinical examination revealed that out of 2448 sheep located at six flocks in Dakahlia governorate 105(4.9%) were suffering from nervous manifestation. The varied clinical signs recorded such as dullness, depression, disorientation and head pressing against solid objects. Walking in compulsive circling, drooping of the ears and eyelids , head tilt, twisting the head to one side and hanging the food in the mouth for long period were also recorded. Losses of menace response reduced check muscle tones, recumbencey and paddling movements, detected usually before death The obtained results are in agreement with that observed by (Czuprynski *et. al*., 1993), (Radostits et al., 2007) and (Burgere *et. al*., 2008). The .,variations in nervous signs were attributed to the site of *microabcesses* formation either in medulla, pons, anterior spinal cord, or in the *cerebellum* (Burgere *et al*., 2008). The postmortem examination revealed that *necrotic foci* in congested liver, highly congested kidney, cloudiness of the *cerebrospinal fluid*, thickened and congested meninges, congested brain and congestion of blood vessels of brain stem and cerebellum and the obtained results were in agreement with that obtained by (El-Sawalhy *et. al*., 1999) and Seaman *et. al.* (1990). These results disagreed with that observed by (Al-Dughaym *et. al*., 2001): who observed that the postmortem picture of listeriosis of sheep was bilateral *keratoconjunctivitis*, lung showed multiple areas of hemorrhage, marked congestion and turbid and edematous meninges. The difference in postmortem picture may be related to the difference of the clinical form and the pathogenesis of listeriosis in sheep and most probably due to multiple causes of death.

Out of 2448 examined sheep 105 diseased sheep with nervous manifestations forming morbidity rate of 4.29 % as shown in Table 1, these results nearly agreement with that of (El-Sawalhy *et al*., 1999) who reported that the morbidity rate of affected flock of sheep suffering from listeric *meningeoencephalitis* was 5.81%. This result similarity may be due to the same area of examination in the same governorate and under the same environmental conditions. On the other hand, disagreed with (Kumar *et al*., 2007) *et. al.* who recorded three outbreaks of *encephalitic listeriosis* in sheep in India with cumulative morbidity of 7.89% and Gitter *et al*., 1986) who said that *encephalitic listeriosis* is more prevalent among ruminants, especially sheep, with an attack rate of approximately 10–12%. This difference may due to the difference in mangemental system and feeding system, which act as risk factor for sheep *listeriosis*.

The total case fatality rate andmortality rate were 77.14 % and 3.3 % respectively, which in agreement with that obtained by (Gitter *et al*., 1986) and disagreed with that obtained by (Kumar *et al*., 2007) who recorded that the cumulative mortality and case fatality rate of listeriosis were, 7.08 % and 89.85% respectively.

The mortality and fatality rates differences may attributed to the age factor as young age is usually highly susceptible and the *encephalitic* form usually fatal and none responsive to treatment which supported by (Radostits *et al*., 2007).

With special orientation to the age, the group of sheep aging 3-6 months old showed highest nervous manifestations Table 1. The high attack rate in lambs attributed to the age of teeth eruption, teeth loss or injury in the mouth cavity and low attack rate in adult may be due to previous exposure to the disease and development of partial immunity to *listeriosis* and this supported by the result of serodiagnosisg by ELISA test Table 4. The obtained results were in agreement with that obtained by (Wilesmith, *et al*., 1986), they recorded that the *encephalitic* form is the commonest and the attack rate in lambs ranged from 1.1% to 7.5% and the affected lambs were between 4 and 9 months old. (Burdarov *et al*., 1987). recorded an outbreak of *listeric encephalitis* among lambs between 10 days and 6 months of age in Bulgaria. (Barlow *et al* 1985). suggested that listerial *encephalitis* in sheep was most common in winter and early spring in the age groups of sheep, which would be cutting, changing and possibly losing teeth. In INDIA (Kumar *et al*., 2007) et. al. recorded low incidence in sheep below 6 months of age with cumulative morbidity of 3.75% as compared to 9.5% in sheep above 6 months of age. The variation in age susceptibility may be due to variation in environment and the nature of feeding material, which make a risk factor in *encephalitic listeriosis*. and this supported by (Malik *et al*., 2002), who reported that the *encephalitic* form of *listeriosis* results from trigeminal nerve infection consequent to abrasions of the *buccal mucosa* with feed or infection of teeth cavities.

The prevalence rate of *ovine encephalitic listeriosis* related to the season shown in Table 2. The occurrence of

Table (1): Morbidity, mortality and case fatality rate of nervous manifestations in sheep in relation to the age after isolation of *Listeria monocytogenes*

Age groups	Total No. examined	Diseased No.	Morbidity rate (%)	dead No.	Mortality rate (%)	Case fatality rate (%)
Group I	553	41	7.4	34	6.15	82.92
Group II	1202	51	4.24	39	3.24	76.47
Group III	693	13	1.88	8	1.15	61.53
Total	2448	105	4.29	81	3.31	77.14

Group I: Sheep aging 3 months to 6 months old
Group II: Sheep aging 6 months to 12 months
Group III: Sheep aging over 12 months

Table (2): Morbidity, mortality and case fatality rate of nervous manifestations in sheep in relation to season after isolation of *Listeria monocytogenes*:-

Season	Total No. examined	Diseased No.	Morbidity rate (%)	Dead No.	Mortality rate No.(%)	Case fatality rate(%)
Winter	508	36	7.08	30	5.9	83.33
Spring	1392	46	3.3	36	2.6	78.26
Summer	381	13	3.41	8	2.1	61.53
Autumn	167	10	5.98	7	4.19	70
Total	2448	105	4.29	81	3.31	77.14

N.B
* Management system is indoors "rearing in closed system"
**Management system is free grazing system

listeriosis all round the year explained as *Listeria monocytogenes* tolerate the adverse environmental conditions and survive at temperature of 4 - 40^{0}C. The high percent of encephalitic listeriosis in winter season attributed to several risk factors including, climatic changes, overcrowding, feeding of stored food and *fecal* contamination of feed during winter. This explanation were supported by (Scott *et al*., 1993) who mentioned that, *encephalitic listeriosis* can occur at any time of the year, but most frequently in winter season, predisposing factors in winter are likely to include housing of animals, which increase animal density and fecal contamination in the environment, feeding of stored feed and stress associated with adverse weather, in the UK. While in autumn months, which comes after summer months where as feeding on stumps of crops of wheat, barley, cotton and corn, which may be causing injurious to the oral cavity, which are predispose cause to ovine listeriosis,while in summer feeding of dry stored feed may facilitate the occurrence of *listeriosis*. These results in agreement with that obtained by (Scott *et al*., 1993), (El-Sawalhy et al., 1999), (Malik *et al*., 2002), and (Burgere *et al*., 2008).

Concerning to locality, the result revealed that Aga flocks had the highest percent of listeriosis (14%) Table 3. The high prevalence of *listeriosis* in *Aga* and *Mansoura* flocks attributed to the managemental system and hygienic measures, where they confinement indoors, feeding in closed pens and over crowded. In addition most of these flocks were yearling sheep (150 out of 250) and (74 out of 108) for Aga and Mansoura flocks respectively, which are common susceptible age to *encephalitic listeriosis*. These results coincided with that of Kumar *et al*., 2007) who reported that outbreaks of *listeriosis* can occur without feeding the silage and poor quality pastures (unhygienic) is considered the source of infection in addition the rotten vegetables or faecal contaminated pastures are posing great threat to the sheep industry. (Radostits *et al*., 2007) et. al. recorded that predisposing factors to *listeriosis* which have been observed or proposed to cause lowering of the host's resistance to infection include a poor nutrition state, a sudden change in the weather, poor access to grazing pastures, stress of advanced pregnancy, over crowding and unhygienic conditions.

In the present study, the antibody titers were measured in diseased and apparently healthy and control sheep from free flocks of listeriosis using Enzyme Linked *Immunosorbent* Assay (ELISA). There was a significant increase in antibodies titers in diseased sheep than incontact apparently healthy ones at P<0.001. These results were in agreement with Low *et al*., 1991) and (Chaudhari et al. 2004) Table 4. The detection of anti *Listeria monocytogenes* antibodies in the apparently healthy and diseased sheep revealed signifi-

Table (3): Morbidity, mortality and case fatality rate of nervous manifestations in sheep in relation to locality after isolation of *Listeria monocytogenes*:-

Locality	Total No. examined	Diseased No.	Morbidity rate %	Dead No.	Mortality rate %	Case fatality rate%
Belkas flock**	799	35	4.38	30	3.75	85.7
Dikernis flock*	167	8	4.79	6	3.6	75
Sherbeen flock **	524	6	1.14	4	0.76	66.66
Mansoura flock *	108	9	8.33	6	5.55	66.66
Sinbellaween flock **	600	12	2	9	1.5	75
Aga flock **	250	35	14	26	10.4	74.43
Total	2448	105	4.29	81	3.31	77.14

N.B
* Management system is indoors "rearing in closed system"
**Management system is free grazing system

cance difference between diseased and healthy sheep. Out of 50 serum samples of diseased sheep with nervous manifestations due to *Listeria monocytogenes* there were 15 (30%) positive for listeria antigen and out of 60 serum samples of healthy sheep there were 9 (15%) positive for listeriosis

Serological tests, such as the most widely used agglutination methods and complement fixation test lack the sensitivity to detect the weak antibody response frequently seen in confirmed *listerial* infection (Miettinin *et al*., 1990). Moreover, this was obvious clear from table 4 where the use of ELISA cannot be a reliable method to be used in *serodiagnosis* of *listeriosis*. The *seroconversion* in apparently healthy sheep may be attributed to the different clinical forms of *listeriosis* in sheep and there is another source of infection, which is clinically undetected (Weber *et al*., 1995).

The genetic basis for serotype identification is not well defined, genetic analysis indicates that the evolution of somatic and flageller antigens have paralleled that of many other genes Call, D. R., M. K. Borucki et al., 2003) therefore, serotyping by PCR primers may be designed as an alternative to the slide agglutination method of serotyping (Borucki et al., 2003). The isolated Listeria monocytogenes isolates were typed by polymerase chain reaction, which revealed that 3 out of 8 isolates were serotype 1/2a representing (37.5%), 3 out of 8 isolates were 1/2b in a percent of (37.5%), and 2 isolates were untyped (25%) Table 5. These results coincised with that obtained by (El- Sawalhy *et al*., 1999). who succeeded in isolating Listeria monocytogenes types 1/2a and 4 b from an outbreak of *listerial meningeoencephalitis* of sheep using the *serological* identification in the same area.

The obtained results were go in parallel with results of The *Listeria monocytogenes* serotypes 1/2a and 1/2b were isolated from ovine encephalitis by (54.16%) and(20.83) (Low et al., 1993). Thus it is obvious that serotype 1/2a and 1/2b the most common isolated serotypes from ovine meningoencephalitic listeriosis as recorded by (Miettinin et al., 1990), Low, 1993), (El-Sawalhy *et al* 1999). Moreover, in agreement with (Borucki et al 2003) who reported that identification of *Listeria monocytogenes* by using PCR primers would further increase the ease and accessibility of this classification system.

In ruminant species *cerebrospinal* fluid collection and analysis provides rapid and in some situations instant information to the veterinary clinician investigating a disease problem in the living animal. CSF analysis is particularly useful with respect to confirming the presence of inflammatory lesion involving bacterial meningoencephalitis. CSF can be obtained from living animal and analysis results are available within 1 hour of submission to the laboratory, where essential as an accurate diagnosis as soon as possible was obtained in order to expedite preventive and control measures.

The chemical and cellular parameters of CSF of listerial *meningoencephalitis* of sheep showed in Table 6. Physical examination revealed that cloudiness of CSF with increased viscosity as compared with normal CSF of control or in-contact sheep, these results are in agreement with those of (Scott, *et al* 1992) which could be attributed to the increased CSF protein concentration and *pleocytosis*.

Cellular examination of CSF of diseased and control healthy sheep as shown in Table 6, revealed significance ($p<0.0004$) elevation of total cell count, increase of polymorphic cells and *monomorphic* cells in encephalitic *listeriosis* in comparison to control sheep, which in turn suggests bacterial meningitis or suppurative none *septic meningitis*. This results in concern with that reported by (El-Sawalhy *et al* 1999), (Scott *et al* 1993) and (Scott *et al* 1992).

Chemical examination of CSF revealed increase of total protein of diseased sheep (2.7±0.46 g/dl) in comparison to incontact healthy ones (1.84±0.27 g/dl). these results are in agreement with that reported by (Gronstol, *et al* 1979), (El-Sawalhy *et al* 1999), (Scott *et al* 2004) *et. al*. who measured total protein in CSF in diseased sheep as (2.25g/

Table (4): Optical density means in serum of diseased, contact and healthy control sheep against Listeria monocytogenes antigen by using indirect ELISA.

State of animals	No. of animals	+Ve ELISA		Mean O.P.D ± SD	+Ve ELISA	
		No.	%		No.	%
Randomly selected contact sheep	60	9	15	432.6±321.21	51	85
Diseased sheep from which Listeria monocytogenes isolated	8	8	100	517.9±344.43	0	0
Diseased sheep without trial of isolation	50	15	30	445.52±272.9	35	70
Control from free flocks	10	0	0	89±119	10	100

Table (5): Typing of isolated *Listeria monocytogenes* using PCR

No. of isolated listeria monocytogenes	L. monocytogens 1/2a	L. monocytogens 1/2b	L. monocytogens Untybable
8	3	3	2
%	37.5	37.5	25

dl). The increased CSF proteins could be due to increased permeability of blood brain barrier or increased intrathecal globulin production or interruption of CSF flow and / or absorption.

None significant increase of total protein in some cases could be attributed to the early stages of the disease, and this supported by (Scott, *et al*., 1992) and (Kumar, *et al*., 2007) who recorded that the total protein in CSF in advanced stages of the disease was 11mg/dl and 3.5mg/dl in initial stages of meningoencephalitis *listeriosis* in sheep. The increase of CSF *albumin* in diseased sheep could be due to damage of the blood brain barrier or *hemorrhage* as reported by (Baily, *et al*., 1997). Creatinine cytokinase (CK) showed significant elevation (18.4 ± 3.05 IU/L) in diseased than control (12.8±3.96 IU/L) ($p<0.0110$) Table 8. The increase of CK in the CSF is due to the nervous tissue damage and *necrosis*. These results are go hand in hand with that obtained by (El- Sawalhy *et al*., 1999). CSF glucose level was non-significant increased (42.0±4.39 mg/dl) Table 6. This result similar to that obtained by (Kumar, *et al*., (2007) who detected increase of glucose level in diseased sheep (97.83mg/ dl) than healthy ones (56.16 mg/dl), While 18 showed decrease in the glucose level in diseased sheep (31.05±1.18mg/dl) than healthy sheep (51.76±1.75mg/dl). This reduction in glucose level could be attributed to the *glycolytic* activity of the infectious microorganisms or to increased use of glucose by bacteria and WBCs.

