ISSN 1597-1651

NVRI SEMINAR SERIES

2012

National Veterinary
Research Institute, Vom

NVRI SEMINAR SERIES 2012

This seminar series is a publication of seminar papers presented by staff and visiting scientists to the National Veterinary Research Institute, Vom during 2012

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ISSN1597-1651

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INTRODUCTION

This publication "NVRI Seminar Series 2012" is a compendium of seminar presentations for the year 2012. Laboratory research findings, field trials, postgraduate research and review of topical issues were presented by staff of the Institute during seminar sessions. The presentations were moderated by carefully selected experts who usually add candour and bring out the best in each presentation. Research staff, visiting scientists and students on industrial training was the usual audience. Their questions and contributions were inspiring and improve the quality of the presentations. The seminar sessions were usually held in a calm and friendly environment conducive for cross fertilization of ideas.

In this series, an update on a devastating malady of small ruminants in Nigeria, Peste des Petits ruminants commonly referred to as PPR was presented. Molecular characterization and phylogenetic analysis of the etiologic agent of the disease was undertaken using modern research techniques. The high genetic relatedness of the circulating virus in animals and vaccine strain currently used in the country provides evidence for the suitability and efficacy of the PPR vaccine produced by the Institute. Similarly, the result of field evaluation of some vaccines used for the immunization of chickens against Fowl Typhoid (FT) was presented. The result showed that NVRI Fowl Typhoid (FT) Vaccine is highly immunogenic at recommended dose and is protective against field challenge. However, there is the need to improve the existing FT vaccines by in-cooperating persistent field strains of *Salmonella gallinarum* and *S. pullorum* in the vaccine seed used by the Institute. The use of nanotechnology and the challenge for food safety regulation in the era of canned and fast food outlets as well as the public health implication of bovine mastitis and molecular epidemiology of *Staphylococcus aureus* in Plateau state were presented and discussed. To improve indigenous breed of cattle for increased milk production, a report of efforts by veterinarians in the Livestock Investigation Division (LID) to use fresh semen from Holstein-Friesian bulls to upgrade the local stock was presented with promising results. Parameters that influence forage crop production, medicinal plants used for management of diarrhoea in animals and the use of histo-structure of skeletal muscle of sheep to assess productivity in relation to age and nutritional level were also presented in this series.

This compendium attests to the undaunted spirit of staff of the Institute who strives for research excellence despite the prevailing financial squeeze. The research designs were vivid; the methodologies were current using state of the art facilities in the Institute and abroad.

Dr P. A. Okewole
Chairman
Seminar and Publications Committee

[1]MOLECULAR CHARACTERIZATION AND PHYLOGENETIC STUDY OF PESTE DES PETITS RUMINANTS VIRUSES FROM SOME NORTH CENTRAL STATES OF NIGERIA

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Introduction

Peste des petits ruminants disease (PPR), caused by PPR virus (PPRV), is a highly contagious disease of sheep and goats that has been widely reported in Sub Saharan Africa (Roeder *et al.*, 1994). Morbidity and mortality can be as high as 100% and 90%, respectively, depending on the endemic status of the disease in an area (Roeder *et al.*, 1994).

This disease was first described in Nigeria in 1975. Earlier studies have suggested that PPR might have been around for quite some time in different countries but was wrongly diagnosed or confused with other diseases. Recently developed molecular biology tools have made it possible to diagnose this disease rapidly and with great sensitivity compared to conventional techniques (Forsyth and Barrett, 1995).

Etiological agent of PPR is a member of the genus morbillivirus. The viral genome is 15,948 nucleotides long and contains six genes encoding six major polypeptides (Bailey *et al.*, 2005). Although PPRV has been known to occur as one strain or serotype, partial sequence analysis of the fusion protein gene, indicates occurrence of four lineages (1, 2, 3 and 4), of which three have been reported in Africa and one in Asia (Shaila *et al.,* 1996). Phylogenetic analysis is helping in the epidemiological understanding of the spread of the disease among animal populations. In Nigeria there is continuing pockets of outbreaks of PPR in small ruminants. The aim of the present study was to determine the phylogenetic relatedness of PPR viruses from outbreaks in two North central states of Nigeria.

Materials and Methods

A total of 33 (7 from Kaduna and 26 from Plateau State) clinically suspected PPR tissue samples of sheep (*n*=20) and goats (*n*=13) from outbreaks were collected in 2007 and 2009 by field veterinary officers, respectively. The samples were kept at the Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom Plateau State. PPR vaccine strain (Nig 75/1) from Jordan Bio-Industries Centre (JOVAC), Jordan was used as a reference strain virus in this study. Standard procedure for permission and shipment of biologicals were followed.

[1] **Seminar presented 2nd February 2012 at NVRI auditorium**

One gram of tissue was homogenized to make 10% tissue suspension. Viral RNA was extracted using the QIAamp Viral RNA Mini extraction Kit® from the suspension following the manufacturer's instructions. PCR was carried out and product resolved as described by Kerur *et al.,* (2008)

PCR amplicons of the F gene were sequenced, analysed and sequence similarity searches were conducted using BLAST. Complete alignment of nucleotide sequences was performed using ClustalW and amino acids deduced. The phylogeny was inferred using neighbour-joining method in MEGA version 4.1 by aligning 322 bp nucleotide segment of the F gene.

Results

Of the 33 clinical samples collected from sheep (*n*=20) and goats (*n*=13), 17 were positive for PPRV. All the 7 samples collected from Kaduna were positive while 10 of the 26 samples from Plateau were positive.

Ten amplicons were randomly selected for sequencing; nine sequences were generated (7 from Plateau and 2 from Kaduna). One sample (VRD/348/09, from Plateau) did not yield any sequence. (Accession number: HQ317871 - HQ317879).

A sequence comparison of the nine 322 bp F gene sequence fragments showed 98-100% nucleotide homology. The two sequences from Kaduna (HQ317873 and HQ317874) showed 99% nucleotide homology while the seven sequences from Plateau showed 98-100% similarities. Two sequences from Plateau (HQ317876 and HQ317879) were entirely identical in their nucleotide. One sequence sample from Kaduna (HQ317873) and one from Plateau (HQ317877) were also entirely identical among them. All the nine field sequences also showed 93-95% nucleotide similarity with the vaccine. Identity levels of deduced amino acid sequences of the 9 samples ranged from 96.3-99.7%.

The results of phylogenetic analysis of the sequences (n=9) obtained from this study all clustered into lineage 1 together with the vaccine strain Nig 75/1 and Nig 76/1. None of the sequences clustered with Cote d'Ivoire which belongs to lineage 2. There were no sequences of lineage 3 in the GenBank to be used in generating the tree.

Discussion

PPR is a very serious economic disease that has persisted in Nigeria for decades. One of the most important outbreaks occurred in 1975 and 1976 (Taylor and Abegunde, 1979). Since then the disease has continued with outbreaks occurring sporadically (Banyard *et al.,* 2010). Due to the endemic situation in Nigeria, control has been mainly hinged on vaccination (Mai *et al.,* 2004). In the present study, PPRV was detected by F gene based RT-PCR in 17 (51.52%) of the 33 clinical samples tested, which confirmed PPRV in the two North central states. Previous survey by Obi and Ojeh, (1989) in Nigeria reported a positivity rate of 86.8% and 81.6% from tissue homogenate using dot ELISA and Indirect ELISA, respectively, from the Southern states of Nigeria. Our findings suggest that PPR is less prevalent in the study area (Kaduna and Plateau) as opposed to the Southern states (Obi and

Ojeh, 1989). Arguably, the differences in positivity rate may also be related to the detection method, sample size and the presence of other exacerbating diseases which can occur concurrently. Nonetheless the PPR positivity rate we found was similar to that reported recently in an endemic region in India (50%) using RT-PCR (Kerur *et al.,* 2008).

A sequence comparison showed high level of homology (98-100%) of the circulating viruses suggesting that these viruses do not undergo rapid genetic changes in the F gene. Our data appear to be in agreement with other studies (Kerur *et al.,* 2008) who reported that the PPR virus is more prone to mutations on the N gene compared to the F gene. Between States, the PPR viruses were also highly homologous. This suggests criss-cross movement of infected small ruminants between States. Geographically, the two states share boundaries but also human related activities and movement of post recovered animals can shade the virus (Ezeibe *et al.,* 2008).

Interestingly, all the Nigerian strains from this study demonstrated 93-95% homology to the vaccine strain currently used for the control of the PPR in Nigeria. Our study revealed that the Nig 75/1 vaccine is indeed the suitable one for use in Nigeria. Based on our findings, the continued occurrence of outbreaks in Nigeria may not be attributed to the choice of vaccine but rather on other factors such as inadequacies related to the control strategy. Indeed Annatte *et al.,* (2006) have reported lapses in Veterinary extension services in relation to PPR control in Lagos State, the situation may not be different in other states.

A consensus Phylogenetic tree based on 322bp F gene sequence segment was constructed with the help of MEGA 4 program. In accordance with the previous studies (Banyard *et al.,* 2010), all the Nigerian strains including the vaccine strain Nig 75/1 and Nig 76/1 isolate clustered together reinforcing the findings that these isolates are homologous. Epidemiologically these data suggest that there has been no introduction of any new PPRV strain into Nigerian small ruminant populations.

Conclusion and Recommendation

The strains of PPRV involved were genetically related to the vaccine strain (Nig 75/1) used in the country. Based on the data obtained, the continued outbreak in the country maybe due to other factors other than the efficacy of the vaccine. To achieve effective control and possible eradication of PPR in Nigeria, we recommend that proper control strategies should be put in place.

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[2]MODERN TECHNOLOGICAL ADVANCEMENTS IN FOOD/FEED/DRINK INDUSTRIES: NANOTECHNOLOGY CHALLENGE FOR FOOD SAFETY REGULATION

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Introduction

Food has been identified globally as not only a biological need but also an economic and political instrument whose safety and quality is very essential for sustenance of life and ensuring food security. "Food safety" implies complete absence or where impractical, acceptable and safe levels of contaminants, adulterants, naturally occurring toxins or any other substance that may make food injurious to health on an acute or chronic basis. Food quality on the other hand can be considered as a complex characteristic of food that determines its value or acceptability to consumers. Other quality attributes besides safety include nutritional value, organoleptic properties including color, texture, taste, and functional properties. For a nation like Nigeria projected to become the 5th most populous nation in the world, with population estimated to reach >250m by 2030 (UNDESA, 2011), the matter of food security and indeed food safety certainly is very important.

The changing eating habits of consumers who are ever demanding fast, ready to eat (RTE) foods, as well as globalization leading to greater import and export trade between nations has placed unprecedented burden on food business operators (FBOs). The food/feed/drink industry must rise to meet these challenges and has continued to promote the invention and adoption of modern technologies thereby transforming virtually all processing technologies. This transformation affects all components of the production chain from “farm-to-fork”. Some of these advancements are especially prevalent in food packaging industry where the demand for food with longer shelf-life has led to the emergence of smart packages. These packages could have the ability to give color indications according to quality and safety loses over time, while others have antibiotics impregnated in them to delay microbial growth preventing spoilage and illness. Silver nanopartciles belong to this latter group.

