NVRI SEMINAR SERIES 2007/2008

This Seminar series is a publication of seminar papers presented by staff and visiting researchers to the National Veterinary Research Institute, Vom during 2007 and 2008

Compiled and edited by Dr (Mrs.) M. Muhammad and Dr. A. T Oladokun

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FORWARD

This is the third in the series of compilations of seminars presented at the National Veterinary Research Institute, Vom. It is aimed at documenting some of the research activities taking place in NVRI. It also aims to acquaint the research community and staff of issues and policies guiding the conduct and administration of research. Topics pertinent to staff and of common interest were also presented.

Continued progress in the quality of publications and research activities is reflected in the increased use of modern technologies in the conduct of research and increasing collaboration with international organizations. The Institute has also witnessed an increasing number of visiting research scientists all of whom have added value to research and production activities of the Institute.

Papers covered in this series include a wide range of topics ranging from diagnostics and disease prevention to project monitoring and planning for retirement. It is hoped that the publication adds knowledge to current issues in veterinary research and disease control.

Dr. (Mrs.) M. Muhammad
Chairman, Seminar Committee
2008

Rabies Virus Antigen in the Brain of Apparently Healthy Slaughtered Dogs in Nigeria: Public Health Implications

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Clinical rabies has historically been regarded as being uniformly fatal because diagnosis could only be confirmed where brain tissue was available for examination (Umoh and Blenden, 1980). However reports have over three decades shown that rabies is not invariably fatal and cases have occurred in which victims did not die but remained carriers (Bell, 1975; Gribencha, 1975; Fekadu and Baer, 1980; Fekadu, 1988).*

The diagnosis of rabies can be achieved by detecting viral antigens in the brain of suspected animals using fluorescent antibody test (F.A.T). Mouse inoculation test (M.I.T) and Microscopic examination for Negri bodies (M.E.N) otherwise called Sellers staining technique could also be diagnostic tools (OIE Manual, 2000).

This paper discusses the presence of rabies virus antigen in the brain of apparently healthy slaughtered dogs from two states in North Western Nigeria and its public health implications.

Materials and Methods

The study was carried out at Sokoto and Katsina States in the north western region of Nigeria. Fifty heads of apparently healthy dogs were collected at 'mami markets' of the Army Barracks in Sokoto and Katsina States where bars and restaurants exist in which dogs are slaughtered as a delicacy for human consumption. Samples of Hippocampus were removed as described by Atanasiu (1975) and stored at -20^{o} C until analyzed. Specimens were subjected to F.A.T and M.I.T and those that were positive by the two methods were then subjected to M.E.N.

Fluorescent antibody test (FAT) was carried out as described in OIE Manual (2000) with minor modifications. Briefly, the brain smears were fixed in cold acetone, stained with Light diagnostic antinucleocapsid monoclonal antibody labeled with Fluorescein-isocyothionate rabies fluorescent antibody assay DFA (monoclonal antibody FITC-conjugate) catalog No.5100 reagent from Chemicon International Inc. 1-800-437-7500. The stained smears were then observed for apple green fluorescence under Leitz Ortholux fluorescent microscope.

For the mouse inoculation test (MIT), six 21 day-old specific pathogen free (SPF) albino Swiss laboratory mice, weighing 14g average were inoculated intracerebrally with 0.03ml of a suspension of each brain sample at 10% of phosphate buffered saline (PBS) pH 7.2. Two drops of 500 I.U penicillin/ml and 1560 I.U Streptomycin/ml were then added to the suspension. The mice were observed daily for 30 days. The appearance of the symptoms (circling & spinning movement, bristling of the fur, agitation, paralysis and death) were monitored and recorded.

* Paper presented on 15th March, 2007 at NVRI auditorium

Microscopic examination for Negri bodies (MEN) was carried-out from impressions of the hippocampii using sellers' stain (two part of 2% methylene blue in methanol to one part of 1% basic fuchsin in methanol). Slides were washed in running tap water, air-dried for 30 minutes at room temperature and observed under oil immersion(x100) using a light microscope. Negri bodies were observed in the cytoplasm and dendrites of the neurons, presenting an acidophilic stain, with basophilic internal granulations.