Hematological examination of blood of sheep suffering from *listerial meningoencephalitis* as shown in Table 7 which revealed that increased total *leucocytic* count with increased number of *neutrophils* in the examined blood of diseased sheep and this is in agreement with that obtained by (Baily, *et al*., 1997). Increased PCV and MCH in diseased sheep could be attributed to the dehydration of the animals due to off food and salivation and loss of *bicarbonate* in saliva. The biochemical values of blood in *encephalitic Listeria monocytogenes* infection observed that the level of CK, AST, glucose and total protein was non significant increased when compared with that of control healthy sheep, this is supported by (Morin, *et al*., 2004) and (Scott, *et al*., 2004).

Histopathological examination of sheep with meningoencephalitic listeriosis clarified typical lesions of listeriosis in sheep, which described by (Jubb,*et al*., 1985). who reported that the brain show edema and small accumulation of *lymphocytes* and *macrophages* and sometimes few neutrophils around or in close proximity to blood vessels of the brain. The results were also in agreement with that obtained by (El-Sawalhy, *et al*., 1999), (Campero, *et al*., 2002) and (Soumaya, *et al*., 2002). revealed that all cases showed multiple microabscess in the brainstem between the midbrain and upper cervical spinal cord and added that microscopic findings consisted of single or multiple *microabscess* with *necrotic* and *liquifactive* changes with infiltration by *neutrophils* and mononuclear cells located either in grey and or white matter.

From this study, we can conclude that, *Listeriosis* is one of the important diseases of sheep causing deaths and economic losses in area of study. Attention must be paid to young age sheep as they are more susceptible to listeriosis and separation between youngs and adults in grazing order is advisable. The common serotypes of *Listeria monocytogenes* isolated in Dakahlia Governorate were 1/2a and 1/ 2b. Although ELISA test cannot be a reliable method for *serodiagnosis* of *listeriosis* but can help in control strategies in handling the flocks. CSF cellular and *biochemical* examination may help in addition to *histopathology* and bacteriological examination in diagnosis of listeriosis in sheep.

Table (6): Cellular examination and *biochemistry* of CSF from diseased and incontact sheep (Mean ± SD)

Group	TCC	Mono	poly	CK	AST	Glu	TP	AL	A/G
Control	36.00 ± 7.08	26.8 ± 4.43	9.2 ± 1.92	12.8 ± 3.96	25.4 ± 4.98	36.6 ± 4.21	1.84 ± 0.27	0.018 ± 0.004	0.01 ± 0.00
Diseased	620.2 ± 36.03*	282.8 ± 38.69*	337.4 ± 40.29*	18.4 ± 3.05*	29.8 ± 4.15*	42.0 ± 4.39*	2.7 ± 0.46*	0.066 ± 0.018*	0.025 ± 0.005

TCC: Total cell count
Mono: Monomorhpic cells
Poly: polymorphic cells
CK: Creatinine cytokinase (u/L)
AST: Aspartate aminotransferase (u/L)
TP: Total proteine (gm/dl)
AL: Albumin (gm/dl)
A/G: Albumine globuline ratio

Table (7): *Hematological* examination of blood samples of diseased and incontact sheep.

Group	RBCs	HB	PCV	MCV	MCH	MCHC	TLC	Lymph	Neutro	Mono	Eosino	Band
Control	12.5236 ± 2.65	8.19 ± 2.87	30.6 ± 2.408	25.52 ± 5.77	6.55 ± 1.42	25.63 ± 7.55	14808 ± 1536.4	7269.8 ± 1227.4	6070.2 ± 1081.19	444.2 ± 339.2	484.2 ± 377.2	404.4 ± 278.3
Diseased	12.312 ± 1.687	12.098 ± 1.487*	35.8 ± 3.114*	28.95 ± 3.79	9.79 ± 1.7*	33.96 ± 4.51*	17140 ± 1465.6*	4567.6 ± 1624.5*	10939.8 ± 1225.44*	483.4 ± 374.7	744.8 ± 263.19	539.8 ± 375.3

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GENETIC EVIDENCE OF ROTAVIRUS IN CHICKEN IN NIGERIA

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PREUVES GÉNÉTIQUES DES ROTAVIRUS DANS POULET AU NIGÉRIA

Résumé

Des études ont été effectuées pour évaluer la présence de rotavirus dans certains troupeaux de volailles à Abeokuta et à Oyo en utilisant la technique de la transcription inverse suivie de la réaction en chaîne de la polymérase (RT-PCR). Un total de 49 échantillons de matières fécales sous forme de prélèvement cloacal a été recueilli de poulets et de dindes appartenant aux groupes d'âge entre 5 jours et 43 semaines. Six échantillons de poulets diarrhéiques âgés de 8 et de 9 jours ont donné un résultat positif pour le virus alors que les autres étaient négatifs. A notre connaissance, il s'agit ici de la première étude ayant donnée un rapport positif pour l'infection de rotavirus dans la volaille du Nigéria. Il est conseillé de faire des enquêtes épidémiologiques pour produire plus de données sur l'infection de rotavirus dans chez la volaille au Nigeria

Mots clés: poulet, Rotavirus, RT-PCR.

Summary

Studies were carried out to evaluate the presence of rotavirus in some poultry flocks in Abeokuta and Oyo, using the reverse *transcription–polymerase* chain reaction (RT-PCR) technique. A total of 49 fecal samples in form of cloacal swabs were collected from chickens and turkeys with age groups ranging between 5days and 43weeks. Six samples from diarrheic chicken aged 8 and 9 days old were positive for the virus, while others were negative. To our knowledge, this is the first study to report rotavirus infection in Nigerian poultry. It is recommended that *epidemiological* surveys be carried out to provide more data on rotavirus infection in poultry flocks in Nigeria.

Key words: Chicken, Rotavirus, RT-PCR

Introduction

Rotavirus gastroenteritis is a worldwide disease affecting primarily infants, young children and a wide variety of young mammalian and avian species (Estes *et al*., 1983, McNulty *et al*., 1984). *Rotavirus* infection in avian species was first reported by Bergeland *et al*. (1977) who found particles *morphologically* indistinguishable from rotavirus in intestinal contents of poults with watery droppings and increased mortality. Since then it has become apparent that rotaviruses infect many species of domestic birds. As in mammals, rotavirus infection in avian species is frequently associated with outbreaks of diarrhoea. The rotaviruses belonging to the family *Reoviridae* contain a genome of 11 segments of double stranded RNA (dsRNA), which can be separated into distinct bands by *electrophoresis*. The migration pattern of the 11 genome segments following electrophoresis of the viral RNA in polyacrylamide gel is called the RNA electropherotype (Estes *et al*., 1984). *Rotavirus* in birds belongs to groups A, D, F and G. (Saif *et al*., 1985). Detailed studies on the *epidemiology* of rotavirus associated diarrhoea in poultry has been performed in advanced countries but none has been reported in Nigeria. Genetic evidence of avian rotavirus in Nigeria is hereby reported.

Materials and Methods

Collection and preparation of samples

Thirteen flocks comprising 5 broiler, 4 local chicken, 2 turkey, and 2 exotic chicken from 3 local government areas were sampled. Faecal samples in the form of cloacal swabs were collected from diarrheic (26) and non-diarrheic (23) birds. The broiler flocks were apparently healthy, while the turkey, pullet and local chicken flocks showed

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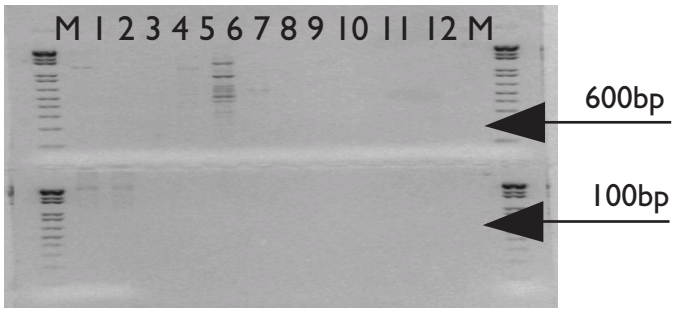
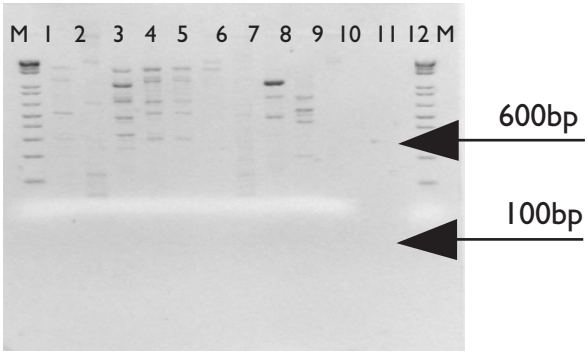
signs of dullness, emaciation and diarrhea. Approximately 3g of fresh fecal samples were collected from the litter and placed in 500µl of viral transport medium (VTM) containing Hank's balanced salt solution, penicillin, streptomycin and fungizone. The cloacal swabs were similarly placed in VTM and clinical signs for each flock were noted. The samples were transported to the laboratory with ice pack and stored at -20^{0}C until used. Polymerase chain reaction (PCR) Isolation and purification of RNA from sample Isolation of Rotavirus RNA from samples and its purification was done using Qiagen RNA minikit (QIAGEN GmbH, Germany), using the protocol for the kit. Reverse transcription was done to generate cDNA from the RNA by mixing 5µl of extracted RNA with 8µl of mix 1 which consisted of 2µl of distilled H_20, 1µl of 10mM dNTPs, and 5µl of 0.03µg/µl random primer and was incubated at 72^{0}C for 10min. Seven (7)µl of mix 2 which contained 1µl of DTT, 1µl of RNAase inhibitor, 4µl of 5x first strand Buffer and 1 µl of superscript III was added and then incubated at 50^{0}C for 1 hour 20 minutes and 70^{0}C for 15 minutes. The polymerase chain reaction for Rotavirus was carried out by adding 2.5µl of cDNA to 22.5µl of PCR mix containing 17.2µl of distilled water, 2.5µl of 10X PCR buffer, 2.0µl of MgCl2 (50mM), 0.5µl of dNTP (10mM), 0.1µl of forward primer NSP4-F30, 0.1µl of reverse primer NSP4-R660 (Pantin-Jackwood, *et al*. 2007) and 0.1µl of taq polymerase (5µ/µl). The PCR reactions was carried out using the following cycling conditions; initial denaturation at 940C for 5min, 35 cycles of amplification at 94^{0}C for 30 sec, 50^{0}C for 30 sec and 72^{0}C for 1min and final extension at 72^{0}C for 10min. The PCR product sizes were visualized by UV illumination in 2% agarose gel stained with ethidium bromide as compared to the 1kb+ size (invitrogen). The positive specimens were detected with band at 630bp.

Fig. 1. Agarose gel electrophoresis for demonstration of RT-PCR product (630bp)



Lane M= DNA Marker, Lanes 5 and 6 are positive samples, Lane 12 Negative control

Fig. 2. Agarose gel electrophoresis for demonstration of RT-PCR product (630bp).



Lane M= DNA Marker, Lanes 3,4,8,9 are positive samples, Lane 12 Negative contro

Results and discussion

Four fecal samples from pullet chicks and two from local chickens were positive for Rotavirus out of the 49 analyzed by the RTPCR technique. These were from 2 farms from two different local government areas. In this study, rotavirus infected birds were found diarrhoeic, dehydrated, anorectic and with low body weight and increased mortality. These observations are in conformity with the earlier reports of McNulty (2003) and Tamehiro *et al.* (2003) who reported that in field conditions, rotavirus infections in poultry might induce subclinical manifestations, or they might be associated with enteritis, dehydration, anorexia, unrest, litter ingestion, low weight gain and increased mortality. Cumulatively, all these can lead to huge economic losses to poultry production systems (McNulty 2003; Villarreal *et al*., 2006). Chickhood mortalities in Nigerian poultry have previously been linked to various factors including disease; none however has been related to rotavirus infection. This study serves as the first report of rotavirus infection of chicken in Nigeria. Since the potential economic resources of the poultry industry may not be fully utilized until the etiological agent of diseases are recognized and possibly controlled. There is need therefore to conduct epidemiological studies to determine prevalence of rotavirus in Nigerian poultry with subsequent genetic characterization of the virus.

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HISTOPATHOLOGY OF THE ORGANS OF BROILER CHICKENS EXPOSED TO FLAME AND FUMES OF KEROSENE BURNING

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HISTOPATHOLOGIE DES ORGANES DE POULET DE CHAIR SOUMIS AUX FLAMMES ET FUMÉES DE COMBUSTION DU KÉROSÈNE

Résumé

On a évalué l'histopathologie des organes de poulets de chair gardés dans un poulailler exposés aux flammes et aux fumées de combustion de kérosène, à des distances variables au cours d'une période quotidienne de 16 heures pendant 56 jours. La combustion du kérosène a été simulée dans un brûleur. La flamme de kérosène dans le brûleur a été respectivement placée à une distance de 4, 8 et 12 mètres des oiseaux, ce qui représente le traitement 1, 2 et 3 alors que le traitement 4 était utilisé pour un autre poulailler qui n'était pas exposé aux flammes et qui était séparé pour servir de témoin. Du début jusqu'à la fin de l'expérience, les propriétaires des poulets de chair les nourrissaient ad libitum. A la fin de la 8ème semaine, trois volailles par traitement étaient abattues par section de la veine jugulaire et les organes viscéraux (le foie, les poumons, le coeur et les reins) étaient extraient pour être examinés en détail en fonction de leur taille et des lésions constatées. Les observations sur l'histopathologie des organes des groupes traités ont démontré plusieurs effets issus des inhalations des fumées de kérosène. Les poumons étaient les plus affectés et présentaient de larges dépôts de carbone (anthracose) avec divers degrés d'inflammation ainsi que d'anthracose du foie, du coeur et des reins.

Mot-clé: Poulets de chair, Flammes et fumées, *Histopathologie*, Kérosène, Organes.

Summary

Histopathology of the organs of broiler chickens exposed to the flame and fumes of refined petroleum product kerosene at varying distances over a period of 16hrs daily for 56 days in a poultry house were evaluated. Kerosene burning was simulated in a designed burner. Kerosene flame in a designed burner was placed 4, 8 and 12 meters from the birds respectively which represented treatments 1, 2 and 3 while treatment 4 was in another poultry house without flame and severed as control Proprietroy boiler starter and finisher diets were fed ad libitum At the end of the 8th week, three birds per treatment were slaughtered by severing the jugular vein and the visceral organs (liver, lung, heart and kidney) were collected and grossly examined for size and lesions Histopathological observations of the organs of treated groups demonstrated various effects of inhaled kerosene fumes. The lungs were most affected showing large carbon deposits (anthracosis) with various degrees of inflammation and necrosis in the liver, heart and kidney.

Keyword: Broiler chickens, flame and fumes, *histopathology*, kerosene, organs.