In view of the above, food regulatory agencies all over the world must continually review guidelines and ensure that regulations are updated and designed in a useful and implementable manner, while ensuring the protection of the consumers’ health and safety. The characteristic features of nanoparticles that make their use beneficial in many sectors of the food industry (and other industries) raises health hazard concerns which differ significantly from those posed by conventional-scale versions of the same materials. Many of these concerns cannot be easily addressed by the knowledge possessed about the conventional-scale counterparts. Using an emerging technology that could dramatically alter how the body absorbs and

[2] **Seminar presented on 19th April 2012 at NVRI auditorium**

metabolizes a product that is largely unregulated creates the potential for risks to consumers in the coming years (Schultz and Barclay, 2009).

General information on nanoparticles

Nanoparticles are discrete entities having three dimensions of the order of 100 nm or less, or can be defined as materials designed and produced to have structural features with at least one dimension less than 100nm (Oberdörster et al., 2005). Compared to a strand of the human hair, this is about 1/100,000th of the width of a strand! The field of nanotechnology has revolutionized industrial advances globally by offering tremendous potential benefits that promises to change just about everything we do from food to agriculture, medicine to environment, energy to security, etc. Indeed the future of technology and modern day industry may well be driven by nanotechnology.

The beneficial antimicrobial property of silver can be said to be a knowledge known long ago (Guillemot et al., 2008; Chen and Schluesener, 2008), and the cooking of food using silver pots is an ancient practice among the Greeks that may well be a consequence of this antimicrobial effect. Claims of medicinal properties have followed silver since the time of Hippocrates. The cost of manufacturing objects entirely from silver is however prohibitive that it is unimaginable to contemplate this as a standard practice. The development and advances in nanotechnology has therefore provided practical ways to exploit the beneficial properties of not only silver, but many other useful metals demonstrating novel properties at nanoscale states. Commonly, these “nano” metals are impregnated in surface linings of other materials where they exert the desired effect (Henig, 2007; Vermuelen et al., 2007; Jung et al., 2008).

Engineered silver nanoparticles have the ability to deliver silver ions (highly reactive and the most fundamental entity of silver) in large doses directly to sites where they effectively attack microbes, are able to block undesirable access for certain compounds in food to parts of the body, or even deliver nutrients to cells that previously could not be reached (Kuzma and VerHage, 2006). From the understanding of this technology so far the process appears to be cost-effective, and one gram of silver nanoparticles for example is sufficient to confer effective antibacterial properties to hundreds of square meters of substrate material (Kuzma and VerHage, 2006).

Silver nanoparticles raise new challenges for risk assessors. Its great potential benefits are accompanied by an equally great potential for risks, posed both by the physical and chemical traits of these “new” or “novel” materials. An understanding of the fate of silver nanoparticles (absorption, distribution, metabolism, and excretion; ADME) following exposure and absorption in the gut will certainly aid the development of appropriate risk assessment strategy by providing necessary information for hazard identification and exposure assessment.

Method

A modified inversed *in vitro* model of the human follicle associated gut epithelium (FAE) was used as a novel bioassay tool to determine the translocation and possible

toxicity effect of the silver nanoparticles. The model is developed from co-culture of human colorectal adenocarcinoma (Caco-2) cell line (HTB-37TM) and human Burkitt's (Lymphoma cells) Raji B line (CCL-86TM) first reported by *des* Rieux *et al.,* (2007). Production of intracellular reactive oxygen species (ROS) was measured using Dichlorofluorescein (DCF) assay adapted from Wang and Joseph (1999) with a modification introducing a lysing step (Wang *et al.,* 2008) for compatibility with the inversed FAE model.

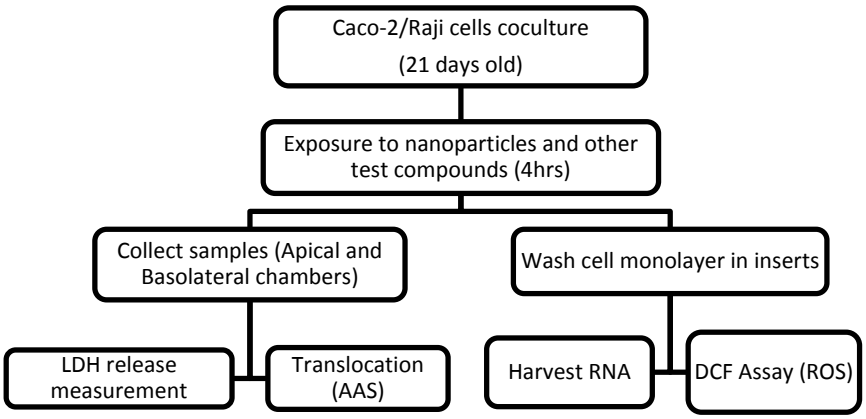

Figure 1.Schematic diagram of the experimental design.

Results and Discussion

Within non-cytotoxic concentration range of silver nanoparticles tested, no statistically significant increase in ROS was observed. Also there was no dose-response formation of intracellular ROS observed with increasing silver nanoparticles concentrations. It is the opinion of Asharani *et al.* (2009) that cytotoxicity studies are limited by the fact that in most cases the dependence of time of exposure and surface functionality remained unexplored. Other reports about AgNP toxicity showed a dose-dependent cytotoxicity effect on cells tested (Asharani, *et al.,* 2009). Depending on the endpoint of toxicity assayed, as well as cell lines and exposure time points used, results also show presence or absence of toxicity

Silver nanoparticles were translocated across the FAE model in a concentration independent manner, with values around <0.05µg.The role of M-cells, already demonstrated to be important for particle transport across *in vitro* models of human FAE (*des* Rieux et al, 2007; Gebert et al., 1996; Kerneis et al., 1997; Jensen et al., 1998) was exploited in this study.

Conclusion

With the promising role of nanotechnology in the current industrial revolution, as well as the attendant consumer concerns with respect to the potential of these novel materials to induce adverse health effect, several strategies will need to be employed to ensure the safe use of nanotechnology. The behavior of nanomaterials in biological matrices needs to be examined. This will help define the most appropriate matrices for hazard characterization and exposure assessment, and will further enhance the investigation of different particle sizes as well as

concentrations on developed models. Translocation and toxicity effects of silver nanoparticles were conducted under sub-acute conditions (4hr), using *in vitro* viability assay to select concentrations. Future target would be to explore the long-term effect of silver nanoparticles on this model. There is need for more *in vivo* studies to validate observations with *in vitro* models to facilitate better translation of results to humans.

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[3]FERTILITY RATE OF BUNAJI CATTLE FOLLOWING AI WITH FRESH HOLSTEIN-FRIESIAN SEMEN FOR DAIRY HERD UPGRADING

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Introduction

Basically nothing has changed about the way in which we get the cows in calf as we still need to bring in a sufficient amount of good quality spermatozoa at the appropriate time, in the appropriate way and at the correct place in the cow to enable fertilization and embryonic growth and development. So the basic factors that have since long been recognized to be important for fertility of a cattle population, are still valid (Opsomer *et al.,* 2006*).* Currently most routine contact by veterinarians with a dairy herd, via herd health visits including fertility the principal aim of which is to assist the herd to achieve its preset targets.

The objective of an organized approach to reproductive management initiated and directed by the veterinarian is to maximize client profit. This goal will be realized if the results of the program increase the number of quality herd replacements, decrease the number of reproductive failures and increase the percentage of the herd in peak production at any given time. In order to justify the efforts and expense involved in an on-going reproductive program, measurement of herd performance must be generated and evaluated and the economic impact of these values determined on a routine basis **(**Morrow, 1986**).** The aims and objectives of this function is to provide fresh Holstein/Friesian (H/F) bull semen from built stations and this source will reduce cost of the farmer managing exotic H/F bulls for the genetic source of upgrading their herd for dairy. The steps essential for producing and maintaining this economic response includes:

1. Client Education
2. Routine examination of all eligible animals
3. Collection and processing of data
4. Assessment of reproductive performance
5. Establishment of herd reproductive goals
6. Implementation of management changes to obtain goals
7. Monitoring herd response.

The western idea of a single-purpose dairy cow producing at least 4000 litres of milk per lactation while her calf is artificially reared is not widely practiced in Nigeria except highly developed farms. It is important however, to help traditional livestock and peri-urban dairy farmers to breed animals that are efficient at using the resources they have at present and those they may gradually attain. The present paper aims to highlight some practical points to keep in mind when advising farmers in their effort to reach an economically optimal dairy production through crosses from heavy Holstein Friesian bulls.

[3] **Seminar presented on 3rd May 2012 at NVRI auditorium**

Materials and Methods

The farm had two locally bred Holstein Friesian bulls weighing 950-1000kg and 650-700kg respectively. Semen collection by electro ejaculator began on them in July, 2003 to assess the sperm status for each bull for fresh semen artificial insemination program.

The bulls are led to cement built mounting supportive crush for restrain and a paraffin lubricated probe introduced into the rectum after evacuating any faeces. The voltage 125-220 of the electro ejaculator is manipulated gently to stimulate erection and the ejaculate collected into a semen collecting cone fixed with a 10ml test tube. The first bull (No. 799) provided 5-7ml semen which was watery with active sperm cells picture (20%) under the microscope; while bull No. 0153 provides 4-5ml per ejaculate, milky or creamy semen with high sperm density and good sperm wave under the microscope with motile sperm cells of 80-90%.

Seventeen cows were identified in normal natural cycling heat from two herds of cattle by herdsmen and the vasectomised bulls between May and November, 2004. Immediately a cow is identified the AI team is informed. The team observes the cow and prepares for semen collection and insemination at the right hour. The semen was collected from bull No. 0153. Electro ejaculation at 12 hours after heat detection for insemination and in absence of electricity the portable electric generator was useful for obtaining the semen.

Two millilitres (ml) or more fresh semen was inseminated into each cow on heat at mid cervix or intrauterine using a five ml syringe and uterine catheter. The inseminated cows were observed for heat by the mounting actions of vasectomised bulls by our herdsmen for a repeated service or were diagnosed at 40 and 60 days after A.I for pregnancy confirmation.

Results

Table 1: Record of AI in Bunaji cows using fresh H/F Semen

S/NO	COW NO	A.I DATES	CALVING DATES	CALF WEIGHT AT BIRTH	SEX	REMARKS
1.	3437	16/5/2004	8/2/2005	28KG	M	
2.	3467	05/5/2004	19/2/2005	27KG	M	
3.	3443	26/5/2004	26/2/2005	28KG	M	Died on 3/3/2005
4.	0040	28/5/2004	26/2/2005	32KG	M	
5.	3430	14/6/2004	17/3/2005	29KG	M	
6.	3046	11/6/2004 30/8/2004	8/4/2005	34KG	M	A.I X2
7.	0033	1/7/2004	29/3/2005	18KG	M	
8.	0008	8/7/2004	11/4/2005	25KG	F	
9.	3489	3/6/2004 26/7/2004	30/4/2005	25KG	F	A.I X2
10.	3525	1/7/2004 25/8/2004	1/6/2005	28KG	F	A.I X2
11.	0011	1/9/2004	8/6/2005	32KG	F	
12.	0009	1/9/2004	13/6/2005	28KG	M	
13.	3549	18/9/2004	20/6/2005	26KG	F	
14.	3554	16/10/2004	14/7/2005	32KG	F	
15.	0002	8/11/2004	15/8/2005	27KG	M	
16.	0029	30/6/2004 19/7/2004 18/9/2004	-	-	-	A.I X3
17.	3521	14/6/2004 7/7/2004 18/11/2004	-	-	-	A.I X3

Fig 1: Offspring of H/F bull x Bunaji Cow using AI technique at LID, Vom

Table1 indicates that seventeen local Bunaji cows on natural heat cycling were inseminated in six months at various dates with fresh undiluted Friesian semen and 15 (or 88%) were fertilized successfully with a single or double insemination service. For the first insemination on the seventeen zebu cows, 12 cows (70.59%) became pregnant, then after the second insemination this number raise to 15 cows (88.23%). At the third insemination there was no increase in conception rate. The period of insemination was from May to November, 2004. All 15 cows calved successfully from February to August, 2005 giving birth to 9 bull calves and 6 heifer calves.