Results

Of the fifty brain specimens tested, 13 (26%) were positive by FAT, while 10 (20%) were positive by MIT. Of the 10 samples that were positive by MIT, only one sample was FAT negative. All the remaining 9 samples were also FAT positive. Fourteen (14 (28%) of the 50 samples were positive for rabies antigen by both techniques. Of the 14 positive samples, only 3 (21.4%) were positive by MEN and only those graded 3+ intensity of fluorescence by FAT as well those positive by MIT showed Negri bodies.

Discussion

The detection of rabies antigen in 28% of samples suggests that rabies in dogs may not invariably be fatal. The presence of rabies antigens may be a result of apparent adaptation some strains of the rabies virus and dogs, manifested in latent infection. Adaptation by the rabies virus has long been known to occur after several transfers of the virus in chick embryo (Koprowski, 1954) and in tissue culture (Wiktor *et al*, 1964). This modification in the reservoir host probably allows for the presence and accumulation of the viral antigen in the brain without apparent effect on the dogs in this study. Gribencha (1975) successively reproduced abortive rabies in rabbits infected intracerebrally with highly pathogenic strain of street rabies virus and in white rats infected with the Challenge virus strain (CVS) strain of fixed virus and suggested that different forms of rabies infection may probably exist in nature. The three samples in this study that showed Negri bodies may have been rabid or were recovering but did not show symptoms at the point of slaughter and were considered apparently healthy. This may be concordance with the finding of Fekadu (1988) who reported that up to 20% of dogs experimentally infected with street rabies virus that were initially showing signs recovered without any supportive treatment and concluded that rabies is not invariably fatal. The public health implication of this finding is that humans and other animals are at a risk of contracting rabies from unrecognized apparently healthy rabies carrier dogs. It is suggested that the norms for human treatment should be carefully re-examined; especially bites involving apparently healthy dogs and bites from apparently healthy dogs should be considered rabies suspect and at least pre-exposure or post exposure rabies prophylaxis be initiated before completion of laboratory confirmation where possible.

Acknowledgement

I am grateful to Prof. S.I. Oboegbulem, Dr. (Mrs.) L.H. Lombin Executive Director National Veterinary Research Institute, Vom-Nigeria and Dr. A.T. Elsa Dean Faculty of Veterinary Medicine Usmanu Danfodiyo University, Sokoto-Nigeria for their support.

References

Atanasiu P; (1975). Animal inoculation and the Negri body. In: The Natural History of Rabies G.M.Baer(Ed). Academic Press Inc. New York Vol.1:Pp373-400.

Bell, J.F. (1975).Latency and abortive rabies. In: The Natural History of Rabies G.M Baer (ed.) Vol.1PP.331-350.

Boulger, L.R. and J. Hardy. (1960). Rabies in Nigeria. West Africa .Med.J. 9(6),223-234.

Fekadu, M. and G.M. Baer. (1980). Recovery from rabies of two dogs inoculated with rabies virus strain from Ethiopia. American Journal of Veterinary Research. 40(10), 1632-1634.

Fekadu, M. (1988). Pathogenesis of rabies virus infection in dogs. Rev. Infect. Dis.10 (4), 678-83.

Gribencha, S.V. (1975). Abortive rabies in rabbits and white rats infected intracerebrally. Arch.Virol.49(4),317-22.

Koprowski, H. (1954). Biological modification of rabies virus as a result of the adaptation to chicks and developing chick embryos. Bull.WHO.10, 709-724.

Office International Des Epizooties. (2000). OIE manual on rabies. Pp277-291.

Umoh, J.U. and D.C. Blenden. (1980). Immunofluorescent staining for rabies antigen in the skin of infected animals. Nigerian. Vet. J. 9, 33-39.

Wiktor, T.J.; Fernandes, M.V.; And Koprowski, H. (1964). Cultivation of rabies in human diploid cell strain WI-38. J. Immunol.93, 353-366.