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Introduction

Petroleum (crude oil) is a remarkably varied substance both in its use and composition. It is often dark, straw colored, black or sometimes green in outlook. Crude oil is the chief source of *hydrocarbons* and when fractionally distilled, the various components are often collected over a range of boiling points (Ababio, 1993). The main fractional distillates of petroleum include natural gas, light petroleum (petroleum ether), ligroin (light naphtha), petrol (gasoline), paraffin (kerosene), gas oil, lubricating oil and asphalt (bitumen).

Refined petroleum product (kerosene) is a mixture of hydrocarbons that contains 12-18 carbon atoms per molecule and it boils between 170 - 250°C. It is a fairly volatile liquid which is used as a fuel for lighting lamps (illumination), heating or cooking, fuel for automobile driving, modern jets and aeroplanes, burning bush, grasses and wood (incineration) (Murray, 1972; Jumoke, 1999). Kerosene is also a good solvent for grease and paints. It is also used as and insect repellant because of its odour. Developing countries with epileptic electricity supply use kerosene in lanterns and stoves for heating and brooding chicks and other livestock. When kerosene burns, it produces a flame which could be blue, luminous flame or yellow sooty flame, depending on the type of burner used. Fumes from sooty kerosene flame are laden with volatile organic carbon (VOC) and suspended particulate matter (SPM), which irritate the respiratory tract when inhaled either by man or livestock. However, .studies on the organs histopathology of broiler chickens exposed to burning crude petroleum is limited. The objective of this study is to determine the effect of burning refined petroleum product (kerosene) on the visceral organs of broiler chickens so as to simulate what happens when kerosene is used in lanterns and stoves for brooding day old chicks.

Materials and Methods

One hundred and twenty (120) unsexed day-old "Aboika" broiler chickens were divided into 4 groups of 30 birds per treatment, replicated thrice with 10 birds per replicate in a completely randomized design trial. The distances from the flame point were 4m, 8m, 12m and the control, represented as treatments I, II, III and IV respectively (Fig. 1). Treatment IV (control) was located in a separate building without flame. The birds were distributed randomly into 12 pens and brooded in an open sided brooding pen on deep litter. Brooding temperatures ranged from 33°C - 3.0.The petroleum product (kerosene) was ignited in a designed metal burner, 22.86cm high, 17.80cm diameter and a thickness of 1.27cm (Fig. 2). The study was done at the Teaching and Research Farm of the Rivers State University of Science and Technology, Port Harcourt, Nigeria.

The birds were fed ad-libitum on a proprietory broiler starter mash containing 2285.6 kcalME/kg and 24.91% crude protein for 5 weeks, and a broiler finisher mash (2304.9kcalME/kg and 20.5% crude protein) for 3 weeks. Water was provided ad-libitum. Feed intake and mortality values were recorded daily. At the end of the 8th week, three birds per treatment were slaughtered by severing the jugular vein and the liver, lungs, heart and kidney were removed and grossly examined for size* and lesions. Statistical analysis was carried out on the organ weights using analysis of variance (Steel and Torrie 1980) and treatment means were separated using Duncan's Multiple Range Test (DMRT) as modified by (Gomez and Gomez 1984). The histopathological examination was carried out at the University of Port Harcourt Teaching Hospital as described by Drury and Wallington (1976).

Results and Discussion

Results of the organ weights of the birds at the end of the experiment are presented in Table 1. Analysis of Variance of the effect of refined petroleum product (kerosene) flame and fum$3on broiler chickens showed that organ weights (liver, lungs, heart and kidney) were not significantly (P>0.05) affected. The weight of lungs ranged between 9.13 ± 2.17g in 4m treatment group and 12.23 ± 1.17 in the control group while the liver of the birds that were 12m away from the flame had the least weight of 32.60 ± 3.41g. It was observed that the kidney weight of birds that were not subjected to kerosene flame were higher (1.97 ± 0.54g) than those exposed to various flame distances.

Mortality rates of the various treatment groups showed no significant (P>0.05) effect. Weekly monitoring revealed that the highest mortality was reported in treatment 2 (8m). Post mortem examination attributed deaths to asphyxiation probably resulting from inhalation of thick smoke. This result tallied with observations made by Williamson and Payne (1978), Bains (1978) who separately reported that broiler chicks mortality is attributable to suffocation, chilling and overheating. The birds in the treatment groups had their feathers darkened by the carbon deposits of VOC and SPM particles; and the dark coloration which also affected the poultry pen roof reduced with increasing distances from the flame. Treatments 1 (4m) and 2 (8m) birds were observed to cough often times because of inhalation of carbon fumes which affected their respiratory tracts. Impacts of crude oil and gas on the organs of animals are many and varied. The effects are manifested as

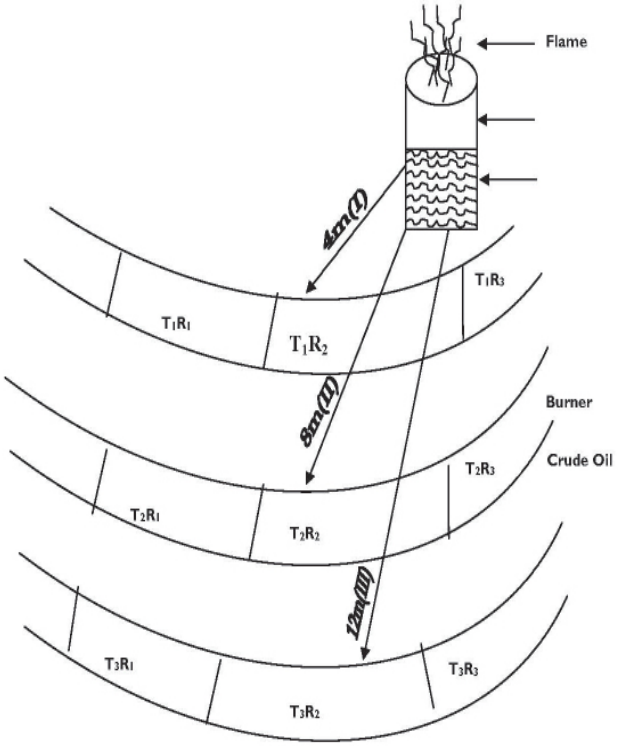

Fig. 1: Poultry pen experimental design, showing the distances from the flame point

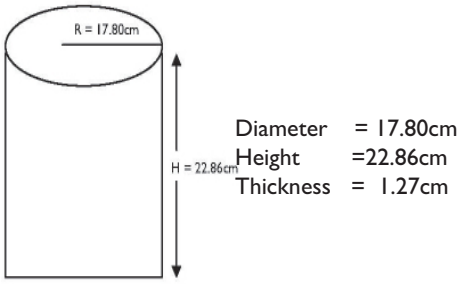

Fig. 2: Design metal burner that was used to burn the kerosene to produce the flame

inflammation and necrosis in the liver (hepatotoxicity), cirrhosis and anthracosis in the lungs, loss of cardiac muscle architecture in the heart and nephrotoxicity in the kidneys (Gillette *et al*., 1987; Cortran *et al*., 1989).

Liver

Exposing broiler chickens to flame and fumes of refined petroleum product (kerosene) was characterized by *focal necrosis* marked by *inflammatory cell* infiltrate and increase in fatty vacuoles in the liver cells. These observations are in agreement with the findings of Durhane and Brovwer (1989) and McConnels (1989). Another form of hepatotoxicity found in the liver was fatty degenerative changes (*cytoplasmic vacuolation*). In this study, fatty degenerative changes (*cytoplasmic vacuolation*) were found in the liver of the treated groups. This suggests an excessive accumulation of triglycerides within the *hepatocytes* resulting in a defect in *hepatic lipid metabolism*, which implies severe injury. This is confirmed by the work of Cotran *et al.,* (1989). Another form of *hepatoxic* changes includes *focal necrosis* and cell inflammation (Ovuru 2002). Presence of liver cytosol enzymes (SCOT and SGPT) normally confirm liver damages (Redlich *et al*., 1990). In the work reported here, inflammatory cell infiltrates and necrosis observed in the treated groups confirm findings in these earlier works. The liver from the control group showed normal hexagonal architecture of the hepatocytes.

Lung

The findings in this study indicated enlarged alveolar spaces. Crude oil constituents ingested or inhaled can produce these effects in the lungs. This was confirmed by the report of Gillette *et al.,* (1987). In this study, another evidence found in the chicken lung exposed to refined petroleum product (kerosene) combustion is the deposition of inhaled volatile organic carbon (VOC) and suspended particulate matter (SPM), within the *bronchioles* called *anthracosis*.

The respiratory system is endangered by the inhalation of noxious gases such as CO, HiS, SO2 and SPM (aerosols), when crude oil is burnt. Evidences of bronchitis and other respiratory diseases are rampant. Inhalation of lead particles gives rise to lead poisoning with resultant irritability of the nose, throat and eyes (Horsfall and Spiff 2001; Ubong and Gobo 2001). In this study, the birds nearest to the flame were seen to cough frequently, confirming the irritability of the air passage. In the control group, the lungs showed normal dilated alveolar channels with thin hyalinized vascular channels with thin alveolar walls. Of all the organs observed, the lungs were most affected by the fumes.

Heart

This study revealed that the heart of chickens exposed to the flame and fumes of kerosene showed waviness of cardiac myocytes, inflammatory cell infiltrate and increased interstitial spaces. These were confirmed by the findings of Benditt and Benditt (1973); Ou and Ramos (1992) and Ovuru (2002). Benditt and Bendit (1973) found that smooth muscles of the heart of animals fed crude oil contaminated feed were affected. Ou and Ramos (1992) reported that aromatic and *polycyclic hydrocarbons* are known persistent environmental contaminants identified as vascular toxins in experimental animals, and are known to initiate atherogenic process in aorta of animals fed crude oil contaminated feed. The findings of Ovuru (2002) showed in addition a loss of cardiac muscle architecture and inflammatory myo-

Table 1: Overall effects of refined petroleum product (kerosene) flame and fumes on mortality and organ weights of broiler chickens.

Treatment		Weekly mortality % (Mean ± SEM)		Organ weights (Mean ± SEM)	
		Liver (g)	Lung (g)	Heart (g)	kidney (g)
4m	0.19 ±0.57	36.40 ± 1.32	9.13 ±2.17 11.	7.90 ±0.81	1.30 ±0.32
8m	0.40 ±0.58	35 .43 ±4.23	70 ±0.70 9.20	7. 13 ±0.63	1.06 ±0.22
12m	0.28 ±0.21	32.60 ±3.41	±1.33	7.13 ±0.64	1.10±0.17
Control	0.00	33.97 ±0.58	12.23 ±1.17	7.23 ± 0.20	1.97 ±0.54

cardial muscle cells. These findings are in agreement with the observations in this study.

Kidney

The findings in this study showed that the kidney exhibited extensive necrosis, inflamed Bowman's capsule and tubule. Kidney weights of the treated groups were similar to the control group. In Ovum (2002) studies, it was demonstrated that graded levels of crude oil in a diet fed to rabbits, elicited eosinophilic cast in the lumen of distal convoluted tubules as well as a dilation of the Bowman's capsules. Monks *et al*., 1994) found similar observations in mice treated with bromobenzene, a hydrocarbon. The presence of heavy metals in crude oil components may cause similar toxicity through the ability to bind to sulfhydryl groups (especially mercury) in animals (Conner and Fowler 1993; Zalups and Lash 1994). Gaseous emissions or pollutants from burnt crude oil may also cause similar damage.

Conclusion

The impacts of refined petroleum product (kerosene) flame and fumes on the organs of broiler chickens as reported in this study are many and varied. Petroleum hydrocarbon effects are manifested as inflammation and necrosis in the liver (*hepatotoxicity*), cirrhosis and anthracosis in the lungs, loss of cardiac muscle architecture in the heart and nephroioxiciiy in the kidneys. Therefore the use of kerosene in lanterns and stoves in poultry production, especially in brooding should be discouraged because of the health hazards to men and livestock.

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INTERCROPPING EFFECT OF CANAVALIA ENSIFORMIS ON REGROWTH ABILITY AND IN-VITRO GAS PRODUCTION OF PANICUM MAXIMUM CV T58

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EFFET INTERCALAIRE DE CANAVALIA ENSIFORMIS SUR LA CAPACITÉ DE REPOUSSE ET LA PRODUCTION DE GAZ *IN VITRO* DE PANICUM MAXIMUM CV T58

Résumé

L'effet de la culture intercalaire de légumineuses sur la capacité de régénération et sur la valeur nutritive à travers la production de gaz *in-vitro* de l'herbe de Guinée a été évalué dans un arrangement factoriel de 2 x 2. L'herbe *Panicum maximum* cv T 58 et la culture intercalaire de *Canavalia* ensiformis ont été étudié pendant six semaines. La hauteur de la taille, le nombre de tailles, le pourcentage de repousse ont été enregistré hebdomadairement. L'herbe a été fauchée à la fin de la sixième semaines. Des échantillons ont été prélevés et séchés au four. La matière sèche produite a été analysée. La composition générale des graminées a ensuite été effectuée. L'état du sol a été amélioré après le traitement. Le pédicelle avait une plus faible quantité de protéines brutes que les feuilles (5,43%). La production de gaz effectuée in vitro a démontré que le gaz méthane produit était plus élevé dans le pédicelle que dans les feuilles. Cela suggère que l'utilisation des feuilles serait plus importante quand elles servent de nourriture pour les ruminants. La culture intercalaire de *Canavalia* ensiformis couplée au *Panicum maximum* a renforcé la capacité de repousse et la production in vitro de gaz réduisant ainsi le besoin de recourir aux engrais chimiques.

Summary

The effect of legume intercrop on regrowth ability and nutritive value via *in vitro* gas production of guinea grass was evaluated in a 2 x 2 factorial arrangement. *Panicum maximum* cv T 58 grass with *Canavalia* ensiformis intercrop was studied for six weeks. The tiller height, tiller numbers, regrowth percentage were recorded on weekly basis. The sward was harvested at the end of six weeks. Samples were taken, oven dried and dry matter yield determined. Proximate composition of the grasses was then carried out. The soil condition was improved after treatment. The stem had the least crude protein content than the leaves (5.43%). *In vitro* gas production carried out showed that methane gas production was higher in the stem fraction than in the leaves. This suggests that leave utilization will be higher when fed to ruminants. Intercropping *Canavalia ensiformis* with *Panicum maximum* enhanced the regrowth ability and *in vitro* gas production thereby reducing the necessity for chemical fertilizer.