Discussion

The number of service per conception (NSC) depends largely on the breeding methods used. It is higher under uncontrolled natural breeding and lower where hand mating or AI is used. NSC values that are greater than 2.0 are regarded as poor. The NSC in the Holstein Friesian X white Fulani crosses and pure Holstein Friesian heifers obtained within Vom environ is 2.0 (+/-1.0) (Ngodigba, *et al.*, 2009) and 1.6 was obtained in indigenous Zebu breed. The first conception rate targets is 60% in AI and 78% was obtained for this fresh semen service.

Artificial Insemination has given tropical dairy cattle breeders the ability to introduced into their herds new genes on a large scale at comparatively low cost. The use of AI in tropical developing countries is still not widespread. Few countries have more than 1% of cows serviced by AI. AI can introduce a better class of animal but this potential will not be realized unless there is a simultaneous improvement in nutrition, disease control, husbandry and project administration (Chamberlain, 1993).

Artificial Insemination is a revolutionary step and it therefore needs to be carefully introduced to the normally conservative tropical community. AI techniques and organization used in developed countries do not necessarily apply in developing areas, where transport and communication services are limited. Apart from the initial cost of equipment the present highest operating cost will be wages for the insemination staff. However, the total costs are unlikely to be as high as importing improved cattle. Semen supply, production, storage and transport are usually responsibilities undertaken by the government. Semen can either be imported frozen or produced locally.

To receive semen from other countries, a liquid nitrogen source is needed and storage equipment, handling facilities and staff must be available. Locally produced semen can be used fresh or frozen. After collecting and diluting semen it can simply be kept in liquid form, but will have to be cooled and used within 72 hours. This programme used fresh undiluted semen on local cows within 24 hours of collection. Chilled semen (cooled to 5^{o}C) has a short life of 3-5 days, but fertility declines with each day of storage. A service based on fresh liquid semen is suitable for an area with a large herd of cows within a short distance of the production centre.

With cheaper equipment the Nigerian Field Veterinarian could upgrade indigenous cattle for the peri-urban small scale dairy farmers by AI with fresh or chilled semen from locally bred H/F temperate bulls. This arm of service can promote the Nigerian small scale dairy industry before any full use of frozen semen for AI in established large scale dairy farms can be practiced in the country. Frozen semen needs more elaborate equipment, motorized transport and good roads for distribution. Semen storage in liquid nitrogen at – 196^{o}C has subsequently became established as the standard medium for long-term preservation of semen over the 40 years for which it has been practiced and has maintained sperm fertility unscathed (Noakes, *et al.*, 2001).

Recent development in Europe showed that, good-quality thawed semen may keep for two hours without deteriorating, allowing two hours traveling time between the centre and the Cow and avoiding the problem of having to carry heavy Liquid Nitrogen Equipment out to farms. This could be important for future use of A.I in the developing tropics (Chamberlain, 1993).

Recommendation

The locally purebred H/F bulls on the Plateau and grassland Savannah region can be trained for semen donation. Those assessed with good sperm cells placed at centres for peri-urban dairy up-grading requirements. Fresh or diluted chilled semen produced should be supplied to peri-urban dairy farmers to inseminate the indigenous cow on natural heat cycle when needed. The revenue coming in from the produced semen and other AI duties can be used to purchase more exotic bulls for these centres or build more service centres for the progress of the small scale dairy industry in the country.

More inseminators can be trained to provide AI services to keep steady progress in AI under the supervision of a Veterinarian. The small scale dairy farmer should be educated on improving stock management nutritionally with good herd health for optimum dairy production with the up-graded stock.

To promote this project, small scale dairy farmers should be encouraged to form cooperative bodies in order to facilitate technology transfer and reduce the cost of service associated with widely scattered individual producers. By disseminating and applying this proven and low-cost technology the productivity of the dairy cattle will be increased to ensure a stable year – round supply of dairy industry raw materials for dairy production.

Acknowledgement
I acknowledge the assistance of the herdsmen, LID Farm colleagues and the farm secretary for typing the manuscript.

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[4]A COMPARATIVE STUDY OF THE EFFICACY OF SOME VACCINES USED IN THE IMMUNIZATION OF CHICKENS AGAINST FOWL TYPHOID IN NIGERIA

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Introduction

Fowl Typhoid (FT) is a serious septicemic disease of domestic poultry with a worldwide distribution caused by the bacterium *Salmonella enterica* Serovar Gallinarum (*S. gallinarum*) (OIE, 2008).The disease is characterized by sudden death, high morbidity and mortality rates and reduction in egg production resulting in large scale economic losses and the persistence of carrier birds after recovery (Berchieri *et al.*, 1997). *Salmonella gallinarum* and *Salmonella enterica* Serovar Pullorum (*S. Pullorum*) causative agent of Pullorum disease (a closely related disease to FT) are serovars of the same species and have been proven to be antigenically similar and cross react (Berchieri *et al*, 1997; OIE, 2008). The first documented report of FT in Nigeria was in the Annual Report of Veterinary Department, Northern Provinces in 1930. "Avian Salmonellosis" is ranked as one of the most economically important diseases of poultry in Nigeria (Abdu, 2007). The National Veterinary Research Institute (NVRI) Vom produces FT vaccine using a smooth live attenuated strain 9R of *S. gallinarum*. Several imported FT vaccines are also marketed in the country.

Material and Methods

a. Experimental Design:

Age (Days) ↓	**Activity** ↓	**Groupings** → **Vaccination**	1 **Vom** (0.5)	2 **Vom** (1.0)	3 **Cam** (1.0)	4 **Sal** (0.5)	5 **UVG** (Nil)	6 **MCG** (0.5)
Day 1 – 42	Screening		Yes	Yes	Yes	Yes	Yes	Yes
Day 43	Vaccination		Yes	Yes	Yes	Yes	No	Yes
Day 51	Seroconversion		Yes	Yes	Yes	Yes	N/A	Yes
Day 67	1st Blood Sampling		Yes	Yes	Yes	Yes	Yes	No
Day 67	Challenge		Yes	Yes	Yes	Yes	Yes	No
Day 79	2nd Blood Sampling		Yes	Yes	Yes	Yes	Yes	No

Note: All groups comprise of 15 birds per group

Key:
Vom $_{(0.5)}$**:** NVRI FT vaccine at 0.5ml/bird
Vom $_{(1.0)}$**:** NVRI FT vaccine at 1.0ml/bird
Cam $_{(1.0}$**:** Lanavet Cameroun FT vaccine at 1.0ml/bird
Sal $_{(0.5)}$**:** Abic, Israel FT vaccine at 0.5l/bird
UVG: Unvaccinated group
MCG: Management control group
N/A: Not applicable

[4] **Seminar presented on 31st May 2012 at NVRI auditorium**

b. Vaccines Tested

1) **NVRI, FT Vaccine:** comprises a smooth live attenuated strain of *S. gallinarum* (9R).
2) **LANAVET, Cameroun FT Vaccine:** a wet inactivated polyvalent vaccine comprising *Pasteurella multocida*, Strain A5, A8, B6 and E6; *S. pullorum* and *S. typhimurium*.).
3) **Group 4 (Sal $_{(0.5)}$) ABIC in Israel FT Vaccine (Salmabic$^{(R)}$):** is an inactivated oil emulsion FT vaccine comprising *S.enteritidis* C8, B3 and *S. typhimurium*.

c. Challenge of Birds and Antibody Detection

Three weeks post vaccination, 12 birds randomly picked from each of groups 1-5 were challenged with 1.0ml each of a virulent Riyom strain of *S. gallinarum* culture containing about 2.5 x $10^{8,}$ cfu/ml (Nwobu, 1993). This was injected directly into the crop of each individual bird. The remaining 3 birds were kept as control for each group and separately housed. A Salmonella Bacillary White Diarrhoea (BWD) antigen supplied by Onderstepoort Biological Products Limited, South Africa containing *S. pullorum* antigen was used to conduct Whole Blood Agglutination Tests (WBAT) on blood samples obtained from individual birds as described by OIE from day 3 post-vaccination to determine the time of sero-conversion (OIE, 2008). Known positive and negative control blood samples supplied along with the antigen were incorporated during testing.

Results

Data collected were reduced into Tables. A two-way Chi square(X^2) analysis was used to investigate the level of association between the 3 vaccines tested at their recommended dosages in terms efficacy and was also used to determine the benefit, if any with double dose over single dose with NVRI, Vom vaccine. All three vaccines studied were immunogenic; evident by the production of detectable antibodies as shown by the WBAT. Efficacy recorded for the tested vaccines was: 66.7% for NVRI, Vom vaccine at Single dose (Vom $_{0.5}$), 75% for NVRI, Vom vaccine at Double dose(Vom $_{1.0}$), 58% for LANAVET Cameroun vaccine (Cam $_{1.0}$) and 75% for ABIC Israel vaccine (table 2).

Table 1: Mortality in Different Groups after Challenge at 14 Days Post-vaccination

S/No.	Grouping/Treatment type	Treatment	Number challenged	Survivors	Deaths	Survival (%)	Death (%)
1.	Vom $_{(0.5)}$	Vaccinated	12	8/12	4/12	67.7	32.3
2.	Cam $_{(1.0)}$	Vaccinated	12	7/12	5/12	58.0	42.0
3.	Sal $_{(0.5)}$	Vaccinated	12	9/12	3/12	75.0	25.0
4.	Nil	Unvaccinated	12	0/12	12/12	0	100
5.	Vom $_{(0.5)}$	Vaccinated (MCG)	0	12/12	0/12	100	0

Table 2: Efficacy Values of the Three Different Vaccines Tested in the Study

S/No.	Vaccine	Survival	Deaths	RR	VE (%)
1.	Vom$_{(0.5)}$	8	4	3	66.7
2.	Vom$_{(1.0)}$	9	3	4	75.0
3.	Cam$_{(1.0)}$	7	5	2.4	58.0
4.	Sal$_{(0.5)}$	9	3	4	75.0

Key: Relative Risk **(RR)** = (a/a+b)/(c/ c+d)
Vaccine Efficacy **(VE)** = (RR-1)/ RR
Vom $_{(0.5)}$: NVRI FT Vaccine at 0.5ml/bird
Vom $_{(1.0)}$: NVRI FT Vaccine at 1.0ml/bird
Cam $_{(1.0)}$: Lanavet Cameroun FT Vaccine at 1.0ml/bird
UVG: Unvaccinated group
MCG: Management control group
Sal $_{(0.5)}$: Abic, Israel FT Vaccine at 0.5ml/bird

Discussion

The lowest efficacy value of 58% recorded with Cam $_{1.0}$ in this study may be attributed to the presence of *Pasteurella multocida* antigen in addition to the fact that it is a killed vaccine. This supports the well documented superiority of live vaccines over killed vaccines in terms of efficacy (Harbourne *et al.,* 1963). Salmabic (Sal $_{1.0}$) an inactivated vaccine gave an efficacy value that was the same as that recorded with Vom $_{1.0}$ (highest value among all the vaccines tested) despite not having either *S. gallinarum* or *S. pullorum* antigens as the case with NVRI, Vom vaccine. This could be attributed to its composition of two Salmonella serovars : *typhimurium* and *enteritidis*, which have been shown to exhibit cross protection properties in Avian Salmonellosis and also capable of giving false positive results in FT and Pullorum disease screening (OIE, 2008). The higher efficacy value recorded with NVRI, Vom vaccine at double dose was not statistical significant when compared with the efficacy recorded with single dosing. However the value this may offer in practical usage cannot be confirmed by this study due to small sample size.