Disease Problems of Exotic *Bos Taurus* Cattle and their Crosses in the West African Geo-Climatic Region

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Ticks and tick-borne diseases such as Babesiosis, Anaplasmosis, and Heart-water are known to be major constraints in up-grading cattle production using imported Bos-Taurus breeds in this sub-region (Callow, 1978; Uilenberg, 1982; Ajayi *et al*, 1983; FAO, 1984). A number of diseases are also additional impediments if not planned for in health programme. These include trypanosomosis, dermatophilosis, besnoitiosis, Lumpy Skin Disease, Foot and Mouth Disease (Davies, 1991; Koney, 1996; Sumpton, 1996).†

Bovine babesiosis is caused by *Babesia bovis* and *B.bigemina*, which are transmitted mainly by Boophilus ticks. It is characterised by fever, haemoglobinuria, anaemia, icterus and emaciation in the chronic stage.
In *B.bovis* infection, central nervous system involvement is present. The disease is managed using **c**linical, parasitological monitoring and regular tick control. While diminazene aceturate (Berenil) will take care of *B. bigemina* infection, Imidocarb dipropionate (Imizol) is needed for *B.bovis* infection.
Bovine anaplasmosis is caused by a rickettsial organism, *Anaplasma marginale,* which is transmitted biologically by ticks, mechanically by biting arthropods and unsanitary operations. It is characterised by fever, parasitaemia, progressive anaemia and emaciation. The disease is managed by good and regular tick control, grazing management using improved and fenced paddocks. Also, clinical monitoring of the herd including parasitological evaluation and treatment using oxytetracycline formulation.

Cowdriosis (heart-water) is caused by *Ehrlichia* (Cowdria) *ruminantium* transmitted by *Amblyomma variegatum* ticks. It is characterised by high fever, anorexia, listlessness, dyspnoea restlessness, intermittent convulsions and death. The disease is managed by strict tick control, clinical monitoring of the herd and chemotherapy using long acting oxytetracycline.

Bovine trypanosomosis is caused by *Trypanosoma vivax, T.congolense*, and rarely *T.brucei* transmitted by Glossina species. It is characterised by intermittent fever, intermittent parasitaemia, anaemia, progressive emaciation in addition to production and reproduction losses. The disease is managed by using trypanocidal drugs which is now beset by drug-resistance problems. However, control using chemotherapy and/or chemoprophylaxis, tsetse surveillance and tsetse traps will minimize the problem since there is no vaccine available.

Dermatophilosis is caused by *Dermatophilus congolensis* bacterium associated with *Amblyomma* tick infestation, trauma from biting flies and rainfall. It is characterised by exudative dermatitis, crust formation which drop off to leave permanently damaged skin. There are also characteristic paint-brush lesions. The disease is managed using strict tick control, long acting oxytetracycline plus culling. Efforts are in progress to find alternative treatments.

Bovine besnoitiosis (globidiosis) is caused by a protozoan parasite *Besnoitia besnoiti* and possibly transmitted by ingestion of isosporan-type oocyst. It is characterised by fever, progressive inappetence, severe respiratory problem and listlessness. There is scleroderma

† Paper presented on 29th March, 2007 at NVRI auditorium

with hyperkeratosis alopecia and thickening of skin while infected bulls become sterile.

Lumpy Skin Disease (LSD) is caused by an African Capri pox virus and transmitted by biting insect vectors. It is characterised by fever which may persist for one week, anorexia, rhinitis and conjunctivitis. There is severe reduction in milk yield, and characteristic nodules develop over the body particularly on the head, neck, udder and perineum (Davies, 1991).The disease is managed by culling since there is no treatment.

Foot and Mouth Disease (FMD) is caused by a highly contagious virus SAT1, SAT2 and O. The disease is transmitted by direct contact between infected and susceptible animals or exposure to excretion and secretion of infected animals. It is characterised by fever, anorexia, depression and severe drop in milk yield.
There are vesicles on the tongue, udders and feet. The lesions can lead to lameness and mastitis (OIE, 2004).
FMD is a major constraint on effort to up-grade livestock production as it imposes severe restriction on internal and international trade on livestock products.