Keywords: *Canavalia ensiformis*, Regrowth ability, *In vitro* gas, *Panicum* maximum

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Introduction

Plants depend on the soil minerals for growth while ruminant animal depends on plants for food. Most naturally occurring mineral deficiency in herbivores (e.g ruminant animal) are associated with specific region and are directly related to soil characteristics (McDowell, 1983; Underwood, 1981). Legumes have being reported to have the ability of fixing nitrogen into the soil thereby affecting soil mineral level. It has being revealed that legume based pasture give higher animal performance for growth, production, reproduction and wool production. Ruminant live weight gain has been related positively to the proportion of ingested legume and has a considerable advantage over feeding sole grass pasture (Humphery, 1994). Incorporation of protein rich forage legume into tropical farming system has improved livestock production system (Van Soest, 1994). As consequences, a range of tropical legumes have being tested for agronomic performance in tropical environment. The high level of production on a grass plus nitrogen system, demand much higher input of fertilizer than on legume based pasture.

Some creeping legumes such as Canavalia ensiformis, Centrosema pubescence, Pueraia phaseliodes are high in crude protein and well adapted to varying weather. Apart from being relished by ruminants, farmers often use these legumes for soil reclamation (Babayemi and Bamikole, 2006). Legume represents the better first choice for pasture development in most tropical countries (Humphery, 1994). The majority of tropical livestock have access only to low quality pasture, crop residues or by- product of relatively low nutritive value. Nevertheless, there is a variety of plants, seed and fruits which remain still relatively unexplored as a feed supplement for ruminant production. Many plants being high in protein could be suitable as food supplement for ruminant animal. Milk yield and growth of ruminant are largely determined by forage quality (Minson, 1990).

The importance of the *in vitro* gas production system for feed evaluation in animal nutrition has long been recognized. The determination of intake and digestibility of feed stuff *in vitro* is time consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large scale feed evaluation (Babayemi, 2007; Makkar *et al*., 1995). Therefore, many attempts have been made to predict the intake and digestibility using laboratory techniques. One of the methods in which the digestibility of feeds can be estimated biologically is the digestion process with rumen micro-organisms (Terry and Tillery, 1963). This is the gas method which known as *in vitro* gas production method. Guinea grass is one of the most productive forage in the tropics. It is a valuable pasture, good for green silage and hay. It is palatable to animals especially in their younger stage and tends to be coarse and less readily eaten as it matures (Reed, 1976). But generally, guinea grass has a lower nutritive value. They hardly supply the minimum energy requirement of ruminants and seasonal variation affects it quality. The consequence is irregular animal growth pattern and low milk yield (Akingbade *et al*., 2004). Therefore legume grass - mixture as feed for ruminant will be beneficial.

The objective was therefore to determine the effect of legume inter crop on soil mineral level, dry matter production and nutritive profile of guinea grass, Panicum maximum cv T 58, via *in vitro* gas production technique.

Materials and Methods

Location of the experiment

The experiment was conducted at the Teaching and Research farm of Ladoke Akintola University of Technology, Ogbomoso in the derived savanna zone of Nigeria and in- vitro gas production was conducted at the department of Animal science, University of Ibadan.

Selected Forages Species

A newly introduced variety of Guinea grass (Panicum maximum) cvT58 was selected for the experiment. Urea was first examined in 1997, 98, 99 planting seasons. The sward was later cut back to the uniform height of 15cm.

Experimental Design and Procedure

The experiment was a factorial design with three replicates making nine- sub- plots. The field measured 22m x 16m in length and breadth respectively having 7m x 5m of each replicated plot with 0.5m spacing between the rows. Soil sample was taken before and after treatment for laboratory analysis. Each treatment was applied once. The three treatments received two seeds per hole per four grass stands. The grass was harvested after six weeks. The samples were harvested from all 9 sub-plots, flip sampled and weighed. The flip samples were taken to the laboratory and dried at 70-80^{0}C for 48hrs before being milled in laboratory in a stainless milling machine using 1mm sieve.

Dry Matter Yield Evaluation

At harvest, the grass from all the sub plot was cuts at 15cm above the ground level, weighed, mixed and sub sampled. The sub sample was put in a polythene bag, taken to the laboratory and then separated into leaf blade and stem. Each fraction was weighed and oven dried, at 70 - 80^0 C for 48hours, and reweighed to obtain dry matter yield.

In-vitro gas procedure

Rumen fluids were obtained from three West African dwarf goats. The method for collection (Babayemi and Bamikole,

2006) as using suction tube from goat previously fed with 40% Concentrate feed. The rumen liquor was collected in the thermo flask that has been pre warmed to a temperature of 39^{0}C. Incubation procedure by Menke and Steingass, (1998) using 120ml calibrated transparent plastic syringe with fitted silicon tube. The sample weighing 200 mg (n=6) was carefully dropped into syringe and thereafter 30ml inoculums containing strained cheese cloth. Rumen Liquor and buffer (g/litre) under continuous flushing with $C0^2$ was dispended using another 50 ml plastics calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube on the syringe was tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 39 ±10c and the volume of gas was measured at 3, 6, 9, 12, 15, 18, 21, 24, hrs. At post incubation period 4ml of NaOH was introduced to estimate methane production (Fleez *et al*., 2005).

Data Collection

Data was collected on sub sampled plant materials harvested, weighed and oven dried. It was kept and preserved for proximate and mineral analysis. Parameters measured included: Weekly regrowth percentage, Tiller height, Dry matter yield, Proximate composition, metablizable energy, amount of gas production, organic matter digestibility and short chain fatty acids.

Statistical Analysis

Data collected were subjected to 2 x 2 factorial arrangements in a completely randomized design using (SAS, 2003).

Table 1: Effect of legumes intercrop on soil nutrient availability after six weeks of regrowth.

Nutrient	Treatment	Control
N %	0.16^a	0.11 ^{b}
Av P(ppm)	7.18^a	5.85^b
K (cmol)	0.20^a	0.12^b
Ca (cmol)	2.29	2.37
Mn (mol/kg)	88.17^b	96.75^a
Cu (mg/kg)	0.37^b	0.55^a
Zn (mg/kg)	1.74 ^{a}	1.42^b

a b; Means value with the same super script are not significantly different at (P<0.05).

Results

The results show that available P, K, Ca, Mn, Zn and total nitrogen in the soil increased after intercropping with the legume. There was higher nitrogen (0.16) in the treatment plot than in the control (0.11). The control plot had a lower phosphorus level (5.85) which may result to low herbage and seed production low voluntary intake in animal. Both the treatment and the control plots had higher value of calcium (2.29, 2.37) showing that enough calcium will be available for plant which will help the animal in formation of bone, if fed to them. The Manganese, copper and zinc levels in the soil were at their optimum. The mean of all tiller height from plot 2 to 3 (0.87, 0. 78.0 87) in Table 2 was higher than the control of tiller height (0.7, 0.6, 0.8). The mean of tiller number in treatment plots (82) was lower than the control value (92) but plot 2 and 3 (88 and 99) has higher value than control value (75 and 88). The percentage regrowth increased from week 1 to week 4 (46.0, 49.7, 60.00, 68.00). It remained the same in weeks 4 and 5 (68.00, 68.00) and greatly increased in value (80.00) in weeks 6. This shows that legume intercrop caused a higher regrowth before the 8th week which is considered as the best period for defoliation for feeding.

Table 2 shows the higher dry matter value in plot 1 (1527.94) which was higher than plot 2 (1218.58) and plot 3 (1503.86). Table 3 above shows that metabolizable energy of the leaf (8.46) was higher than the stem value (8.27). The organic matter digestibility of leaf (57.24) was higher than organic matter digestibility of stem value (55.59). There were no differences in the SCFA of grass leaf and stem.

The table 4 above shows the time of degradability, it potential of degradable fraction the intercept and rate of degradation of b. The initial gas produced in stem (8.50) is higher than the leaf (7.50). The leaves (35.0) had higher potential degradable fraction than the stem (34.0). The rate of degradation of leaf (0.07) was higher than the rate of degradation of stem (0.05). The time at which the leaf was degraded (95.17%) was higher than the time at which stem was degraded (25.50%). Table 5 shows the crude protein, crude fibre, Ash, E.E., NFE and Dry matter of leaves and stem. The CP of leaf (8.75) was higher than CP of stem (5.43). CF of stem (31.97) was higher than CF of Leaf (24.75). EE and Ash of leaf (2.88 and 12.08 respectively) were higher than stems. NFE of leaves (51.54) is higher than the stem (51.22). Dry matter value was higher in stem (90.15) than the leaf (89.76). All these compositions were higher than control except the CF of leaves and stem (24.75 and 31.97 respectively) which were lower than CF in the control (39.29). The CP and ASH of stem (5.93) and (9.69) were lower than control (6.89 and 11.3).

Table 2: Effect of Legume Intercrop on the mean tiller height (m), tiller (No), Regrowth Percentage (RP) and Dry Matter Yield (DMY) of Guinea grass, Panicum maximum cvT58 after 6 weeks of Regrowth

	Plot 1		Plot 2		Plot 3	
	m2	ha	m2	ha	m2	ha
Mean Tiller Height	0.87[a]		0.78b		0.87[a]	
Control Height	0.70[b]		0.6c		0.8[a]	
Mean Tiller Number	82[a]	23428.6	88[b]	25442.8[b]	99[a]	28285.7[a]
Control Tiller Number	92[b]	26285.7[a]	75[c]	21428.6	88[b]	25142.9[b]
Legume intercrop(DMY)	5.35[a]	1527.94[a]	4.26[b]	1218.58[b]	5.26[a]	1503.86[a]
Control (DMY)	7.40[a]	2115.60[a]	5.79[b]	1655.6[b]	7.75[a]	2215.35[a]
Grass regrowth/ week	80.00[a]	7619.2[a]				
Control regrowth/ week	50.23[b]	4783.8[b]				

a b, bc Means value with the same super script are not significantly different at ($P<0.05$).

Discussion

It was shown from the result that *Panicum maximum* cvT58 intercropped with the legume (*Canavalia ensifomis*) can regenerate easily. Based on tiller height, the plot had different values i.e. plots 1 and 3 were higher than plot 2. This confirms previous research report by Akangbe, (2007) that different legume can associate with different grasses. Tiller production and dry matter production increased as the age of the grass increased. It may be due to the level of mineral taken up by plant for their metabolism. The tiller numbers also varies in plot which is due to variation of soil mineral level of the plot and also it can be due to amount of nitrogen uptake. This shows that plant ages as the week progresses. But on weeks 4 and 5 the value remained the same. There was great increase in the 6th weeks of regrowth which is due to proper usage of soil mineral. Regrowth percentage value at sixth week was higher than the control. This shows that the mineral uptake help the production of the grass.

The mid-rib showed that there is variation in the storage of foods i.e. the rate at which the nutrient is distributed in the leaves varies. The mid-rib which has the highest value 0.043 stores more food. The higher the leaf is broad, the higher the dry matter of the leaf as been confirmed by the findings of Akangbe, (1999). The dry matter of plot 4 is higher which might be due to level of minerals in the soil. The proximate composition of grass shows that the crude protein of leaf is higher than the stem. The leaf with high CP will stimulate the intake of forages and its utilization by animal. It also enhances microbial multiplication in the rumen which in turn determines the extent of fermentation. The stem and control had high crude fibre and therefore reduce the utilization of forage compared to the leaves. The Ash content of the leaf is higher than the stem and control. The amount of ether extract stored in the leaf is higher therefore more fat stored in the leaf. The metabolizable energy was 8.27MJ/kgDM in stem and 8.46 MJ/kgDM in leaf. They are not significantly different ($P<0.05$). The organic matter digestibility in leaf and stem was 57.24 OMD and 55.59 OMD. The short chain fatty acid of both leaf and stem is 0.95 mmol which does not exceed 4.5 mmol. Therefore, there will be increase in level of pH and increase in microbial activity as supported by the report of Beuvink (1993). The leaf has high potential degradable fraction than stem, Hence, high nutritive value. Figure (1) shows graph of *in vitro* gas production of Guinea grass, *Panicum* maximum cvT58. There was an increase in volume of gas has the time progressed. Figure 2 showed less volume of methane gases produced. The graph showed that leaf produces lower methane than the stem. This indicated that the stem had high energy loss compared to the leaves when fed to ruminants as being confirmed by the findings of Babayemi and Bamikole (2006); and Babayemi and Bamikole (2004).

Conclusion

Panicum maximum cvT58 can be established and inter cropped with legume like *Canavalia ensiformis* to increase mineral uptake by plant for their metabolism and reduce the use of inorganic fertilizer.

Impact

The intercropping *Canavalia ensiformis* with Panicum maximum enhanced the regrowth ability and in vitro gas production thereby reducing the necessity for chemical fertil-

izer. It also shows that utilization of Canavalia ensiformis on the regrowth ability of Panicum maximum suggest higher in vitro gas production of methane gas production were higher in stem fraction than in leaves. This is an indication that stem fraction of most of the plants will produces more gases than leaves fraction of the plant.

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L'ÉLEVAGE PASTORAL FACE AUX POLITIQUES COLONIALES, POSTCOLONIALES ET DE RÉGIONALISATION DANS L'ESPACE CEDEAO

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Résumé

L'élevage et l'agriculture sont les deux principales activités humaines de l'espace de la Communautés Economique des Etats d'Afrique de l'Ouest (CEDEAO). Mais, la disparité des ressources pastorales entre pays sahéliens et côtiers accentue les mouvements de bétail à l'intérieur de la CEDEAO et occasionne souvent des conflits socioéconomiques entre les pasteurs transhumants et les populations des pays d'accueil. La présente étude vise à (i) contribuer à une meilleure appréhension de la transhumance transnationale en Afrique de l'Ouest, (ii) mettre en perspective cette forme de pratique d'élevage en relation avec la libre circulation des personnes et des biens dans l'espace CEDEAO et (iii) identifier des mesures d'intégration de la transhumance transnationale dans cet espace. Elle est basée sur une revue critique des politiques coloniales et post-coloniales en matière d'élevage. Ceci est complété par une étude de cas dans un échantillon de trois pays dont le Burkina Faso, représentant les pays de départ d'une part et d'autre part le Ghana et Togo comme pays d'accueil des transhumants. Les résultats révèlent que les politiques coloniales française et anglaise, puis post-coloniales, essentiellement basées sur les transformations technico-économiques, n'ont pas totalement
réussi à sédentariser l'élevage transhumant. Les mouvements des animaux des pays de départ (Burkina Faso) vers les pays d'accueil (Togo et Ghana) ne sont plus en réalité des transhumances

Mots clés : Transhumance, politiques publiques, politique d'élevage, CEDEAO,

Summary

Livestock production and agriculture are the two main human activities in the Economic Community of West African States (ECOWAS) region. But the disparity in pastoral resources between sahelian and coastal countries accentuates livestock movements within the ECOWAS region and often causes socioeconomic conflicts between transhumance pastoralists and people of the receiving countries. The present study aims at (i) contributing to a better understanding of cross border transhumance in West Africa, (ii) putting an emphasis on that form of livestock production in relation with the free movement of people and goods in ECOWAS region and (iii) identifying transnational transhumance integration measures in the ECOWAS region. This study is based on a critical review of colonial and post colonial policies in terms of livestock production. This is completed by a case study in a sampling of three countries of which Burkina Faso, representing departure countries on one hand and on the other hand Ghana and Togo as receiving countries for pastoralists. The results reveal that French and English colonial, then post-colonial policies, essentially based on technico-economic development, did not completely succeed in keeping to one place the pastoral livestock breeding. Livestock movements from departure countries (Burkina Faso) toward receiving countries (Togo and Ghana) are no more in reality a seasonal transhumance, but a migrationevasion. The social acceptance of pastoralists in the receiving zones seems to be the consequence of a social anthropological and local politics factors than a technico-economic development. Therefore there is a need to amend livestock production and public policies such as land-use policies in order to harmonize them at the level of ECOWAS region. ECOWAS should further involve itself in the definition of regional public policies and promote the convergence of national policies in terms of livestock production and management of natural resources.