Conclusion and Recommendation

This study was able to prove the immunogenicity of the 3 vaccines tested. The study further showed that proper vaccination against FT may reduce flock losses but may not entirely prevent infection from field strains. There was no significant statistical difference in protection levels between NVRI vaccine at single dose and at double this dose.

This study thus recommends the following:

1. Re-evaluation of the vaccine seed potentials of *S. gallinarum* (9R) presently used in producing FT vaccine in NVRI, Vom with the aim of inclusion of persistent field strains of *S. gallinarum*, *S. pullorum* and/or any other Salmonella serovars as vaccine antigens with the aim of improving protection levels and spread.
2. Attempts should be made towards the production of an oral or parenteral multivalent vaccine against the most prevalent poultry diseases in Nigeria such as FT, Fowl cholera and Newcastle disease or some other antigen as presently obtains in some laboratories across the globe.

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[5]INFLUENCE OF CROP SPECIE AND MONTH OF HARVEST ON PROXIMATE PARAMETERS OF FORAGE CROPS

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Introduction

The fast growing pasture grasses *Bracharia* and *Digitaria decumbens* and legumes *Stylosanthes guianensis* and *Centrosema pubescens* have appreciable crude protein(CP) concentration(over 10% for grasses and up to 15% for legumes) in the rainy season; are suitable for grazing, feedlot utilization, hay and silage making due to high herbage yields and relative ease of propagation(Ogedegbe *et al.*,2012). Their fast growths enable early harvest maturity that result in significant drops in quality. Maturation decreases crude protein content and organic matter degradability; increases neutral and acid detergent fibre including lignin contents of forages (Cone *et al.*, 1999). Generally, changes in forage nutritional composition depend on plant maturity, species, season, moisture and grazing system (Rinehart, 2008).

In the tropics, forage conservation through silage production and hay making could be used to preserve excess rainy season forage for dry season feeding. For a myriad of logistic reasons, conservation practices which require timely execution are often carried out quite late into the dry season enhancing further deterioration of forage quality and quantity. In Thailand, the concentrations of crude protein, ash, calcium and phosphorus in *Bracharia humidicola* showed a curvilinear decrease (quadratic $p<0.05$) with advancing maturity (Chobtang *et al.*, 2008). Adequate quantities of green forage can supply most of the energy and protein needs of ruminants (Rinehart, 2008) whereas poor quality forage characterized by dryness, bleaching and high-cellulosic materials (Umunna and Agishi, 1988) retards the growth and reproductive rates of ruminant livestock (McDowell, 1985).Since many pasture crops in the tropics are harvested late in the dry season, it is imperative to know their quality at different months of harvest. This information will enable informed management decisions regarding supplementation especially where over-mature forages represent the bulk of dry season feeding as is common in most tropical countries. The objective of the research therefore was to evaluate the influence of crop specie and month of harvest on the proximate composition of four tropical forage crops.

Materials and Method

The experiments were conducted during the dry seasons of 2003 and 2004 at Dagwom Farm at the National Veterinary Research Institute(NVRI), Vom located at latitude 09°

[5] **Seminar presented on 17th May 2012 at NVRI auditorium**

44'E and longitude 08° 44'N with an elevation of 1,239.4m above sea level. The acidic soil (pH 5.5-6.1) of the experimental plots was of ferallictic cambisol developed from volcanic rocks (Enwezor *et al.*, 1990). Four perennial forage species, namely: *Bracharia decumbens, Digitaria decumbens, Stylosanthes guianensis*, *Centrosema pubescens* and five harvest months of November, December, January, February and March were factorally combined as treatments. The crops were previously established on 4m x 2m plots in 2002 in a Randomized Complete Block (RCBD) arrangement with three replications giving a total of 60 plots in the experiment. To simulate natural conditions, fertilizer was not applied on the plots. On the last working day of each sampling month, herbage within a 1m x 1m quadrat was cut from each plot at 20cm stubble height and sub-sampled for proximate analysis; carried out on two replications to save costs. The sub-sample was dried to constant weight in a Fisher Isotemp oven at 70°C and ground with a Christy and Norris laboratory mill (Christy and Norris Ltd, England) to pass through a 1mm screen, then, labelled for proximate analysis. Proximate parameters determined according to AOAC (1990) methods were moisture, crude protein, crude fibre, ash and nitrogen-free-extract. To determine the significance of treatment effects (Snedecor and Cochran, 1967); proximate analysis data for both years were combined and subjected to one way statistical analysis of variance using SAS version 9.0 software (SAS, 2002); and the means were separated by Duncan's Multiple Range Test (DMRT) at the 5% level of probability (Steel *et al.*, 1997).

Results and Discussion

Crop specie significantly (p=0.01) affected all the proximate parameters (Table 1). Moisture content of the crops was much lower than required in hay which implies that by November when the study began, the crops had dried beyond the level required for good quality hay and were technically crop residues because rice straw in Egypt contained 3.6% moisture(Nour,2012) similar to the values in the current study. Moisture content of hay ranges between 10-15% (Philipp and Jennings, 2012) and excessively low moisture (<10 %) in hay indicates brittleness and low palatability (Ball *et al.*, 2012).The crude protein concentration of Centrosema (14.2%) was significantly superior to that of other crops and both legumes produced significantly higher CP concentration than the grasses. This supports the principle that legumes are the crops of choice for CP supplementation. It also indicates that ruminants fed on the grasses (average of 7.4%CP) require high levels of supplementation because McDowell (1985) reported that fattening and lactating cattle require 15% and 11% CP respectively. On the other hand, the quality of the proteins (amino acid balance) may be lower than the values actually present because of excessive drought and high temperatures associated with dry season could denature some proteins.

Generally, the crude fibre (CF) values (35.1-44.3%) were higher than ideal for hay which could have negative impact on digestibility. The rate of Rhodes grass digestibility declined from 0.08% to 0.15% day^{-1} due to increased fibre concentration attributed to advanced maturity (Keftasa, 2012). Mean CF and total digestible

nutrients (TDN) values for average quality hay fall within the range of 25-32% and 45-55 % respectively. Legumes usually accumulate less fibre than grasses (Ball *et al.*, 2012) but in this study, Centrosema and Bracharia were at par and this could be attributable to late harvest and differences in drought tolerance which may affect CF concentration. The grasses produced similar but significantly (p=0.01) higher ash and nitrogen-free-extract (NFE) concentrations than the legumes. The total ash concentration in the herbage is of lesser consideration for ruminants than the actual quantities of Ca, P and their ratio which was reported for these crops by Ogedegbe *et al.* (2012).However, the result for non-structural carbohydrate indicated by NFE is consistent with common assumption that grasses provide more energy than legumes a reason for their intensification in mixed pasture systems for ruminants.

Except for ash concentration, month of harvest did not reveal a consistent trend on its effect on proximate parameters (Table 1). This inconsistency is buttressed by the correlation analysis (Table 2) which revealed highly significant negative correlations among CP, Ash, CF and NFE. This suggests that great tact is required for supplementing livestock whose basal diets are poor quality crop residues gathered at various intervals; because case by case feed formulation may be difficult to achieve under tropical systems. The only positive and highly significant correlation (0.418) between ash and NFE can be interpreted to mean that these forages may provide livestock with higher quantities of both nutrients concurrently. Table 3 shows the interaction between crop specie and month of harvest for percent crude protein concentration. When crop specie is held constant at varying months of harvest, the highest CP concentration was obtained in November and except for Stylosanthes, decline in CP concentration was not consistent beyond December because roughages have a wide variability in protein content (Hamilton, 2012). At a static month of harvest, the significantly highest CP concentration was produced by Centrosema; and Stylosanthes produced significantly higher CP than the grasses, month of harvest not-withstanding. The implication of this interaction is that, after November, a blanket supplementation program will not suffice since the quality of the forage fluctuates unpredictably and poor quality affects intake which necessitates higher supplementation (Ball *et al.*, 2012). Higher forage intake and digestibility allow lower supplementation rates without reducing milk production and animal growth (Rayburn, 1977).This interaction also shows that legumes are superior to grasses in CP and Stylosanthes has a more predictable drop in terms of CP than Centrosema hence it may be more suited to systems where late harvest is the norm.

Conclusion

The study has shown that forage crops harvested after November are unsuitable as hay and may be better utilized and treated as crop residues for maximum animal benefit. Beyond November, most of the quality parameters are inconsistent; therefore, late-harvested roughages should be stored in forage lots to make supplementation easier to apply.

Acknowledgements
The authors appreciate staff of Pasture Development Unit (Dagwom Farm) NVRI-Vom and Analytical laboratory (Department of Animal Science), Ahmadu Bello University-Zaria, for experimentation by the former and proximate analysis by the latter groups of staff who provided the useful data that made this write-up a possibility.

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Table 1: Effect of crop specie and month of harvest on proximate parameters

	Proximate Parameters (%)				
Treatment	**Moisture**	**Crude protein**	**Crude fibre**	**Ash**	**Nitrogen-free-extract**
Crop specie					
Stylosanthes	3.4a	9.7b	44.3a	3.9c	37.7c
Centrosema	3.4a	14.2a	37.0b	6.0b	40.1b
Bracharia	3.3b	6.6d	36.9b	10.1a	45.2a
Digitaria	3.4a	8.1c	35.1c	10.4a	45.3a
SE	0.02	0.05	0.24	0.14	0.46
Significance	**	**	**	**	**
Month of harvest					
November	5.9a	11.7a	36.9c	8.1a	40.3c
December	2.2d	9.1c	39.6b	6.3b	43.2b
January	4.1b	8.6d	36.7c	8.1a	44.4ab
February	2.2d	9.9b	42.2a	8.2a	37.4d
March	2.5c	9.1c	36.1c	8.1a	45.1a
SE	0.02	0.06	0.27	0.16	0.51
Significance	**	**	**	**	**
Interaction					
Crop x Month	**	**	**	**	**

Mean within a column of any set of treatments followed by different letters are significantly different at 5% level of probability using Duncan's Multiple Range Test(DMRT).**,*=Significant at 5% and 1% levels of probability respectively; NS=Not significant.

Table 2: Matrix for Correlation coefficients among the proximate parameters

	1	2	3	4	5
1	1.000	0.281	-0.243	0.099	-0.075
2		1.000	0.083	-0.482**	-0.499**
3			1.000	-0.537**	-0.835**
4				1.000	0.418**
5					1.000

**=Significant at 1% level of probability.

1=Moisture, 2=Crude protein, 3=Crude fibre, 4=Ash, 5=Nitrogen-free-extract

Table 3: Interaction between crop specie and month of harvest on crude protein concentration (%)

	Month of harvest				
Crop specie	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
Stylosanthes	13.1d	9.6g	8.9h	9.1h	8.1j
Centrosema	15.6a	13.3d	12.7e	14.5c	14.9b
Bracharia	8.1j	6.6n	5.4o	7.2l	5.7o
Digitaria	10.2f	7.1m	7.5k	8.5i	7.2l
SE			0.12		

Mean within a column of any set of treatments followed by different letters are significantly different at 5% level of probability using Duncan's Multiple Range Test (DMRT).

[6]MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BOVINE MASTITIS MILK USING PULSE FIELD GEL ELECTROPHORESIS (PFGE)

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Introduction

Mastitis is the most significant cause of economic loss in the dairy industry, and *Staphylococcus aureus* is as an important causative agent of the disease all over the world. It causes both subclinical and clinical mastitis (Cabral *et al.*, 2004, Pradeep *et al.*, 2003). Clinical cases of mastitis are characterized by the presence of one or more of the following: clots and/or blood in milk, udder swelling and systemic signs including an elevated temperature, lethargy and anorexia (Erskine, 2001). Sub-clinical cases show no visible changes in the appearance of the milk or the udder, but milk production decreases. Composition is altered and is characterized by high somatic cell count (Erskine, 2001).

In Nigeria, Ameh *et al.* (1999), reported 34.6% *S. aureus* recovery rate from bovine mastitic milk in Maiduguri; while prevalence rates of 31% and 3.2% were reported by Umoh *et al.* (1990a) from settled and nomadic herds respectively in Zaria. It is well documented that *S. aureus* is a heterogenous (polymorphic) species (Fitzgerald *et al.*, 2001a) and was found to have a clonal population structure (Feil *et al.*, 2001). It is believed that *S. aureus* does not undergo extensive recombination; rather it diversifies largely by nucleotide mutations, and shows a high degree of linkage disequilibrium. In order to distinguish strains within a heterogenous species for local epidemiologic or outbreak investigation purposes, a highly discriminating genetic marker that detects variation rapidly, such as pulsed-field gel electrophoresis (PFGE), is required (Enright and Spratt, 1999). For studying longer-term or global pathogen epidemiology and population genetics, such as the worldwide distribution and frequency of bacterial lineages, virulence properties associated with certain lineages, a highly discriminating genetic marker that accumulates genetic variation relatively slowly is desired (Enright and Spratt, 1999). However, no single technique had been clearly shown to be efficient in both outbreak and global investigation due to the different requirements for rates of accumulating genetic variation.

Most of the staphylococcal virulence factors are not required for growth and multiplication of the organism, and are often encoded by accessory genetic elements such as plasmids, prophages, and chromosomal pathogenicity islands (Baba *et al.*, 2002). Carriage by these elements implies a spread of virulence genes among

[6] **Seminar presented on 28th June2012 at NVRI auditorium**

staphylococcal strains, causing a non-uniform distribution among strains. There are a number of *S. aureus* lineages possessing unique combinations of genes that enhance ability to cause infection (Booth *et al.*, 2001).

Materials and Methods

The study was conducted in the northern senatorial district of Plateau State.

Milk Sample Collection

The study involved 339 quarter milk samples collected from 98 lactating cows, because 53 cows had blocked teat canals and 11 milk samples could not be identified in laboratory. Prior to sampling, the udder, teats and adjacent flank areas were thoroughly washed and dried with single-service sanitary paper towel. The teats were disinfected with 70% alcohol before sampling. Prior to sampling, CMT was conducted on all the quarter milk directly from the cows. About 15ml of CMT positive milk samples were then collected in sterile universal bottles from the udder.

Isolation and Identification of *Staphylococcus aureus*

Staphylococcus aureus were isolated from milk samples in dairy herds according to the protocols of the National Mastitis Council (Harmon *et al.*, 1990).

Identification and Characterization of *S. aureus*

DNA was isolated from the *Staphylococcus aureus* using the DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions.

PCR Amplification of Staphylococcal Genes

PCR amplication of the following genes 23SrRNA (specie specific gene), coa, nuc, spa (IgG)and spa(x-region) were carried out according to the protocol of Struab, *et al.,* (1999), Hookey, *et al.,* (1998), Seki, *et al.*, (1998) and Brakstad *et al.,* (1992).

Pulsed Field Gel Electrophoresis (PFGE)

Pulsed field gel electrophoresis was conducted according to the method of Maslow *et al.* (1993).

Results

California Mastitis Test

The results of CMT conducted on 339 milk samples for the presence of sub clinical mastitis are presented in Table 1

Table 1: California Mastitis Test of milk obtained from different LGA, from northern part of Plateau State.

Location of herds	No. Tested	CMT Results					Σ(CMT+)	%
		-	±	+	++	+++		
Jos South	61	33	11	4	10	3	17	27.9%
Barkin Ladi	61	39	4	5	10	3	18	29.5%
Bassa	47	27	3	7	3	7	17	36.2%
Riyom	66	42	2	3	6	13	22	33.3%
Jos East	34	18	3	0	6	7	13	38.2%
Jost North	70	51	1	3	4	11	18	25.7%
Total	339	210	24	22	39	44	105	30.9%
Percentage		61.9	7.0	6.4	11.5	12.9	30.9	

Key:
% = Percentage prevalence of subclinical mastitis in herds in the study area
CMT= California Mastitis Test
-, ±, +, ++, +++ = CMT scores
LGA= Local Government Area

Isolation and Identification of *S. aureus*

From the 105 mastitic quarter milk samples, 103 *S. aureus* were isolated. All the isolates were gram positive cocci, catalase positive, showed a typical growth on Baired Parker medium, were coagulase and clumping factor positive. All fermented manitol. Forty (39%) of the isolates showed alpha haemolysis, 20(19%) were beta haemolytic and 43(42%) were gamma haemolytic. Eighty (78%) of the isolates demonstrated a DNase activity and 90(87%) of the isolates were Voges-Proskauer positive. All the 103 Isolates were confirmed with the staphytect plus agglutination test system (Oxoid) as coagulase positive *S. aureus*.

Molecular Characteristics of the isolates

The results of the PCR amplification of certain genes of *S. aureus* are present as follows; Amplicons of the *23SrRNA* gene, *nuc, coa* and *spa* (*x–region*) had uniform sizes of 1250bp, 280bp, 600bp and 300bp respectively. Amplification of the 1gG – binding region encoding fragment of protein A gene *spa*, yielded two amplicons of different sizes of 900bp and 750bp for 18 (90%) and 2 (10%) strains respectively.

Pulsed Field Gel Electrophoresis

The results of PFGE showed that isolates in lanes 2 and 8 were different from all other isolates based on the number of restricted fragment using smaI enzyme, Figure 1

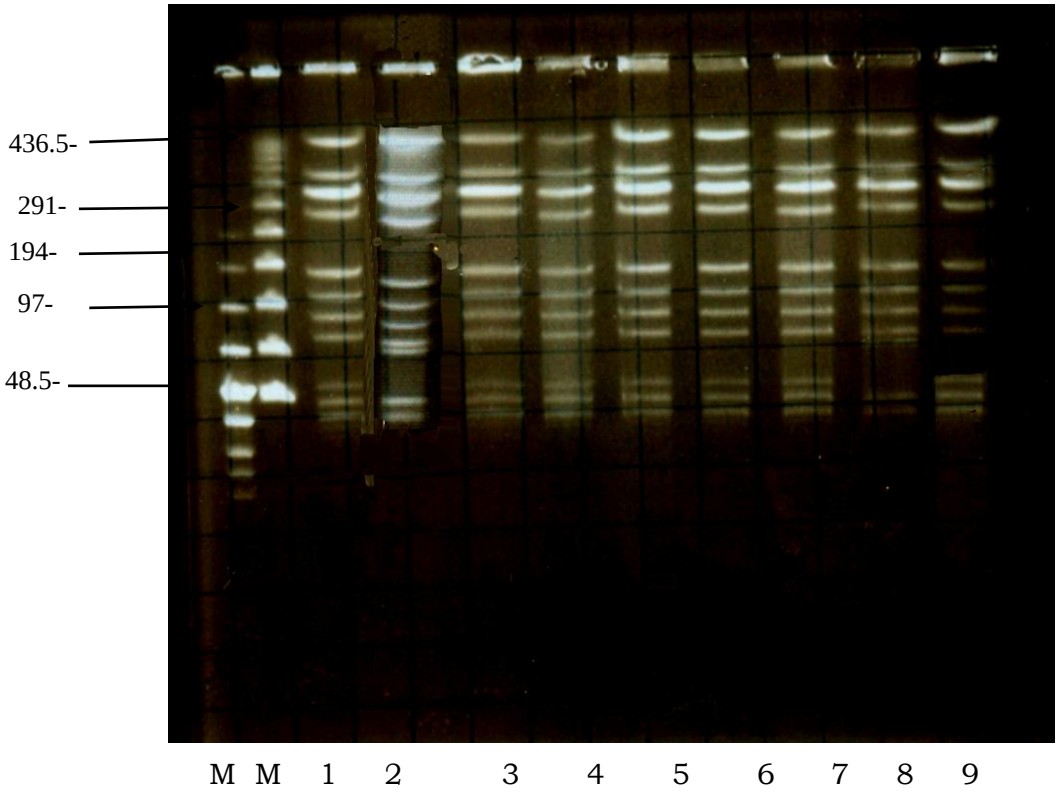

Figure 1: Pulsed-field electrophoretic restriction patterns of chromosomal DNAs of *S. aureus* isolates with DNA restriction patterns 1a (lanes 1(JES11), 3(BFR 9A), 4(JNT 3A), 5(BAKD 4), 6(JVM 9), 7(JES 94) and 9(PSC 10b)), 1b (lanes 2(JEF 4) and lane 8(BAKD 17). M 0.1- to 200-kb ladder (Low Range PFG Marker; Biolabs, Schwalbach, Germany) and a 50-to 1000-kb ladder (Lamda Ladder PFG Marker, Biolabs) served as molecular marker.

Discussion

In this study the prevalence of subclinical mastitis based on CMT, was found to be 30.9%. This is much higher than the prevalence of 3.2% among nomadic herds reported by Umoh *et al.* (1990a). This study demonstrated an association between CMT scores and *S. aureus* intra-mammary infection (IMI). The fact that, in the present study, 98% of the samples in which *S. aureus* was isolated had CMT scores ≥ +1, suggest CMT values as indicator for the presence of *S. aureus* IMI, in the dairy cows studied. Accordingly, it can be said that CMT score of +1 corresponding to 500,000 somatic cells m/s^{-1} is less than one-tenth as likely to come from infected quarters as from non-infected quarters. These results further support the use of CMT as screening tool for subclinical mastitis in low-yielding quarters at herd level. The low proportions of false positives found in the present study, suggests that CMT and microbiological screening are the most reliable indicators of on-going IMI as the CMT-positive and culture-negative samples could be explained partly due to udder trauma or effect of antibiotic treatment or infection due to other pathogens (Menzies and Ramanoon, 2001).