Key words: Transhumance, public policies, livestock production policy, ECOWAS.

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Introduction

L'agriculture et l'élevage sont les deux principales activités humaines de l'espace de la Communautés Economique des Etats d'Afrique de l'Ouest (CEDEAO). Elles occupent 60% des 242 millions d'habitants de l'espace (CEDEAO, 2004), pour un disponible en terres arables d'environ 236 millions d'hectares, la Mauritanie y comprise. Mais, seulement 55 millions d'hectares sont mises en valeur, avec une moyenne de 2 hectares par habitant. Les moyens de production restent rudimentaires ; ce qui a pour conséquence la faible productivité en terme de rendement à l'hectare et par actif agricole. D'après les données statistiques agrégées par la CEDEAO (2004), les populations animales étaient estimées en 2001 à environ 47 millions de bovins, 58 millions d'ovins ; 68 millions de caprins ; 3,4 millions d'ânes ; 2,1 millions de chameaux ; 1 million de chevaux ; 8 millions de porcins ; 360 millions de poules traditionnelles et améliorées et enfin 265 000 canards. L'élevage de ruminants (bovins, ovins, caprins) est donc prédominant dans l'espace. Il est généralement pratiqué de manière extensive sur environ 170,4 millions d'hectares.

Cependant, la dégradation des parcours fortement soumis au surpâturage, à la variation climatique et l'inadéquation des techniques d'élevage et agricole a entamé environ 80% des pâturages dans les pays sahéliens. En revanche, les pays côtiers disposent encore d'une relative abondance en ressources pastorales. Cette modification de la distribution des ressources pastorales occasionne des mouvements de populations d'éleveurs et de leur bétail vers les pays disposant encore de pâturage. Ces mouvements concernent particulièrement les Fulbè semi-nomades. Dans leurs pérégrinations, ils sont souvent accusés d'être à l'origine de dommage causés aux ressources physiques. Par ailleurs, la rencontre des cultures nomades et sédentaires engendre également des conflits sociaux à différents niveaux de la vie sociale économique et politique. C'est ainsi que l'on enregistre des conflits socioéconomiques entre les pasteurs et les communautés d'accueil ; entre les pasteurs eux-mêmes ou encore entre les pasteurs et d'autres groupes nomades que sont les pêcheurs et les charbonniers. Il n'est pas aussi rare que des conflits éclatent entre les administrations des différents Etats de l'espace CEDEAO dont le principe fondateur est pourtant « la libre circulation des personnes et des biens ».

La présente investigation porte sur l'élevage pastoral, un mode de vie et de production animale fondée sur l'exploitation itinérante des ressources naturelles (eau, pâturages, saline etc.). Elle vise à (i) contribuer à une meilleure compréhension de la transhumance transnationale en Afrique de l'Ouest, (ii) mettre en perspective cette forme de pratique d'élevage en relation avec la libre circulation des personnes et des biens dans l'espace CEDEAO et (iii) identifier des mesures d'intégration de la transhumance transnationale dans cet espace.

Approches méthodologiques

Pour mieux cerner les contours socio anthropologiques et politiques de la transhumance transnationale, il est important de recourir à une combinaison d'approches méthodologies. Ainsi, une revue critique de la littérature et des données secondaires a permis d'évaluer le degré d'intégration des dimensions socio anthropologiques et politiques dans les connaissances sur la problématique de la transhumance transnationale dans l'espace CEDEAO. Ensuite, un échantillon de trois pays (Burkina Faso, Ghana et Togo) a été tiré pour une investigation approfondie du sujet avec toutes les parties prenantes. Ces pays sont représentatifs des mouvements de bétail regroupés en quatre axes principaux empruntés par les pasteurs transhumants au sein de la CEDEAO (figure 1). Il est qualifié d'axe central de la transhumance transnationale et se compose du Bénin, Burkina Faso, Cote d'ivoire, Ghana, Mali, Niger et le Togo. Dans cet axe, le Burkina Faso représente un des pays de départ des transhumants transnationaux (zone aride à subaride), tandis que le Ghana et le Togo sont représentatifs des pays d'accueil (zone humide à subhumide). Dans chacun de ces pays, des entretiens de groupes et individuels ont été conduits.

Des outils et méthodes de recherche historique, sociologique et anthropologique ont été utilisés. Ces entretiens ont permis de collecter auprès des services publics, des pasteurs transhumants et des populations des zones d'accueil, des informations sur la transhumance et les questions qu'elles posent dans un contexte de l'État nation et d'intégration régionale. Enfin, les résultats des différentes réunions de groupe de travail tenu à Abuja, Lomé, Ouagadougou entre 1998 et 2004 sur le sujet de la transhumance ont été exploités. L'étude s'est déroulée entre 1998 et 2004.

Résultats

Les résultats obtenus révèlent entre autres, une diversité régionale mal valorisée dans les politiques d'élevage, une persistance de la transhumance comme mode de vie des Fulbè et le relatif échec des politiques coloniales de sédentarisation, des conflits liés à la transhumance et de déficits informels.

Les diversités dans l'espace CEDEAO

La CEDEAO a été instituée en 1978 avec 16 Etats. Elle compte actuellement 15 Etats, après le retrait de la Mauritanie. Elle couvre une superficie de plus de 5 millions de

km² (Groupe Jeune Afrique, 1993). La majorité des États membres de la CEDEAO ont accédé à l'indépendance au cours de la décennie 1960, à l'exception du Ghana en 1956, de la Guinée en 1958 et enfin le Liberia en 1847 (Coquery-Vidrovitch, 1999). Cet espace est caractérisé par une forte diversité linguistique en terme de langues locales que celles adoptées du colonisateur. Ainsi, outre le français, l'anglais et le portugais hérité de la colonisation, chaque pays constitue une richesse linguistique variable. Cette diversité linguistique est tributaire de celle ethnique et socioculturelle. Enfin, les modes de vie et de production tributaires des ressources naturelles sont la synthèse d'une riche diversité sociale, ethnique, culturelle et politique endogène.

Sur le plan agro climatique, la CEDEAO est caractérisé par un gradient pluviométrique inférieur à 400 mm au Nord, et supérieur à 3000 mm par an dans certaines zones du golfe de Guinée (CEDEAO, 2004). On rencontre du Nord au Sud, une zone aride (≤500 mm), semi-aride (500-1000 mm), subhumide (1000-1500 mm), humide (1 500-2000 mm) et forestière (≥ 2 000 mm). Ces caractéristiques écoclimatiques basées sur la pluviométrie ont été élaborées successivement par Chevallier (1933), Aubervilliers (1949) et Keay (1959). Outre la variation du gradient pluviométrique, la CEDEAO est également caractérisée par une variabilité des jours de croissance (jc) de la végétation (Janhke, 1984) : zone aride (≤ 90 jc), semi-aride (90-180 jc), subhumide (180-270 jc) et, zone humide et forestière (270-365 jc).

Développer des nouvelles approches

Il est important de noter que les pays membres de la CEDEAO ont également expérimenté diverses approches de développement économique en général et du secteur de l'élevage en particulier. Ainsi, les puissances coloniales ont diversement appliqué des politiques de transformations technico-économiques visant l'agriculture et l'élevage. Pour ce dernier, l'imposition du modèle de sédentarisation par les autorités coloniales visait surtout le contrôle des populations humaines et animales avant de s'attaquer au problème de développement proprement dit. Sur le prémisse qu'il est difficile d'introduire des innovations (cultures fourragères, suivi zootechnique, amélioration génétique du cheptel) si les acteurs ne sont pas fixés, des centres de recherches zootechniques ont été créés (Landais, 1990 ; Bocco, 1990). Des éleveurs ont été installés autour de ces centres pour faciliter le transfert des résultats de recherche. La mise en œuvre de la politique de sédentarisation diffère toutefois entre les français et les anglais. Alors que les premiers privilégiaient la création de points d'eau permanents (Merlin, 1991) et la vaccinations contre les épizooties (Landais, 1990), les seconds mettaient l'accent sur le développement des ranchs. Les politiques françaises ont évolué à partir de 1950 pour inclure les aménagements pastoraux (Touré, 1991).

La CEDEAO est donc un espace de diversités d'origine endogène et exogène. L'élevage pastoral dans cet espace est le plus touché par ces diversités de part sa grande mobilité. Cette mobilité véhicule des pratiques, des politiques et des modes de vie différents d'un pays à un autre pouvant engendrer des conflits d'intérêt entre les utilisateurs des ressources naturelles. L'importance des enjeux national et communautaire de la transhumance exige de replacer l'élevage dans ses contextes évolutifs anthropologique, politique et socioculturel. L'utilisation de nouvelles approches méthodologiques serait d'une contribution importante à la compréhension de la transhumance transnationale que certains auteurs qualifient souvent d'invasion zébus ou Fulbè (Bernardet, 1984 ; Arditi, 1990).

Pourquoi l'élevage pastoral persiste-t-il ?

Les systèmes d'élevage pastoral sont divers et représentent des alternatives d'exploitation des ressources rares dans un environnement ou les ressources pastorales sont variables tant du point de vue de la quantité que de la qualité. Ces systèmes sont prédominants dans le contexte sahélien. Ces pratiques productives mettent en rapport, l'homme, l'animal et le milieu physique. Dans ce «triptyque» géré pour les besoins monétaire, social et alimentaire de l'homme, l'animal est à la fois moyen et instrument de production, tandis que le milieu physique en est le support. Les comportements sociaux et spatiaux et la mobilité des pasteurs constituent des réponses conjoncturelles aux nombreux facteurs environnementaux, sociaux et culturels. Bonfliglioli (1991) les regroupe en facteurs d'ordre économique, écologique et social. Autant les facteurs sont divers, autant les réponses le sont. Ainsi, les pasteurs, éleveurs de bovins et de petits ruminants composés surtout de Fulbè, pratiquent la transhumance nationale ou transnationale qui peut aboutir souvent à une migration-fuite définitive. Plusieurs éleveurs des zones sahéliennes ont fui leurs zones après les sécheresses de la décennie 1970 et 1980 dont les origines remontent aux années 1969 (Piguet, 1998). En revanche, les éleveurs de camélidés (chameaux, dromadaires) pratiquent le nomadisme pastoral même si cette pratique productive devient de plus en plus rare du fait de la sédentarisation des peuples Touaregs qui sont concernés par ce type de production.

Historiquement deux types d'élevage pastoral ont coexistés : nomade, transhumant ou semi-nomade. Mais, la majorité des tribus nomades a tendance a une quasi-sédentarisation comme le note Bernardet (1984) : « Jusqu'au 16ème siècle, la société peule peut encore être considérée comme étant composée de pasteurs nomades qui

migraient pour les raisons suivantes : recherche de l'eau et de l'herbe, contraintes politiques, apparition des premiers empires liés au développement du commerce avec le monde Arabe et à l'Islamisation, transformant ainsi les transhumances en de véritable migration ». Avec la délimitation définitive des territoires étatiques, les nomades sont confrontés à des problèmes d'identité territoriale. En revanche, le pastoralisme transhumant ou semi-nomadisme persiste à travers des va et vient à l'intérieur des Etats et entre des Etats voisins. Cette forme de production animale à moindre coût, est fondée sur l'alternance de la saison des pluies et la saison sèche et elle vise à exploiter alternativement des pâturages et des points d'eau (Touré, 1990). Dans certains pays, les pasteurs transhumant sont tellement influencés par les facteurs sociaux qu'ils deviennent agro pasteurs. Au Burkina Faso, plusieurs Fulbè fortement intégrés au sein des communautés Bobo-Bwa et Bobo-Mandarè produisent parfois plus de coton que ces derniers (Somda *et al*, 2006).

Comment l'élevage transhumant a-t-il résisté face aux politiques coloniales et post-coloniales ?

Les politiques de sédentarisation conduites par les puissances coloniales et poursuivies après l'indépendance ont visé à assurer un contrôle par les pouvoirs publics des biens publics et privés. Si les ressources pastorales pouvaient à l'époque être considérés comme des biens publics dont l'accès n'était soumis à aucune appropriation individuelle, il n'en était pas de même pour le bétail. Le succès de telle politique publique dans des économies non encore structurées était déjà voué à l'échec. Les différentes mesures appliquées (construction de points d'eau permanent, pratique de vaccination ou développement des ranchs, etc.) n'ont pas convaincu les pasteurs de leur meilleure efficacité. La vision techniciste a été prédominante dans l'élaboration des politiques d'élevage tant coloniale que post-coloniale. Si le déplacement des pasteurs était seulement lié à la disponibilité en ressources pastorales et hydriques, alors ces politiques auraient trouvé du répondant. Hélas, des facteurs sociaux, culturels et même économiques sont autant de mobiles de transhumance pour les pasteurs, constitués essentiellement de Fulbè.

Les Fulbè sont des peuples en mouvements, des pasteurs nomades, semi-nomade mais qui peuvent aussi se sédentariser. Le processus de migration-fuite de l'Est africain des Fulbè a commencé au 8ème siècle après Jésus-Christ (Doutressoule 1947). On voit les Peuls reprendre un mouvement contraire qui les avait amenés dans le Fouta-Toro. Ils remontent vers l'Est jusqu'au pays Haoussa, à l'Adamaoua, au Tchad et au Ouadaï au 19ème siècle. Ils arrivent successivement au pays de Nioro au 13ème siècle, au Massina au 15ème siècle, au pays de Khasso fin du 17ème siècle, au Fouta-Djallon et au Ouassoulu au 18ème siècle». D'autres migrations de Peuls sont rapportées dans le Nord-Nigeria dans l'Etat actuel de Kano dans la même période que leur avènement dans le pays de Nioro au Soudan occidental, (Mali actuel). Ces migrations n'étaient pas seulement à la recherche des pâturages qui ont une implication économique pour eux et leur famille. Il y a eu certainement d'autres enjeux tels que politique et religieux à partir de leur conversion à la religion musulmane.