The characterization of *S. aureus* of 20 representative isolates by PCR amplification of various genes was carried out in order to gain better insight into the genotypes of the

isolate. All these target genes allowed a rapid identification of this species with high sensitivity and specificity, as was reported by Straub *et al.* (1999). The PCR product of the gene encoding staphylococcal protein A (1gG binding region) displayed minor gene polymorphism and allowed a genotypic characterization of the bacteria. Comparable *spa* gene polymorphism was similarly observed by Schwarzkopf *et al.* (1993). Amplification of the X region of the protein A (*spa*) gene yielded a single amplicon of 300bp for all the 20 isolates. This differs with the findings of Akineden *et al.*, (2001) who observed size polymorphism. The 20 *S. aureus* isolates were further analyzed for epidemiological relationship by macro restriction analysis of their chromosomal DNA by PFGE. This has been successfully used for investigating mastitis isolates of this species (Annemuller *et al.*, 1999). By means of SmaI macro restriction analysis, the isolates yielded 2 different PFGE patterns, which differed from each other in one fragment (1a and 1b) and thus displayed a clonal relationship as previously reported (Tennover *et al.*, 1995). The PFGE patterns, the size polymorphism of protein A (IgG binding region) substantiates the existence of a single clone of *S. aureus* responsible for the observed cases of bovine mastitis among the various herds in the study area. The result of the present study is in agreement with previous studies (Annemuller *et al.*, 1999).

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[7]THE DEVELOPMENT AND VALIDATION OF A BACTERIOLOGICAL SCREENING TEST FOR ANTIMICROBIAL RESIDUES IN EGGS

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Introduction

Poultry and poultry products including eggs are comparatively cheap sources of protein in many countries for people who cannot afford the more expensive beef, pork or fish. Residues of drugs given to birds during treatment may be found in tissues without the observance of withdrawal periods. Consumers may suffer from health risks like skin allergies, anaphylactic reactions or even toxicity if they consume eggs containing antimicrobial residues (AR), (Donoghue & Hairston, 2000). Because of these concerns, maximum residue limits (MRL) have been set for AR in food items to safeguard the consumer.

For a screening method to be of use, it must meet the European Commission Decision 2002/657/EC standard which stipulate that; "a screening method is used to detect the presence of a substance or class of substances at the level of interest. These methods have the capability of a high sample throughput and are used to sift large number of samples for potential non-compliant results. They are specifically designed to avoid false compliant results" (EC, 2002). The test must be capable of detecting most if not all possible bacterial inhibitors at the MRL in animal products. However, in Africa, even the cheapest tests can prove to be too expensive for mass application. For this reason, the new in-house bacteriological screening test was developed to screen eggs for AR.

Materials and methods:

Quality control

Bacterial culture, bacterial count checks, morphological identification, biochemical tests and Gram stains were carried out on the sub-cultured *Bacillus megaterium* ATCC 9885 and *Geobacillus stearothermophilus* ATCC 12980 to ensure Quality Control, viability and purity of cultures as described by Koneman *et al.,* in 1992.

Testing using the new bacteriological screening test for antimicrobial residues in eggs

Working in a Class II Biological Safety Cabinet to avoid contamination, sterilized nutrient broth was supplemented with 1% glucose, 0.04% phenol red indicator and seeded with approximately 10^7 colony forming units (CFU)/mℓ of *G. stearothermophilus* (equivalent to a 0.5 Mac Farland standard) was used as the new

[7] **Seminar presented on 14th June 2012 at NVRI auditorium**

test solution. 900µℓ of the test solution was tested with 100µℓ of homogenised egg sample at 80^{0}C for 10 minutes in a water bath to inactivate the natural inhibiting substance-lysozyme and to allow the bacterial spores to germinate. The temperature was reduced from 80^{0}C to 65^{0}C and incubated until the colour of the growth control changed from red to yellow indicating a negative result for AR. Each of the 18 antimicrobial test samples were run five times and in triplicate for reproducibility and repeatability of result (Table 1) (OIE, 2008).

Testing using the 36 Hen trial

A preliminary trial was conducted on 36 hens that were given therapeutic oral doses of over-the-counter antimicrobials daily for seven days based on the manufacturer's recommendations (Table 1). Eggs were collected during and after treatment and tested for AR. Several performance criteria and minimum detection concentrations were estimated and discussed.

Testing using the Kundrat micro screening four-plate test

The principle of the Kundrat micro screening four-plate test is similar to other agar-plate tests like the STAR® test. It was later used by an independent laboratory that has been accredited by the South African National Accreditation Standards (SANAS) to screen 40 treated hens and field egg samples. Results of the in-house test were compared with that of the Kundrat test (see Table 1.)

Determination of sensitivity (in aqueous solution)

A range of aqueous concentrations of 18 analytical grade antimicrobials were purchased from Sigma-Aldritch and prepared. Each antimicrobial solution was tested in the test solution several times until the minimum limit of sensitivity for each antimicrobial was obtained; which is the point where the lowest concentration of antimicrobial does not support bacterial growth (OIE, 2008).

Determination of sensitivity (in egg solution)

Temperatures of 65^{0}C, 70^{0}C and 75^{0}C were tried to inactivate lysozymes in eggs, but these temperature ranges did not work until 80^{0}C for 10 minutes was tried; fortunately, the temperature of 80^{0}C for 10 minutes did not affect the sensitivity of the antimicrobials when eggs spiked with different antimicrobials (and later field eggs) were tested over a period of five weeks.

Determination of detection capability

The minimum detection concentration (MDC) of the antimicrobials in µg/ℓ was considered to be the lowest concentration of antimicrobial at which growth was not detected (Fig: 1). The mean MDC for each of the tested antimicrobial using *B. megaterium* and *G. stearothermophilus* is shown in Table 1 and compared to the MRL and published MDC of the Premi®Test.

Survey of antimicrobial residues in commercial chicken eggs in Tshwane Metropolitan Area of Gauteng Province, South Africa

A two seasonal survey was also conducted to determine the prevalence of antimicrobial residues in randomly purchased commercial chicken eggs in Tshwane area of Gauteng Province, South Africa using the new test method. The first survey was in October/November 2008 (spring) and the second survey was in April/May 2009 (autumn).

Results and Discussion

Quality control

Geobacillus stearothermophilus ATCC was chosen as the test bacteria because it was able to detect all the 18 tested antimicrobials better than *Bacillus megaterium* ATCC 9885.

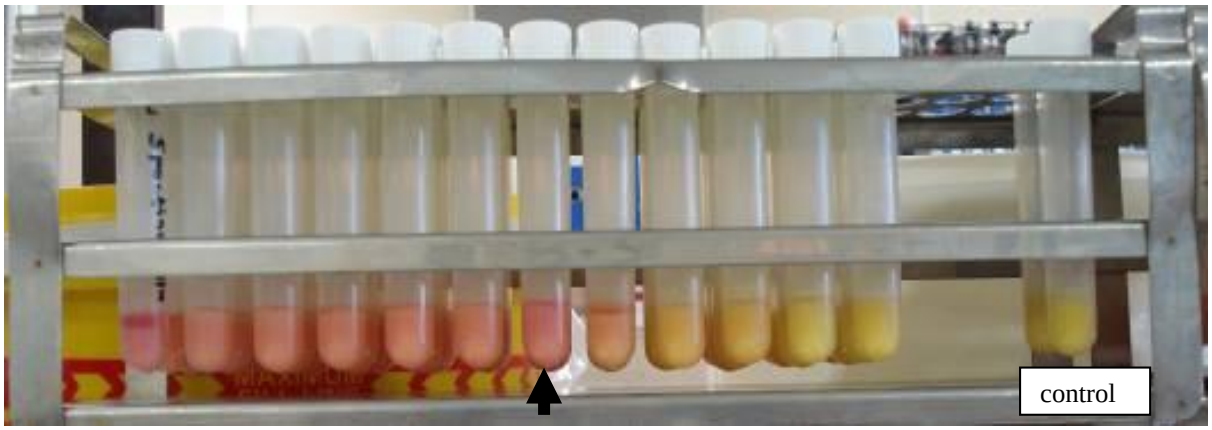

Figure 1: Broth cultures showing the minimum detection concentration of spectinomycin indicated by growth inhibition of *G. stearothermophilus* in tube 8 (indicated by black arrow).

Table 1: Average sensitivity of In-house test results for aqueous solutions of antimicrobials compared with published Premi®Test and E.U maximum residue limits-MRL

Antimicrobial	*B. megaterium* µg/ℓ	*G. stearothermophilus* Average In-house test µg/ℓ	Standard deviation of the In-house test µg/ℓ	Premi®Test µg/ℓ (published results)	MRL µg/ℓ
Enrofloxacin	30	23	2.35	250	100
Norfloxacin	1 000	247	0.33	-	NE
Neomycin	63	3.8	0.58	600	500
Tylosin	250	3	0.50	50	200
Chlortetracycline	1 028 000	163	1.51	600	200
Florfenicol	8 000	90	1.34	-	NE
Sulfadiazine	1 028 000	88	2.44	25	100
Sulphamethoxyzole	1 028 000	126	1.19	25	100
Trimethoprim	8 000	39.8	1.66	50	50
Spectinomycin	16 000	263.6	0.58	-	200
Ampicillin	2 000	2.34	0	5	50
Gentamicin	15	2.34	0	100	100
Fosfomycin	128 000	4 953	2.61	-	100
Lincomycin	64 000	4.6	1.15	150	50
Tiamulin	>64 000	367.9	1	-	1 000
Colistin	>32 000	10.5	0.58	-	300
Oxytetracycline	8 000	69.4	0.58	400	200
Doxycycline	1 028 000	6.1	1.31	200	200

Sensitivity (in aqueous solution)

The new in-house test method was more sensitive to enrofloxacin, norfloxacin, neomycin, tylosin, chlortetracycline, trimethoprim, ampicillin, gentamicin, lincomycin, tiamulin; colistin, oxytetracycline and doxycycline (see Table 1). Florfenicol and norfloxacin have no established MRL or published Premi®Test values, therefore, their MDC and sensitivity could not be compared to that obtained by the new bacteriological in-house test method. However, the new test method was able to detect and establish MDC values for florfenicol and norfloxacin in this study. Colistin, florfenicol, fosfomycin, norfloxacin, spectinomycin and tiamulin have no published Premi®Test values; therefore, their Premi®Test detection values could also not be compared to that obtained by the new test method. The Premi®Test had much better sensitivity to sulfadiazine and sulphamethoxyzole than the new test method. The new test method is therefore more sensitive than the MRL and Premi®Test of the compared antimicrobials in this study.

Survey of antimicrobial residues in commercial chicken eggs in the Tshwane Metropolitan Area of Gauteng Province, South Africa

Multivariable logistic regression was used to estimate associations of a descriptive factor(s) with AR. The confidence interval (CI = 95%), and the significance test (set at P

<0.05) were determined. Results indicated that certain egg brands, cheaper eggs (6 eggs /sample unit) for the 2 different sampling periods and informal/roadside shops sales outlet had high levels of AR in them compared to the other categories.

Conclusions

The new in-house test for the detection of AR in eggs was evaluated according to the criteria set out in Commission Decision 2002/657/EC (EC, 2002) and was compared with Kundrat test, set MRL and published values of Premi®Test. The new in-house test method was more sensitive to enrofloxacin, norfloxacin, neomycin, tylosin, chlortetracycline, florfenicol, trimethoprim, ampicillin, gentamicin, lincomycin, tiamulin, colistin, oxytetracycline and doxycycline.

Florfenicol and norfloxacin have no established MRL or published Premi®Test values, therefore, their MDC and sensitivity could not be compared to that obtained by the new in-house test method. However, the new test method was able to detect and establish MDC values for florfenicol and norfloxacin in this study.

Colistin, florfenicol, fosfomycin, norfloxacin, spectinomycin and tiamulin have no published Premi®Test values; therefore, their Premi®Test detection values were not compared to that obtained by the new test method.

The Premi®Test had much better sensitivity to sulfadiazine and sulphamethoxyzole than the new test method. Generally, the new test method screens a wider range of the compared antimicrobials.

While it cost about R140 and R100 to screen an egg sample using the Kundrat Test and Premi®Test respectively, it cost about R15.00 with the new test method, making it affordable. 80 triplicate samples (320) can be screened at a time with the new in-house test method and results read in less than 4 hours compared to the Premi®Test which can screen only 10 samples at a time within 4 hours, thus saving time, effort and money.

It is a good screening test for multi-residue testing unlike the other commercial tests that are mostly suitable for targeted or single-residue testing. Other test like ELISA, HPLC and MS are expensive to purchase (thousands of rands) and run requiring skilled personnel to manipulate compared to this cheap method that does not require skilled personnel to perform it.

The storage temperature of -20^{0}C was able to keep spiked egg samples for 60 days without loss of stability. The new in-house test is more sensitive than the Kundrat micro-screening four-plate test for AR in eggs since it screened a wider range of egg samples for AR. Certain egg brands, cheaper eggs and eggs sold at the informal/roadside shops outlets had high levels of AR in them compared to the other categories in RSA. They are therefore important determinants of the odds of finding AR

in eggs in RSA and further studies are indicated to more specifically investigate these factors.

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[8]MEDICINAL PLANTS USED BY FULANI HERDSMEN IN THE MANAGEMENT OF ANIMAL DIARRHOEA IN PLATEAU STATE, NIGERIA

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Introduction

The Fulani tribe found mainly in Central, Western and Northern Africa hold a large number of livestock. In Nigeria and most parts of Africa, mobile pastoralism is the dominant system of livestock management practiced by pastoralists. It is generally believed that Fulani herdsmen have good knowledge of medicinal plants because as they move from one place to another they depend on these plants to tackle their health challenges as well as those of their animals (Abdu *et al.*, 2000).

More recently veterinarians and other scientists in recognition of the fact that livestock owners possess considerable understanding of herbal remedies and their application in disease management have intensified their efforts towards harnessing this knowledge in dealing with livestock diseases. Most livestock diseases present diarrhoea as a symptom with adverse effects reported to include anorexia, weight loss, general malaise and death (Gattuso and Kamm, 1994). Despite the immense technological advancement in modern medicine, many people in developing countries still rely on herbal drugs for the management of diarrhoea. Several investigators have contributed to reports which establish the use of plants in the treatment of diarrhoea in South Africa (De Wet *et al.*, 2010; Appidi *et al.*, 2008; Mathabe *et al.*, 2006), Mozambique (Ribeiro *et al.*, 2010), India (Tetali *et al.*, 2009) and Sokoto State, Nigeria (Etuk *et al.*, 2009). A review of available literature shows that such survey has not been conducted in Plateau State, Nigeria. The state has a large population of Fulani nomads probably due to the favorable climate all year round (FAO, 2009). The need to preserve and transfer indigenous knowledge from one generation to another is imperative in order to prevent the rapid depletion of such knowledge (Prance, 1991; Cox, 1990). This study is therefore intended to record the medicinal plants used for the treatment of diarrhoea by Fulani herdsmen in Plateau State.

Materials and Methods

The data was collected through oral interview of Fulani herdsmen from 9 selected local government areas of Plateau state Nigeria (Figure 1) which spread across the 3 senatorial zones of the state during the months of October to December 2010. The selected LGAs are known to have high population of cattle and favorable environment

[8] **Seminar presented on 29th Nov 2012 at NVRI auditorium**

for livestock production (Bertu *et al.*, 2010). Plants claimed to be beneficial in the treatment of diarrhoea were collected based on the guided field-walk method (Rashid *et al.*, 2010). The plant specimens collected were pressed, labeled with their local names where available and sent to the herbarium of the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, where they were identified, authenticated and voucher number assigned to them.

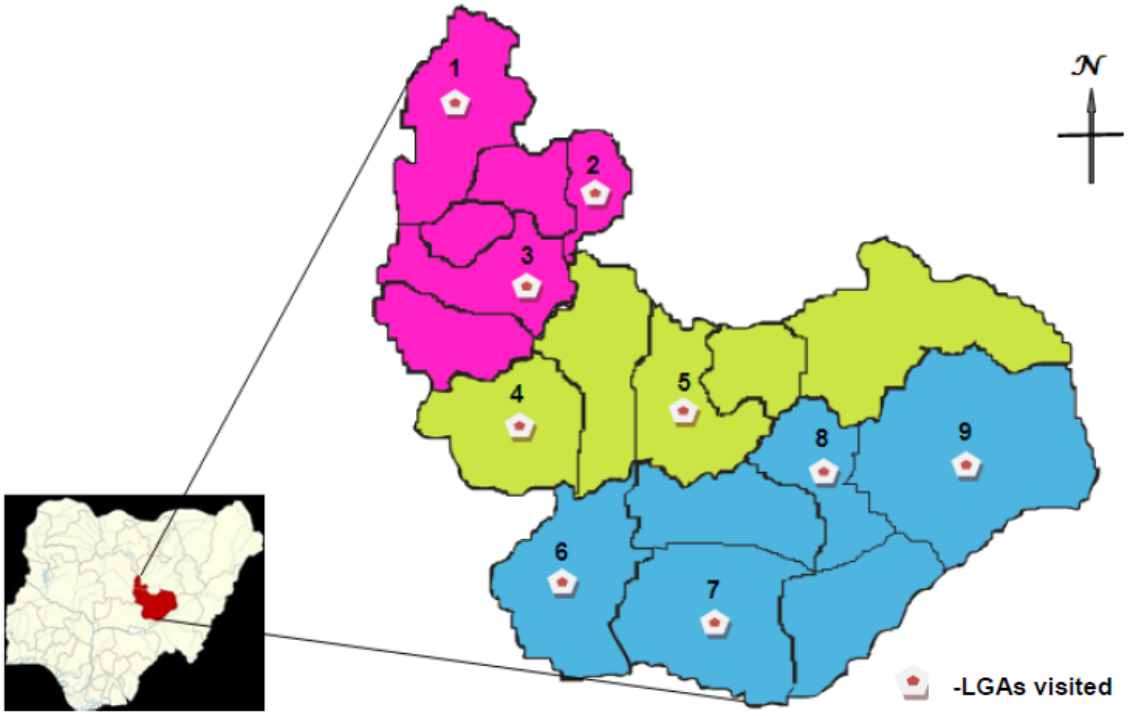

Figure 1. Map of Plateau State, Nigeria showing LGAs visited. Bassa (1), Jos East (2), Barkin-Ladi (3), Bokkos (4), Pankshin (5), Qu'an Pan (6), Shendam (7), Langtang North (8) and Wase (9) LGAs. Pink – North, Green – Central and Blue – Southern geopolitical zones.

Results

One hundred and five questionnaires were administered directly during the survey. A total of 87 (82.86%) respondents admitted having used antidiarrhoeal medicinal plants or were still using them to treat their animals. Eighteen (17.14%) had no knowledge of herbs or medicinal plants used for the treatment of diarrhoea in animals. Most of the respondents were able to give adequate description of the nature of the diarrhoea often seen in their animals. Data generated from the survey indicated seventy-nine (79) medicinal plants as remedies in use for diarrhoea management out of which twenty-eight (28) were properly identified by their scientific nomenclature and local names (Table 1). The 28 plants scientifically identified represents 23 genera distributed among 17 families (Table 1), with the families Fabaceae (21.43%) having the highest frequency of occurrence followed by Combaretaceae (17.86%). Moraceae and Verbanaceae had 2 (7.14%) members each while all other families were mentioned once (3.57%). *Khaya senegalensis* 26 (24.76%) was the most common plant mentioned

followed by *Adansonia digitata* 10 (9.52%). *Vitex doniana* was mentioned 9 (8.57%) times while *Combretum glutinosum*, *Terminalia avicennioiodes* and *Terminalia macroptera* were mentioned 7(6.67%) times each.

Various parts of these plants in use were also indicated (Figure 2), with the leaves being the most commonly mentioned (42.86%). Plant parts to be used are usually prepared by soaking the fresh or dried plant parts in water and the extract administered by drenching. In some cases, the plant materials are mixed with feed and/or potash to improve palatability.

Table 1: Family distribution of medicinal plants used by Fulani herdsmen in Plateau State

FAMILY	FREQUENCY	PERCENTAGE
Asclepiadaceae	1	5.88
Bignoniaceae	1	5.88
Bombacaceae	1	5.88
Caricaceae	1	5.88
Combretaceae	5	29.41
Fabaceae	6	35.30
Leguminoceae	1	5.88
Liliaceae	1	5.88
Meliaceae	1	5.88
Moraceae	2	11.77
Myrtaceae	1	5.88
Rubiaceae	1	5.88
Sapotaceae	1	5.88
Scrophulariaceae	1	5.88
Solanaceae	1	5.88
Verbanaceae	2	11.77

Table 2: Common Medicinal plants used in the management of diarrhoea by Fulani herdsmen in Plateau State

Plant	Common Name	Family	Hausa/Fulfulde Name	Frequency	Part used
Khaya senegalensis	African mahogany	Meliaceae	H: Madaci F: Dalehi-kahi	26	SB
Adansonia digitata	Baobab	Bombacaceae	H: Kuka	10	L
Vitex doniana	Black plum	Verbanaceae	H: Dinya F: Bodilohi	9	L, SB, F
Combretum glutinosum	-	Combretaceae	H: Kantakara, Baushe	7	L, R
Terminalia avicennioides	-	Combretaceae	H: Baushe	7	L, R
Terminalia microptera	-	Combretaceae	H: Baushe F: Bodi	7	L, R
Parkia biglobosa	Locust bean, Monkey cutlass tree	Fabaceae	H: Doruwa	3	SB