Les efforts consentis par les puissances coloniales dans la recherche des connaissances de ce groupe sociale témoignent également d'un intérêt particulier de rente économique. Les enjeux coloniaux étaient claires, le contrôle de ces communautés pour lever l'impôt qui serait réinvesti dans le développement des territoires que ce soit dans les colonies anglaise ou française. Outre l'impôt, les animaux étaient aussi achetés à bas prix pour l'effort de guerre. Ces prélèvements publics sur des biens privés que constitue le bétail ne pouvaient que faire échouer les politiques de sédentarisation. Si les impôts peuvent être justifiés sur le plan économique, il reste que sa raison sociale n'est pas toujours considérée dans son application. Malgré la suppression des impôts sur le bétail dans certains pays de la CEDEAO, la crainte d'un retour n'est pas visiblement dissipée au niveau des pasteurs.

Enfin, la politique territoriale coloniale et post-coloniale apparaît comme une antinomie à la politique territoriale des pasteurs. A la politique de libre circulation des personnes et du bétail chez les pasteurs s'est superposée une politique de contention du bétail et son propriétaire dans un territoire délimité : la sédentarisation des pasteurs. L'élevage pastoral a résisté à cette vision territoriale coloniale et post-coloniale pour deux raisons majeures. D'abord, les politiques en faveur du développement de l'élevage ont depuis toujours été très fébriles dans l'ensemble des pays de la CEDEAO, notamment en matière d'investissement publics (Kamuanga *et al*, 2007). Les instruments mis en œuvre n'ont pu soutenir de façon conséquente les transformations sociotechniques prônées par la politique de sédentarisation. Ensuite, les pasteurs sont dotés d'une excellente capacité d'adaptation tant aux changements climatiques que politiques. Par exemple, dans des territoires fermés ou la pratique de la transhumance n'est pas possible, les pasteurs peuvent se séparer temporairement de leur bétail et se convertir en agriculteur. Le bétail est ainsi localisé dans un territoire différent du propriétaire. Ce qui rend encore plus complexe le contrôle de l'élevage transhumant et de ses effets par les pouvoirs publics.

Enfin, la majorité des pays disposent de législation foncière qui reconnaît l'existence de trois grands domaines : domaine de cultures, les domaine pastoral et domaine forestier. Mais, la propriété foncière varie d'un pays à l'autre.

Au Ghana, la terre appartient aux communautés à la base qui sont sous l'autorité morale de Chefs traditionnels. Au Togo, il y a trois types de domaines (domaine public, les terres incultes relevant du domaine public, les terres détenues par les communautés à la base selon les prescriptions coutumières). Au Burkina Faso, la terre appartient à l'État, mais l'accord des autorités coutumières appropriées est recommandé pour toute aliénation de la terre. Cette diversité de politique foncière laisse de l'espace à l'élevage pastoral, et aux conflits entre transhumants et communautés dans les zones d'accueil.

Les Fulbè : conquérants d'espace ou pasteurs stratégiques dans les pays voisins

Sur les terrains politiques et de la religion, pendant la période anté-coloniale, les Fulbè ont réussi à créer des États en dominant les communautés agraires dans plusieurs régions de l'Afrique de l'Ouest et imposé aux communautés préétablis la religion musulmane. Ainsi, le royaume du Fouta-Djalon a été constitué vers la fin du 17ème siècle au début du 18ème siècle (Diallo, 1972). Sous l'influence de ces premiers succès, sont nés successivement sur la rive droite du fleuve Sénégal, le royaume du Fouta Toro et au cœur du Soudan occidental (actuel Mali) avec des ramifications sur le territoire burkinabè, (djelgodji), l'Etat Théocratique du Massina (Ba, 1963 ; Sanankoua, 1990).

En dehors du Soudan Occidental, dans la partie orientale, les Fulbè « jiihaadiste » ont également réussi à soumettre les populations nigérianes animistes à l'Islam. Les troupes d'Ousman Dan Fodio ont même dépassé la zone sahélienne, le Nord Nigeria pour atteindre le pays Yorobu : « Au Sud, les Peuls gagnèrent du terrain sur les Yoruba en créant l'émirat d'Ilorin et en les repoussant vers la forêt méridionale ; le jiihad fut stoppé moins par les hommes que par la mouche tsé-tsé qui s'attaquait aux chevaux » (Coquery-Vidrovitch, 1990). Les périodes de conquêtes terminées, les Peuls n'ont plus que les activités d'élevage pour justifier en partie leur migration ou transhumance.

Leur distribution actuelle en Afrique de l'Ouest (Stenning, 1951 ; Dupire, 1981 ; Boutrais, 1988 ; Botte *et al.*, 1994) suggère des raisons de production et de développement économique. En effet, les mouvements de fuite se sont accentués avec l'avènement de la sécheresse du début des années 1969 qui ont frappé l'Afrique (Piguet, 1998). Toute la partie septentrionale des pays du Golfe de Guinée a été prise d'assaut par les Fulbè en provenance du Mali et du Burkina Faso (Bernardet, 1984). Les destinations préférées ont été d'abord la Côte d'Ivoire, puis progressivement le Togo et le Ghana. La pratique du pastoralisme est pour les peuples pasteurs une manière d'éviter également le travail épuisant que représente l'exhaure de l'eau d'abreuvement du bétail, autrefois dévolu aux Maccubè, les esclaves en fulfuldé. L'abolition de l'esclavage apparaît aussi comme un facteur aggravant la pratique de la transhumance.

Ce déferlement d'éleveurs transhumants avec d'importants troupeaux vers le Sud a engendré une forte compétition pour l'exploitation des ressources provoquant du coup des heurts entre les pasteurs d'une part et les cultivateurs autochtones d'autre part. Les tensions sociales entre les populations agraires et les populations pastorales se sont alors accrues à l'intérieur de la majorité des pays sahéliens (Burkina Faso, Mali, Niger) puis à l'extérieur entre les pasteurs originaires des ces pays et les cultivateurs autochtones des pays de destination notamment ceux des pays côtiers. Certains pasteurs se sont sédentarisés de leur propre gré en diversifiant leurs activités économiques.

La transhumance, la migration-fuite et la libre circulation des biens et des personnes

Le développement socio-économique procède de l'échange entre nations, peuples et communautés. Cet échange implique une circulation aussi bien des personnes qui échangent que des biens qu'ils échangent. Sur la base de cette prémisse, la CEDEAO a établi depuis sa création le principe de libre circulation des biens et des personnes au sein de son espace. Appliqué à l'élevage transhumant, ce principe semble confronté à la réalité d'un secteur économique particulier. Les produits de ce secteur peuvent être des biens mobiles (le bétail) à la différence des autres secteurs économiques où les biens sont inertes. L'application stricte du principe de libre circulation dans l'espace CEDEAO semble alors caduque si toutes les parties prenantes n'ont pas la même compréhension de la transhumance, de la migration-fuite et de la libre circulation. Les études de cas réalisés au Burkina Faso, au Togo et au Ghana permettent d'élucider ces concepts.

Les informations collectées tant au niveau des pays de départ (Burkina Faso) que d'accueil (Togo et Ghana) indiquent que les mouvements des animaux ne sont plus en réalité des transhumances saisonnières. En effet, la majorité des éleveurs que l'on traite de transhumants au Togo ou au Ghana, y sont établis il y a plus de 20 à 30 ans. Ceux-ci effectuant des transhumances au départ se sont retrouvés alors dans une situation de migration-fuite de la réduction des terres à pâturages dans les pays sahéliens. Mais, ce changement de mode de vie pour devenir sédentaire en territoire autrefois d'accueil est un des motifs de conflits entre les communautés de base et les « transhumants » au Togo et au Ghana. Ces conflits sont également rapportés dans les zones de transit à l'intérieur du Burkina Faso. Même si les autochtones des zones d'accueil reconnaissant des aspects positifs dans ce mélange de communautés, en terme d'échange de connaissances en matière

d'élevage, l'insertion sociale des nouveaux installés n'est toujours sans accroc.

Les conflits inhérents à la transhumance ne constituent pourtant pas un motif de démotivation des communautés pasteurs de poursuivre cette pratique. De même, il n'empêche que des communautés dans les pays d'accueil permettent l'exploitation de leurs ressources naturelles par le bétail transhumant et d'en tirer quelques avantages. Un encadrement approprié de la transhumance transnational tant au niveau des Etats que de la CEDEAO est donc indispensable. La politique de décentralisation engagée dans la plupart des pays concernés par la transhumance peut servir de cadre pour la réduction des conflits et l'augmentation des avantages liés a la pratique de transhumance. Si ce type d'élevage doit survivre comme il l'a fait face aux politiques de sédentarisation mises en place dans les pays de départ, l'absence d'encadrement peut entraîner la déstructuration de la CEDEAO à travers la multiplication de conflits entre transhumants et autochtones. Il est impératif de développer des actons de soutien à la base qui faciliteraient l'insertion sociale des transhumants et leur acceptation par les communautés d'accueil. Mais, ceci requiert une bonne compréhension des causes et effets des conflits, et les modes actuels de résolution.

Les conflits de la transhumance transnationale : causes et modes de résolution

Les investigations ont permis d'établir une typologie des conflits entre les éleveurs transhumants, les agriculteurs sédentaires et certains pêcheurs semi-nomades. Un premier type de conflits porte sur des effets socio-économiques. Ils concernent les dégâts sur les cultures encore au champ, les récoltes détruites par le bétail et les vols de récoltes et/ou de animaux locaux. Un second type concerne les actes ayant des effets psychosociologiques et moraux. Il regroupe les vols et agressions à main armée, les viols de jeunes filles et des femmes, les assassinats, le non-respect des us et coutumes tel que la destruction des bois sacrés. Le troisième type de conflit concerne les actions dont les effets sont négatifs sur l'environnement. Dans ce groupe, on note la coupe abusive et mutilation des arbres, pollutions des eaux de surface, feux de brousse pour profiter de la repousse. Enfin, le quatrième groupe se manifeste sous forme de conflit polico-juridique. On y recense le non-respect des prescriptions et des lois nationales togolaises, l'absence de pièce d'identité et/ou de certificat international de transhumance CEDEAO, la fausse déclaration sur les effectifs réels d'animaux en transhumance, le refus de se déclarer chez les chefs de cantons ou de villages, l'inexpérience et insuffisance des bouviers/bergers qui conduisent les animaux dans les pâturages etc.

Les causes de ses conflits ont été analysées dans deux perspectives : les causes apparentes et sous-jacentes. Les causes apparentes sont surtout liées au conflit ayant des effets socio-économiques. Ainsi, les dégâts sur les cultures et les incursions dans les forêts et domaines classés ont été rapportés comme principales causes de conflits entre les transhumants d'une part et les communautés et l'administration des pays d'accueil d'autre part. Les transhumants relève que l'absence de piste de transhumance (au Ghana par exemple), l'obstruction des passages à bétail par les cultures et la pollution des eaux par les pêcheurs seraient à la base des dégâts causés sur les culture ou

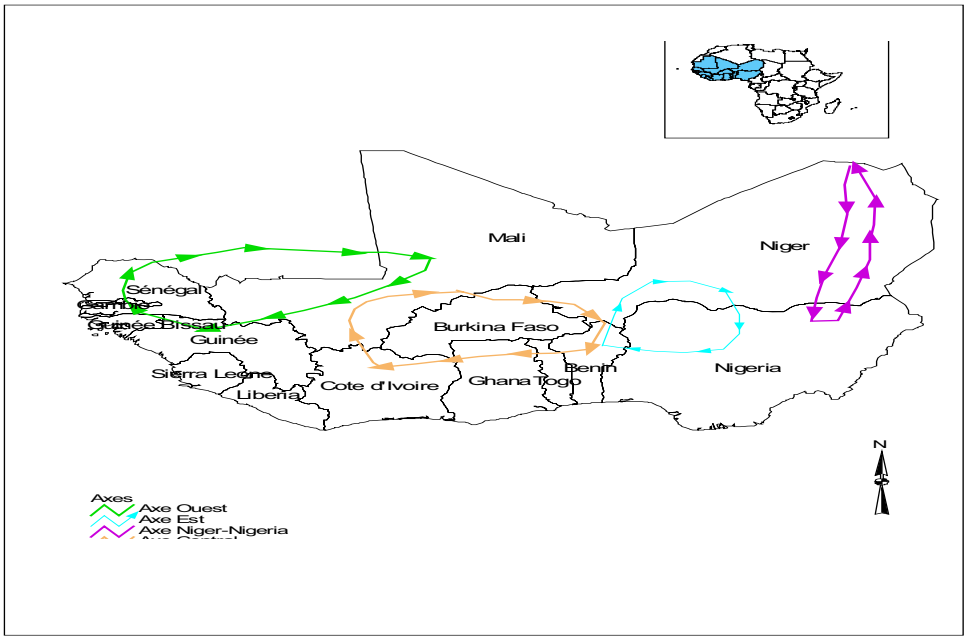

Figure 1 : Les principaux axes de transhumance transnationale dans la CEDEAO

l'incursion dans les domaines forestiers classés. Les causes sous-jacentes semblent être liées à l'absence de consensus social sur le mode de gestion des conflits. Il existe un climat de suspicion importante entre les parties prenantes qui ne favorisent pas le respect des règles d'utilisation des ressources naturelles. Par exemple, l'équipe d'évaluation des dégâts sur les cultures est soupçonnée d'être juge et partie, parce qu'elle est généralement dirigé par un agent de l'agriculture en l'absence d'un agent en charge de l'élevage.

Diverses modes de résolution des conflits ont été recensées au cours de cette étude. Trois groupes de mécanismes coexistent dans les pays visités : endogène, légal et réglementaire. Les informations collectées suggèrent que les mécanismes endogènes sont efficaces dans la résolution des conflits à effets socioéconomique tels que les dégâts sur les cultures. Par exemple, les pouvoirs publics du Togo ont fait faciliter la mise en place d'un dispositif dans lequel les autorités coutumières des localités touchées par la transhumance acceptent la désignation d'un représentant des éleveurs transhumants. Ce représentant joue le rôle de tampon entre les pasteurs et les populations autochtones et facilite les relations et la résolution des conflits sans l'intervention de l'administration judiciaire. Les conflits aux effets psychosociologiques, moraux et politico juridiques trouvent rarement des solutions satisfaisantes par les mécanismes endogènes. Il est généralement fait recours aux mécanismes judiciaires.

Enfin, il existe des dispositifs réglementaires nationaux et régionaux qui règlementent la pratique de la transhumance dans l'espace CEDEAO. Au Burkina, la loi d'orientation sur le pastoralisme précise les modalités des mouvements du bétail à l'intérieur tout comme à l'extérieur du territoire national. Au Togo, un arrêté organise cette pratique itinérante de l'élevage sans qu'il n'y ait des heurts entre les populations d'accueil et les transhumants généralement des fulbè. Au Ghana, ce sont les chefs traditionnels qui autorisent les pâturages des animaux étrangers. Au niveau régional, il existe des accords bilatéraux de réglementation de la transhumance transnationale. Mais, les pays sahéliens semblent plus prompts à la signature de tels accords. On note ainsi un accord entre le Burkina Faso et le Mali, et un autre entre le Niger et le Burkina Faso. La CEDEAO a réussi depuis 1998 à faire signé un accord qui fixe les principes d'une réglementation de la transhumance transnationale entre les pays membres. Il insiste sur la garde des animaux transhumants, leur accueil et le respect des législations nationales propres à chaque État. Cependant, les parties prenantes, en particulier les communautés à la base, ne semblent pas suffisamment informées de l'existence de ce document.