H= Hausa, F= Fulfulde, L= Leaves, R= Roots, SB= Stem bark, F= Fruits

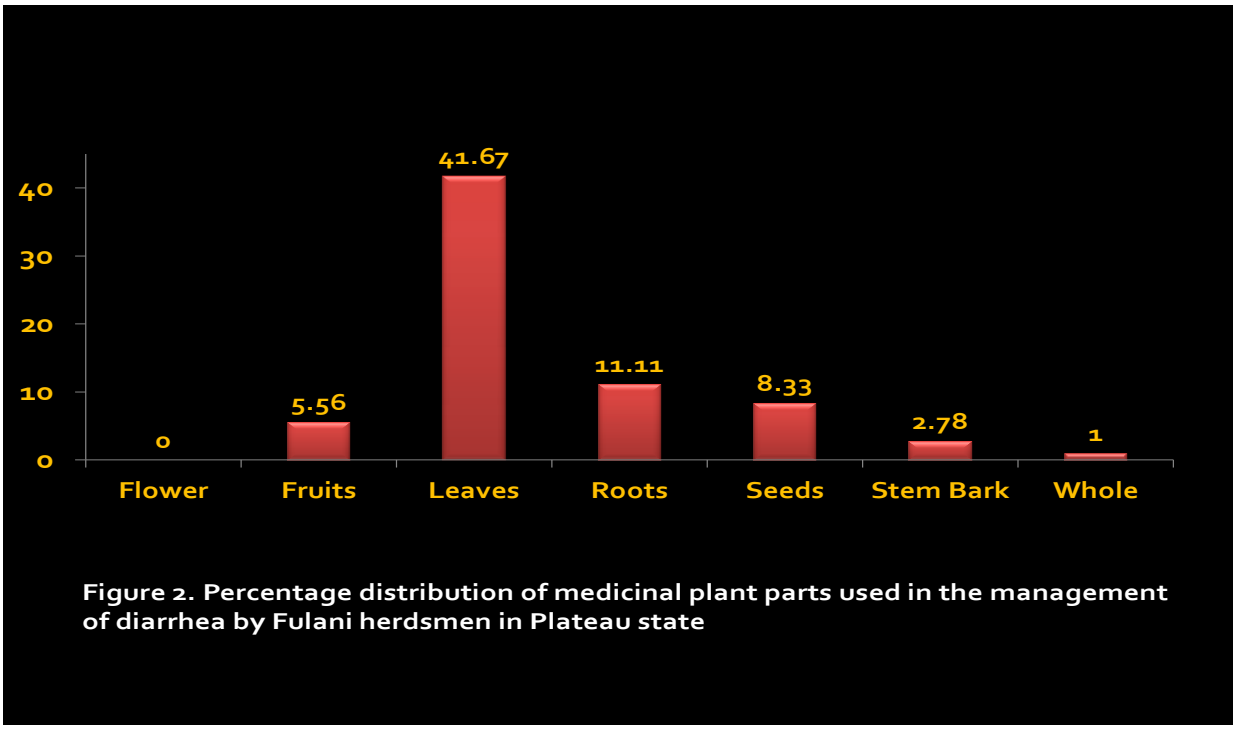

Figure 2. Percentage distribution of medicinal plant parts used in the management of diarrhea by Fulani herdsmen in Plateau state

Discussion

The high proportion, 87 (82.86%) of herdsmen that use herbal remedies to manage animal diarrhoea, agrees with earlier reports on the relevance of different traditional healing practices in Nigeria as well as other parts of the world (Abdu *et al.*, 2000; Mathias, 1994; McCorkle, 1986). The reliance of pastoralists on herbal remedies for

both prophylactic and therapeutic purposes in Nigeria has been reported (Abdu *et al.*, 2000; Kudi and Myint, 1999)

Khaya senegalensis 26 (24.76%) *and A. digitata* 10 (9.52%) are the plants commonly used by Fulani herdsmen in the management of diarrhoea in livestock. Another survey of ethnoveterinary practices of agro-pastoralists in eleven selected states of Nigeria also reported that *K. senegalensis* and *A. digitata* are the most common plants used as remedies for various livestock diseases (Abdu *et al.*, 2000).

Fabaceae is the most common plant family reported in this study, having 6 genera (21.43%), followed by Combaretaceae which has 5 (17.86%). A similar observation suggesting that the Fabaceae family may be more likely to have anti-diarrhoeal property than plants from other families has been made (Appidi *et al.*, 2008).

The observation that the leaves (42.88%) constitute the most frequently used plant part agrees with a survey of plant parts used in Dheera town Arsi zone in Ethiopia which reported that leaves are the most frequently used plant part in herbal preparations (Wondimu *et al.*, 2007). Communities using herbal medicaments have indicated preference for the use of leaves because it is more convenient collecting leaves than root parts, flowers and fruits (Giday *et al.*, 2009). It is known that leaves are actively involved in photosynthesis and the production of metabolites (Ghorbani, 2005). Thus, the numerous constituents found in leaves could explain their efficacy in the treatment of various ailments in both humans and animals. Collection of leaves for herbal preparations ensures sustainability as long as some leaves are left on the parent plant (Yinegar *et al.*, 2007). This is opposed to the collection of roots which could be a threat to rare and slowly producing plants.

Conclusion

The Fulani herdsmen are a relevant source of information on medicinal plants used for the management of diarrhea in animals owing to their nomadic nature. Such plants could be harnessed and used as potential drug sources.

Acknowledgement

The authors are grateful to the management of the National Veterinary Research Institute Vom, for funding the project; the University of the West Indies, St. Augustine, Trinidad and Tobago for releasing Dr. Offiah for this project, Mr Jamo Aliyu and Simon Emmanuel of the Extension services Department for linkage with the farmers and Mr. U. S. Gallah of Biological Sciences Department, ABU, Zaria for identifying the plants. We are grateful also to the Chairmen of LGAs visited and their extension Staff, the Miyetti Allah Cattle Rearers Association, all Village Heads and their subjects for their cooperation and assistance.

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[9]HISTOSTRUCTURES OF SKELETAL MUSCLES OF SHEEP WITH PRODUCTIVITY CONCEPT, AGE AND NUTRITIONAL LEVEL

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Introduction

Meat is an important product which is the main source of valuable protein for human beings. Recently, the selection of sheep for breeding has been geared towards increased meat productivity. With the changing market economy, meat production in sheep has become profitable, whereas the value of wool in relation to its realization, has significant decreased while the cost of its production is many times higher than meat.

Most of the sheep breeding areas in Russia with this aim have started using meat-fat breeds of sheep directed at increased productivity. Meat-fat breeds of sheep have early maturation time and good quality meat. Edilbavskoi sheep, from Kazakhstan is a good example of meat-fat breeds of sheep. Russian Scientist involved in breed selection use imported meat-type breeds from Finland, Netherlands and Australia e.g. Teksel, Polldorset and others. Native Russian breeds are sometimes used for such studies.

In recent times, the increase demand of meat does not depend on the quantity of the indices of meat productivity but also on the quality of the meat itself. Scientist believe that in line with other quality indices, the character of the microstructures of skeletal muscles allow for more objective evaluation of the quality of meat (Hemmond, J. 1937; Bogolyuski, C. H. 1971). The aim of this study is to compare the histological morphology of skeletal muscles of different breeds of sheep. This study also explores the age differences of the muscle structures and the influence of level of nutrition on their formation in Edilbafskoi.

MATERIALS AND METHODS

The muscle samples for the study of the influence of cross breeding on the histo-structures was collected from carcasses of experimental young sheep from experimental farm of a research Institute (Staveropol, Animal and Feed Production), where scientists of that Institute formed four (4) groups of rams (7 per group) for comparative study of the quality of their feeding (Nutritive value). Formation of groups was carried out by raising rams at stalled fattening from 7.5 months of age. They were slaughtered at 9.5 months of age at the end of fattening. The study of the changes in histostructures of skeletal muscles of Edilbafskoi rams with age and the influence of that factor on the level of nutrition also leads to comprehensive research. The

[9] **Seminar presented at NVRI auditorium**

population of the experimental rams of Edilbafskoi breed was supplied by scientists from Moscow Agricultural Academy from the sheep farm of Volgogradski region, Physiological Animal Farm. For the study of histostructures of muscle tissue with age differences, samples were taken from the carcass of 3 rams per day, new born, 7 and 10 months old rams.

For the study of influence of level of nutrition in forming meat products, scientist from the section of rearing and nutrition (Vija) formed 2 experimental groups of animals, 10 animals per group starting from 5 months of age. One group was kept in moderate plane of nutrition, in accordance with standard (Vija), the second group was kept under intensive plane of nutrition, higher than the standard by 23%. They were slaughtered at 7 and 10 months of age.

Histological morphology of muscles in all the experimental animals i.e. longissimus spinosus muscle, since it belongs to the largest muscle of the vertebral column, which also belongs to group of high-grade meat composition and traditionally used for comparative characteristics of microstructure of muscle in different species of animals. Samples were taken from three animals from each experimental group similar in live weight.

In total, twenty seven (27) samples were collected. At the time of separating the longissimus spinosus muscle from the carcass, a piece of the muscle was taken from the last thoracic and the 1st lumber vertebrate size of 1.5 – 2cm and was fixed in 10% neutral formalin. Preparation of the histo-slides and their study was conducted in University Laboratory (PFUR). During preparation, staining and describing histological slides. We applied method indicated by histology of muscle tissue (Romes, D.; Popkov, G. A., 1974).

Results and Discussion

Muscles of hybrid rams compared with pure breeds of Severekafkaskoi but differ less in amount of primary bundles of muscle fibres and has less content of muscle firbres inside them. The average area in the muscle of hybrids CK x TK is 1.5 times, CK x PD is 1.6 times, CK x ED is 1.2 times less than the Severokafkaskoi pure-breeds. The quantity of muscle fibre within the bundles of experiment groups of rams fluctuates at a range of 59 to 74. Most of the muscle content is observed in the muscles of Severokafkaskoi rams and exceeds hybrids CK x TK by 39.8%, CK x PD by 25% and CK x ED by 12%. Muscles of hybrids received from cross-breeding with imported breeds (rams) differ comparatively having thin perimysium between primary bundles of muscle fibres, more developed septation, and endomysium. Width of septum in hybrids CK x TK – 12 microns endomysium – 6.8 microns CK x PD – 15.3 microns and 7.2 microns, CK x ED – 9.8 and 5.9 microns pure breed Severokafkaskoi – 8.1 and 5.4 microns respectively. Diameter of white muscle fibres in the muscles of all the experimental group of rams at an average 2-2.5 times exceeds diameter of red muscle

fibres and 1.5 times – intermediate. On the account of large amount of white muscle fibres (37% against 19% in CK x ED; 18% in CK x TK and 15% in CK x PD) the average diameter of muscle fibres in the muscles of Severekafkaskoi is the largest (32.3 microns) and exceeds groups CK x ED by 2.9 microns, CK x PD by 3.4 microns. Perimysium of hybrids CK x ED differ due to the presence of accumulated lipocytes than in the perimysium of muscles of hybrids with imported breeds (rams), lipocytes are located as a narrow layer or small aggregation. Relative of fatty tissue on the histo-section of rams muscles CK x ED consist of 8.8%, hence it is 1.5 – 1.6 times more than other groups of hybrids. With age, intramuscular structures of edilbafskoi breeds rams undergo significant changes. Increase in area of muscular bundles in 7 months old rams in comparison with newborn lamb is 2.9 times higher. While in 10 months old rams, it is 3.4 times higher. For width of connective tissue layer, the width of the perimysium between the secondary bundles is 9.9 microns in newborn lamb and between prmary bundles is 1.8 microns, while endomysium is 0.9 microns respectively. In 7 months old rams, it was 57.2 microns, 20.1 microns, and 4.7 microns respectively. While in 10 months old ram, it was 149.3 microns, 35.5 microns and 6.3 microns respectively.

The intensive nutrition of edilbafskoi breeds promotes the formation of more quantity of bundles of muscle fibres with predominant contents of muscle fibres in them (in 7 months of age – by 8.5%, in 10 months of age by 17.6%) and with more narrow perimysium (in 7 months or age there was a difference in width of perimysium between secondary bundles which consisted of 40.9%, between primary bundles – 42.5%).

Conclusion

In conclusion, the hybrids rams obtained from crossbreeding meat-wool severekavkaskoi breeds with imported meat-type breeds, Teksel and Poldorset have more optimal internal muscle structures which attest to high quality of their meat products and general utilization of those breeds for commercial breeding. In relation to intensive internal deposition of fat, Edilbafskoi pure breeds and hybrids obtained from crossbreeding with Edilbafskoi breeds, is recommended for meat at 7 months of age with commencement of fattening from 5 months of age under intensive plane of nutrition.

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