Conclusion et implications politiques

Les résultats de cette étude suggèrent que les problèmes de l'élevage pastoral sont congénitaux aux politiques de développement du secteur élevage héritées de l'administration coloniale. Ces politiques ont été orientées essentiellement sur la transformation technico-économique de l'élevage sans prendre en compte les dimensions socio-anthropologiques et les stratégies endogènes d'adaptation des communautés de pasteurs. Face à l'échec de la politique de sédentarisation de cet élevage, les pouvoirs publics se sont résolus à investir modestement dans ce secteur. Pourtant, la réalité indique que les pasteurs peuvent aussi se sédentariser, mais pas sous la pression des seuls facteurs technico-économiques. L'acceptation sociale des pasteurs dans la zone d'accueil semble être un facteur déterminant pour sa sédentarisation et son insertion dans la communauté. Cette acception est plus la résultante de facteurs sociaux, anthropologiques et politique locale que le fait d'une transformation technico-économique. Une certaine littérature évoque d'ailleurs la lente transformation du pastoralisme comme pour magnifier que ce mode d'élevage ne suit pas le rythme du progrès technologique et économique qualifié de rapide. En définitive, l'acceptation par tous du pastoralisme comme un mode de vie en même temps qu'un mode d'élevage contribuera à la réduction des conflits inhérents.

Les implications politiques se situent tant au niveau des Etats que de la CEDEAO. Au niveau des différents Etats, il y'a une nécessité de réviser les politiques d'élevage et publiques telles que les politiques foncières pour les harmoniser au niveau de l'espace. La CEDEAO devrait s'impliquer davantage dans la définition des politiques publiques régionales et promouvoir la convergence des politiques nationales en matière d'élevage et de gestion des ressources naturelles. Dans ce processus, les autres parties prenantes du secteur élevage et des ressources naturelles doivent être impliquées. La décentralisation constitue une opportunité pour les Etats et la CEDEAO pour la mise en place de processus participatif d'élaboration et d'application des politiques publiques. La participation des différentes parties prenantes favorise non seulement la circulation de l'information mais garantie une application socialement acceptable des réglementations arrêtées de commun accord. Il ne s'agit pas de recommencer les politiques en cours mais de permettre leur large diffusion afin d'intégrer les préoccupations des transhumants et des communautés des zones d'accueil.

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SHORT COMMUNICATION

INFLUENCE OF LEVAMISOLE ON ANTIBODY RESPONSE TO NEWCASTLE DISEASE VACCINATIONS IN CHEMICALLY IMMUNOSUPRESSED BROILER CHICKENS

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Levamisole hydrochloride, an imidazothiazole group, was initially introduced as an antihelmintic drug with effects against a variety of nematodes of chickens, sheep, cattle, pigs, horses, dogs and cats (Alexander, 2003). It has a paralyzing action on nematodes that is due to sustained muscle contraction and it also acts as a ganglion stimulant. Levamisole being a widely used veterinary antihelmintic, has been recognized to concurrently enhance immune responses (Jiangmei *et al*., 2006) in stressed, old or chronically ill animals (Mohri *et al*., 2005). Its use in human has also been described in asthmatic (Almeida *et al.,* 1999) and cancer patients (Stevenson *et al.,* 1991) as well as in children (Demirci *et al*.,2005).

Newcastle Disease (ND) is an acute infectious, highly contagious and fatal viral disease of avian species affecting the respiratory, nervous and digestive systems (Nwanta *et al*., 2008). Apart from commercial domestic poultry, a wide range of captive and free-ranging semi-domestic and free-living birds including migratory waterfowl are susceptible and can be a primary source of infection. ND poses serious economic challenge to all segments of the poultry industry. The persistence of the infection may be associated with the relative stability of the virus to adverse environmental conditions and its methods of spread especially by air (Alexander, 2003). Control has been mainly by vaccination but the disease remains a threat to poultry production in industrialized countries and the cause of substantial losses in developing countries (Aldous and Alexander, 2001).

One of the reasons adduced to poor response to vaccination is concurrent infection with immunosuppressive agents e.g. infectious bursal disease virus and chicken infectious anaemia virus as well as stress associated immunosuppressive factors such as harsh environmental conditions and poor quality feed ingredients common to the tropical developing countries. It was therefore considered that levamisole being a recognized immunostimulant (Giambrone and Klesius, 1985), could enhance antibody response to vaccination in immunocompromised poultry birds. Earlier studies have shown non-significant increase in Newcastle disease virus (NDV) antibody response when levamisole was administered to healthy birds (Porchezhian and Punniamurthy, 2006; Sanda *et al*., 2008). This study was carried out to assess clinically the effect of levamisole on ND vaccinal antibody response in immunosuppressed broilers using hydrocortisone as an immunosuppressing agent.

Sixty (60) Arbor acres broilers purchased at day-old were reared at the Teaching and Research Farm of the University of Ibadan, Ibadan, Nigeria for a period of eight weeks. The broilers were vaccinated with Newcastle disease vaccine (NDV) Hitchner B1 strain intraocularly at day-old before they were subsequently grouped into four in separate pens i.e. groups H, L, HL and C at 10 day-old. Broilers in groups H and HL were administered Hydrocortisone sodium succinate (LABOHYDROR) at a dose of 10mk/kg body weight at 14 days of age. Groups L and HL were administered levamisole at 16 days of age at a dose of 15mg/kg body weight while broilers in group C were not medicated and served as the control group. All the groups were administered NDV LaSota strain on day sixteen. The experiment was approved by the ethical committee of our faculty

Prior to separation into groups, ten broilers were randomly selected and bled via jugular venipuncture into plain McCartney bottles at 5 and 9 days old. Blood was allowed to clot; serum was harvested into eppendorf tubes and immediately subjected to Newcastle disease (ND) haemagglutination inhibition (HI) test. After the broilers were separated into groups, ten randomly selected members of each group were also bled at 14, 21, 28, 35 and 42 days old, serum was harvested and similarly subjected to ND HI test.

The β procedure of HI test was used as described by Thayer and Beard (1998). Fifty µl of 4 haemagglutinating (HA) units of ND antigen was dispensed into all the wells in a 96-well U-bottom microtiter plate using 2 rows per serum sample. Serum samples (50 µl) were dispensed into the first wells in each row of wells and mixed. Fifty µl was passed on into the next well and so on, resulting in dilutions of 1:2, 1:4, 1:8, etc after which 50µl from the last well was discarded. Fifty µl of 0.5% washed chicken red blood cell was dispensed into each well and the plates

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were shaken on the microshaker for 3 seconds. The microtiter plates were left at room temperature for 30 minutes before the results were read. ND lyophilized vaccine (LaSota) from the National Veterinary Research Institute (NVRI) Vom, Nigeria, was used as the viral antigen. HI titer for each sample was noted and the geometric mean titer (GMT) for each group per sampling time was calculated. Mean ND haemagglutination inhibition titers in the groups of broilers were compared for significant difference using the least significant difference (LSD) method of multiple comparison.

An increase in GMT was observed in the broilers from 2.9 at 5 day-old to 3.2 at 9 day-old. Thereafter, there was a general decline in ND antibody titers in all the four groups up till 21 day-old. From 28 day-old, an increase in GMTs was observed particularly in the hydrocortisone, levamisole and control groups up till 42 days of age. The decline in GMT observed in the hydrocortisone-levamisole group from 14 day-old (3.5) extended up till 35 day-old (1.0) and thereafter showed a sharp rise to 3.0 at 42 day-old.

In general, GMTs in the levamisole group were significantly higher ($p<0.05$) than those of hydrocortisone and control groups. Hydrocortisone-levamisole group had non-significantly higher (p.0.05) titers than the levamisole group at14 to 21 day-old but had significantly lower titers ($p<0.05$) at 28 and 35 days old. However, the hydrocortisone-levamisole group ended up with a higher titer of 3.0 than 2.25 obtained for the levamisole group. This study has been able to assess the effect of levamisole on ND vaccinal immune response in chemically immunosuppressed broiler. Following the administration of ND vaccine to all the broilers intraocularly at day old, they all sero-converted and the antibody titre increased up till 9 day-old. By 14 day-old a decline was already being observed. This shows that the conventional administration of ND vaccine LaSota strain at 3 weeks of age might leave a window period during which birds could be exposed to ND virus infection.

A consistent decrease in ND antibody titer was observed in all groups from 9 day-old reaching a minimum at 21 day-old except in the hydrocortisone-levamisole group whose decrease in titer was observed after 14 days of age reaching a minimum of 1.0 at 28 and 35 day-old. The decrease in antibody up to a non-detectable level by HI test at 21 day-old in the hydrocortisone group shows that the hydrocortisone administered at 14 day-old indeed resulted in immunosupression which the group was able to recover from by 28 day-old. The mechanism of its action has been suggested to be as a result of induction of lymphopeania with a selective depletion of T-cells from circulating blood (Sukhinina *et al*., 1990; Alimkhodzhaeva *et al*., 2002). The explanation for the modification of circulating lymphocytes is not yet clear but pharmacological concentration reduced lymphocytes responses in chemotactic tests. It has been recognized that interference with T-cell recirculation diminishes the probability of lymphocyte:antigen interaction (Beck and Browning, 1983).

The increased antibody titer observed from 28 day-old in the hydrocortisone, levamisole and control groups as well as at 42 day-old in the hydrocorticone-levamisole group is an evidence of the broilers' responsiveness to the ND (LaSota) vaccine administered at 16 day-old. The generally higher antibody response observed in the levamisole group than in the hydrocortisone and control groups shows the enhancing effect of levamisole in non-immunocompromised broilers. This observation is in agreement with reports by other workers (Mohan *et al*., 1987; Singh and Dhawedkar, 1993) while it is in sharp contrast with the report of Sanda *et al*.,(2008) who reported no significant stimulation by levamisole. However, this stimulating effect was more marked in broilers in the hydrocortisone-levamisole group which were earlier immunosuppressed by hydrocortisone before the administration of levamisole. Thus, agreeing with previous reports that levamisole enhances immune response in stressed, old or chronically ill animals (Mohri *et al*., 2005). It is noteworthy that at 42 day-old when the experiment was terminated, the HL group had the highest ND antibody titer. Levamisole as a known immunostimulator acts by the restoration of cell-mediated immune function in the peripheral T-lymphocytes and stimulation of phagocytosis by monocytes (Renoux, 1980). It also has the ability to increase the

H Control	L	H+L	Control
2.9	2.9	2.9	2.9
3.2	3.2	3.2	3.2
1.5	3	1.5	3.5
0	1	2	0.5
1	1.d	1	1
1.25	1.d	1	2
1.5	2.25	3	1.d

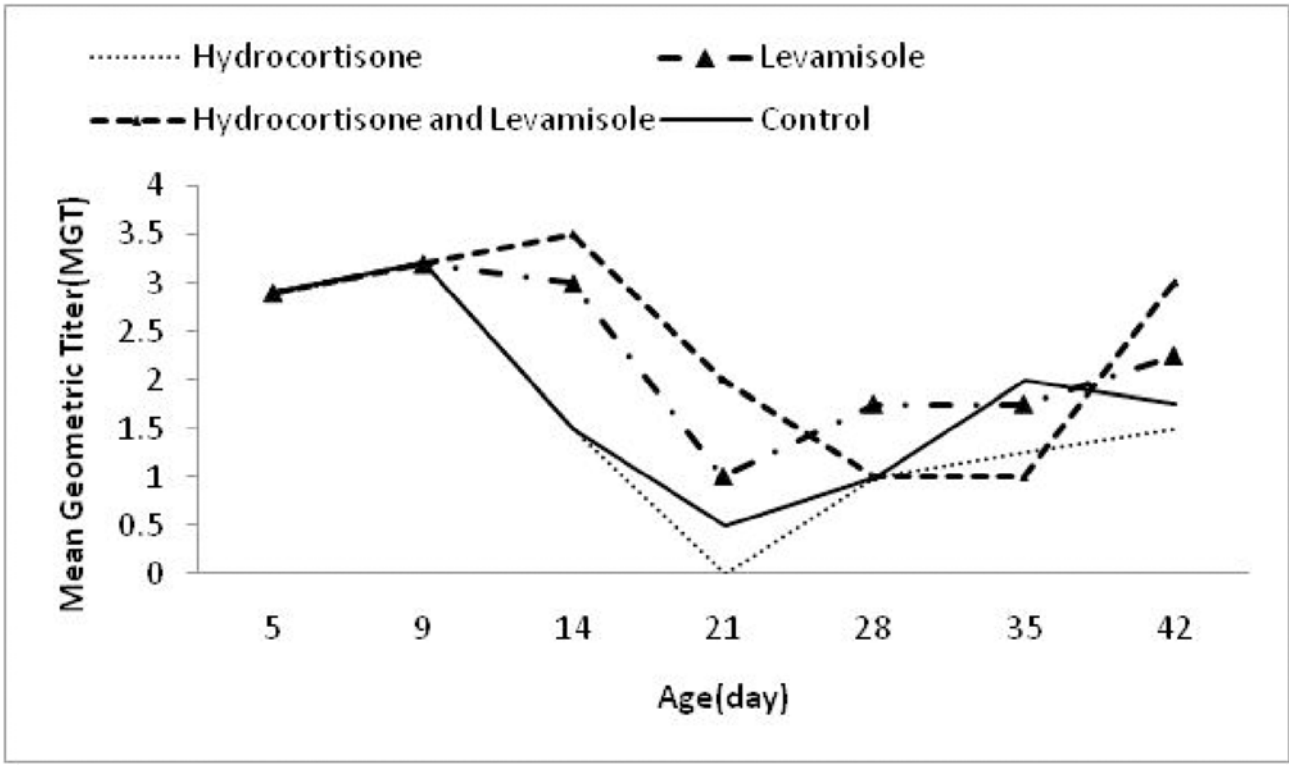

Figure 1: Newcastle disease vaccinal antibody response in broilers administered *hydrocortisone* / and or levamisole

level of T-cells in circulating blood by stimulating precursor T-lymphocytes to differentiate into mature T-cells (Symoens and Rosenthal, 1977). This has been properly elucidated in the control of coccidiosis in chicken (Soppi et al., 1979; Onaga et al., 1984; Giambrone and Klsius, 1985) and in asthmatic (Almeida *et al*., 1999) and cancer patients (Pavlovsky *et al*. 1981; Stevenson *et al*. 1991). Also, one of the major subsets of T-lymphocytes, the T-helper cells (CD4+) are necessary for B-cell differentiation (Hirota et al. 1981) with direct effect on antibody production.

Conclusion

The study has revealed that the immunosuppressive effect of hydrocortisone when combined with levamisole was mild. Thus, levamisole has the ability to reverse the immunosuppresive effect of hydrocortisone and possibly that of pathogens like infectious bursal disease and chickens infectious anaemia viruses as well as harsh environmental conditions and poor nutrition. It is therefore established that levamisole could be used as an immunostimulant for immunocompromised broilers.

Impact

This study highlight the fact that levamisole is an immunomodulator and it can be useful in reverting the immunosuppresive effect of agents like infectious bursal disease and chickens infectious anaemia viruses as well as harsh environmental conditions and poor nutrition.

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SHORT COMMUNICATION

PRELIMINARY STUDY ON BOVINE TUBERCULOSIS IN NEKEMTE MUNICIPALITY ABATTOIR, WESTERN ETHIOPIA

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Introduction

Bovine tuberculosis (TB) is an infectious disease of cattle characterized by the formation of tubercle in any tissue or organ of the animal. According to Cosivi *et al*. (1998), 60 % of the African 47% of Asian, and 38% of the American and Caribbean countries have reported the occurrence of bovine TB from sporadic to enzootic level. Thus, bovine TB is one of the devastating diseases of cattle in developing countries throughout the world (Berrada and Barajas, 1995). Bovine TB has severe implication for animal welfare and animal health since it causes reduced productivity and premature death in cattle and affected farms suffer severe economic losses (Zinsstag *et al*., 2006). Studies have reported that the prevalence of bovine TB ranging from 3.4% in small holder production system that keeps zebu cattle to 50% in per-urban (intensive) dairy production system in Ethiopia (Ameni and Roger, 1998; Ameni *et al*., 2001; Laval and Ameni, 2004; Ameni *et al*., 2007; Regassa *et al*., 2007; Ameni *et al*., 2008; Demelash *et al*., 2008). However, the effects of bovine TB on reproduction and productivity have not yet been studied in Ethiopia so far. Clinical tuberculosis in cattle is typically debilitating illness characterized by progressive emaciation and development of tubercle in any tissue (Radostits *et al*., 1994). Such excessive loss of body condition could lead to reduction in milk production and reduced reproductive efficiency, which can be estimated by measuring different reproductive and productivity variables including age at puberty, age at 1st calving, number of service per conception, calving interval, milk yield, milking days etc. Thus, the objective of this study is to investigate the effects of bovine TB on the reproductive efficiency and productivity of *Holstein* dairy cows.

Material and Methods

Study farm

The Farm which served as a source of data was first established in 1955 and started with 120 in-calf Holstein heifers, which had been imported from the United States. In addition, some pure Holstein heifers were introduced from Kenya in 1959. After about 20 years, an additional 120 in-calf Holstein heifers were donated by the Government of Cuba in 1980 and formed the nucleus of the present Farm. The Farm is located 43km west of Addis Ababa at Holetta Town on the main road to western Ethiopia. Because of the occurrence of clinical signs suggestive of bovine TB (e.g. respiratory distress, coughing, weight loss, emaciation) and post mortem lesions in animals that had died, the herd was tuberculin skin tested in 2002. On the basis of the result of this test the Farm was divided into positive and negative herds, which were physically separated but kept under a similar cattle husbandry and management.

Study animals and data collection

A total of 100 pure Holstein cows, 50 bovine TB positive and 50 negative, were used for this study. The reproductive records including age at puberty, age at first calving, number of service per conception and calving interval were analyzed. In addition, the number milking days and milk yield were analyzed. Both positive and negative cows belong to a single farm. But they were kept physically separate while they were managed uniformly under similar management practices. All records of each of the cow including date of birth, calving date and the first date of service were kept and thus used to calculate the age at puberty, the age of 1st calving, and calving interval. The 1st service date for the first lactation was considered as the age at puberty of the animal.

Data analysis

Means (Mean±SEM) were calculated for all reproductive parameters. Student t test was used to compare the means of the measured variables of negative and positive cows. Statistical significance was set at a P-value of <0 .05.

Result

The analyzed comparative reproductive variables for positive and negative cows were presented in Table 1. The mean age at puberty (26.36± 0.93 vs. 28.26±0.93) and mean age at 1st calving (36.09±0.94 vs. 36.23± 1.37) did not differ between negative and positive cows. Similarly, the means of number of service per conception for the first calving did not differ between positive (1.62±0.14) and negative (1.56±0.15) cows. Nevertheless, although not statistically significant, the number of service per concep-

tion for the first calving was slightly higher in positive cows as compared to negative cows. Further as the number of calving increases, the difference in means of number of services per conception between the positive (2.02±0.18) and negative (1.25±0.16) cows was evident (P<0.05). Although the mean milk yield did not differ between the positive and negative cows, the mean milking days did show difference across the different calving (Table 1). From Table 1, it can be extracted that milk production was reduced by 10%, 13%, and 5% during 1st, 2nd, and 3rd calving, respectively in TB positive cows when compared with that of the negative cows.

Discussion

A comparative assessment of the variables of reproduction and productivity was made in bovine TB positive and negative Holstein cows to estimate the effects of bovine TB on the reproductive efficiency of dairy cows. The result showed that there was no difference between positive and negative cow in reaching age at puberty and age at 1st calving. The reason could be the fact that bovine TB is affected by age of the animals i.e. young animals are less likely to be infected and the risk of infection increases as the age of the animal increases. Furthermore, as bovine TB is a chronic disease, even if younger animals are skin test positive (infected), as the disease is at its earliest stage its effects could not be so much serious and hence the consequences could be then be milder.

On the other hand, the mean of number of service per conception was greater in bovine TB positive cows than in negative cows particularly as the number of calving increases. As explained above, the effects of bovine TB increase as the age of the animal increases because of its chronic nature. Its effects could be direct on the reproductive organs like uterine and ovaries thereby hindering their physiological functions (Andrews, 1992), or indirect including loss appetite, loss of condition and emaciation, respiratory distress, which could affect cow's reproductive efficiency and productivity. Infection of the uterine with M. bovis causes tuberculous metritis with characteristic feature of chronic purulent discharge that affect the conception rate of infected cows.

At all the three (1st, 2nd, and 3rd) calving the milk yield was slightly greater in bovine TB negative cows than in positive cows although not statistically significant. The absence of statistical significance could be due to the low sample size. Otherwise, obvious difference in milk yield was observed between positive and negative cows. The mean number milking days in each calving was significantly greater in negative cows than in positive cows. Thus, as the total number of milking days has a direct effect on the total volume of milk production i.e. the lower number milking days in bovine TB positive cow causes reduction in the total volume of milk production. Similarly, previous studies in Germany (Meisinger 1970) reported 10%±2.5% loss of milk production and 4%±2% loss of meat production attributed to bovine TB. In Hungary, milk losses were assumed to 12% of the total yield, and loss caused by sterility at 5% in infected animals (Danes, 1986). Further in Spain, Bernues *et al.* (1997) assumed losses of meat production at 10% in calves born from infected cows.

On top of its effect on the reproductive efficiency and productivity, its control has huge cost implications. Efforts

Table 1: Tuberculous lesions by sex, age and body condition score of Horro breed cattle slaughtered at Nekemte Municipality abattoir

Variable	No Examined	Positive	% Positive	95% CI	X2	P-value
Total	940	48	5.1	0.0369-0.061	4.48	0.11
Sex	862	37	4.3	0.029-0.56	14.21	0.000
Male	78	11	14.1	0.062-0.220		
Female						
Age	57	62	10.5	0.15-0.97	4.74	0.094
<5	624	7	4.3	0.21-1.49		
6-10	259	15	5.8	0.13-1.05		
>11						
Body Condition	34	0	0	0.50-1.80	5.33	0.069
score	701	32	4.6	0.5-3.09		
Poor	205	16	7.8	0.467-2.99		
Medium						
Good						

Table 2: Comparison of routine and detail meat inspections for the detection of carcasses with *tuberculous lesions*

Routine Meat Inspection	Detailed Meat Inspection		Total
	Positive	Negative	
Positive	5	0	5
Negative	43	892	935
Total	48	892	440

Kappa = 0.18

to control bovine TB were initiated in 19th century (Bang, 1884), and have occupied substantial part of human resources and institutional capacity of the veterinary sector in the 20th century (Zinsstag *et al*., 2006). Many of the developed countries have put enormous efforts into the control of bovine TB, and finally declared free of the disease, as they could accomplish the important aspect of the control of bovine TB, which is the compensation of farmers for the culled animals. For example, the cost/benefit analysis of M. bovis eradication in the United States showed an actual cost of $538 million between 1917 and 1992 (Frye, 1994). By reducing the number of cattle lost from 100,000 to less than 30 per year, the program saves $150 million per year in replacement costs alone, and as a consequence of such efforts, farmers have benefited by eliminating the indirect cost of losses in milk and meat production, stock replacement, decontamination procedures. Similarly, in the Republic of Ireland, the present value of the benefit of control exceeded the present value of the costs by 85% and the rate of return of the scheme was 15.5% (Sheehy and Christiansen, 1991). Nonetheless, in the United Kingdom, negative results were obtained in the economic evaluation of the control campaigns because of the wildlife reservoir of M. bovis (Power and Watts, 1987).

However, most developing countries could not undertake such control scheme. And hence bovine TB could be considered as a disease of poverty since the rural communities of most developing countries are not only at higher risk of its zoonosis but also vulnerable to poverty resulting from the loss of livestock productivity.

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OVARIAN MASSAGE: A SIMPLE, BUT USEFUL TOOL TO MANAGE OVARIAN ACYCLICITY IN DAIRY COWS

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Ovarian acyclicity deters successful running of a dairy enterprise by prolonging the inter-calving intervals and thereby affecting overall milk yield. It is one of the major drawbacks in cattle productivity with a prevalence rate of 10 -30 % among dairy herds and has been widely investigated (Fagan and Roche, 1986; Zdunczyk *et al*., 1992). Resumption of ovarian activity postpartum is influenced by many factors such as nutrition, milk yield, suckling or presence of calf, uterine diseases and environmental conditions (Chenoweth, 1983; Richards *et al*., 1989). Milk production peaks at 7 – 8 weeks after calving and then drops 1.5 – 2.5 % per week for the remaining lactation, therefore finding a cost effective methods to bring about early resumption of ovarian activity is necessary. This study investigated the efficacy of massage in pluriparous cows with acyclic ovaries 60 days postpartum.

In the study, 67 of the 178 cows diagnosed with ovarian acyclicity were used. These were of the Holstein-Friesian breed, in at least their second lactation and with an average milk yield of 4000 litres per lactation period of about 310 days. The animals were mainly fed on grass and corn silage, hay and concentrates during winter and on pasturage in summer. All experimental animals were selected during routine herd-health visits done every fortnight and were later subjected to double clinical examinations of both ovaries at 10-day interval at which blood sample for progesterone levels analyses were collected from the coccygeal vein into heparinized test-tubes. The collected blood was, within three hours of collection, centrifuged and plasma transferred into sterilized test-tubes and stored at -2^0 C until assayed using RIA. Progesterone was analyzed using RIA, according to Hoffman *et al.,* (1973). Low progesterone levels (less than 1ng/ml) on both examinations were indicative of acyclic ovaries. Thirty-five (35) of the acyclic animals were treated with ovarian and uterine massage while 32 served as controls .The fertility indices were analyzed statistically by student t-test and chi-square.

Following treatment, twenty-eight (80.0%) of the 35 animals came into heat, a result significantly ($p \leq 0.05$) higher than the control. Twenty-seven (77.1%) of the 35 cows became pregnant with 16 (57.0%) conceiving on first insemination. The artificial insemination index between treated and control groups was insignificant. However, the first service and total conception rates, and service period length in the massaged group were significantly better ($p \leq 0.05$) than in the control group as shown in Table 1. Average days from treatment to conception of treated animals were significantly shorter than the control group. The mechanism by which ovarian massage positively influences resumption of inactive ovaries is not clearly understood, but is probably due to activation of intrinsic intra-ovarian factors and enhancement of blood circulation to the ovaries and uterus (Campbell *et al*., 1995; Monget and Monniaux, 1995; Spicer and Echternkamp, 1995). Usually, the characteristic features of inactive ovaries include reduced size and flaccidness, thereby increasing the possibilities of poor blood circulation to the organ. It is on this understanding upon which the principle of ovarian massage is based, in that massage would enhance blood circulation to and from the ovary thereby playing a positive role in ovarian activity resumption through increased hormonal substances availability to or leaving the organ.

Studies have shown that the process of ovarian function is controlled by a complex of interactions between multiple mechanisms involving GnRH in the *hypothalamus*, gonadotrophins from the pituitary gland and intra-ovarian steroid hormones (Lobb and Dorrington, 1992; Campbell *et al*., 1995). Also follicular proteins, and other endocrine hormones such as growth hormones, Insulin-like growth factors-inhibitor (IGF-I) and insulin as well as locally produced ovarian factors have a functional influence on the ovary and may in part be regulated by various factors such as nutrition or level of milk production (Gospodarowicz and Ferrara, 1989; Lobb and Dorrington, 1992). It has been suggested that changes in metabolic hormones such as growth hormone (GH), insulin and insulin growth factor (IGF), which consistently accompany the nutrition-induced alteration in body energy and protein balance can affect ovarian function, either directly or by modulating gonadotrophin actions at the ovarian level (Pell and Bates, 1990). It is therefore most likely that reduced *intra-ovarian* activity as well as (or) due to defective ovarian circulation could

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lead to ovarian inactivity, hence no follicular growth on the ovary which manifests in form of anoestrus. However, increased availability of endocrine substances to the ovary as a result of improved blood supply to the organ, better still coupled with adequate energy level intake, could reduce the incidence of ovarian acyclicity in cattle. *Ovarian* massage may also influence ovarian function through stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation, and in case of misdiagnosis of ovaries with corpus luteum as inactive ovaries, oxytocin could lead to corpus luteum luteolysis and subsequent estrus manifestation.

Although the actual mechanism that brings about ovarian activity following massage is not well understood, the obtained results in this study show that this method could be positively exploited by veterinary practitioners and other livestock managers in managing cases of ovarian acyclicity disorder. Its low financial implications and the ease at which it can be applied in easy-to-handle cattle breeds makes it the first choice method to managing ovarian inactivity in cattle. However, where large numbers of animals are to be treated it may just prove to be tedious and or tiresome therefore a well planned and staggered treatment approach is recommended.

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
September 2010

Preamble

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states.

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1. On page one of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, place where the work was carried out, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion and References.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the findings presented and the conclusion(s) reached. Up to six keywords should be provided. The abstract should contain the objectives, brief description of materials and methods, highlights of significant results, conclusions and recommendations.
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Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double space lines spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
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- Books: Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.
- Chapter in a Book: Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- Reports: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- Conference Proceedings: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- Thesis: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- Web links: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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