Exotic and cross bred animals ought to be vaccinated in this region.

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References

Ajayi, S.A., J.P. Fabiyi and I.Umo (1983) Clinical Anaplasmosis and Babesiosis in Friesian cattle. World Animal Review. 36, 68.

Callow, L.L (1978) Ticks and Tick-borne Diseases as a barrier to the introduction of Exotic cattle to the tropics. World Animal Review 28, 20-25

Davies, F.G. (1991) Lumpy Skin Disease. An African capripox virus Disease of cattle. Brit. Vet. Journal 147, 489-503.

F.A.O (1984) Prevention of loss from tick-borne diseases and ticks in cattle imported by developing countries. In Ticks and Tick-borne Disease Control. A practical field Manual Vol. 11, 597-621. Rome

OIE (2004). Manual of Diagnostic Tests and Vaccines for terrestrial Animals 5th edition. Featuring Foot and Mouth Disease, Lumpy Skin Disease, Heartwater, Anaplasmosis, Bovine babesiosis, tsetse borne Trypanosomosis and dermatophilosis.

Sumpton, K.J (1996). The control of heartwater in West Africa- present and future. Trop. Anim. Hlth. Prod.28, 655-735

Uilenberg, G (1982) Disease problems associated with the importation of European cattle in the tropics. In proceedings 12th world congress on diseases of cattle. Amsterdam vol 11.1025.

Enhancing Information Service Delivery in N.V.R.I. Library

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NVRI library is a special library designed to provide access to specialized or subject information, packaged to address the needs of a special clientele. Special libraries are distinguished from other libraries by their emphasis on the information function. They serve a particular group of people, such as employees of government agencies, research organization, members of professional group, e.t.c. [°]

The goal of the library is to support the institute's programme of research, diagnostic investigation services and training. It does this by collecting, organizing, storing, retrieving and disseminating recorded knowledge in the forms of books, journals, reprints, reports, video and audio visual materials, maps and other materials of research value to veterinary medicine and allied fields of sciences and technology. The library is designated for research purposes only. It is to serve mainly, the research officers in the Institute and clients on referral visits.

The objectives of the N.V.R.I. library include

1. To provide the research scientists access to the right information, in the right form at the right time.
2. To establish a computerized information system for easy access to the current information on animal health and production from all over the world
3. To provide an opportunity for all states of the Federation gain access to scientific information on the country's animal health and production activities.
4. To create and manage local databases on the country's animal health and production research activities.
5. To provide extension workers nation-wide access to expert systems which cover a wide range of problems and solution, to enable them transfer this knowledge to end-users.
6. To provide scientific information services to university researchers ad academics, polytechnics, other institutions of higher learning, businessmen and policy makers.
7. To link up with international computerized information systems such as

Database of the Commonwealth Agricultural Bureau (CAB Abstracts), Agricola Database, Medline Database, FAO Agrindex and Agris Databases, CD ROM by the consultative Group on International Agricultural Research. Biosis reviews, Chemical Society Abstracts, Food Science and Technology Abstracts on Tropical Agriculture.

The E-library

An e-library is a library whose holdings have been digitized and made available to users via terminals installed on site. In other words, it is a collection of journal article, reports etc made available for online or off-line reading or download. It is a facility that makes it possible for the users to conduct research over the internet or on a standalone computer. In response to this development in information service provision and the need for our researchers to keep abreast with current development in research, an electronic library has been established in the Institutes' library through Management initiative.

CD-ROM Services

The following are some of the Off-line resources available in the library.

[°] Paper presented on 7th June, 2007 at NVRI auditorium

CAB Abstracts
Commonwealth Agricultural Bureau International CAB Abstract is the most comprehensive bibliographic database covering agriculture, forestry, human nutrition, veterinary medicine and the environment.

Medline
Medline is the database version of Index Medicus and it covers many aspect of veterinary medicine, particularly small animal.

Beast CD
It contains information mostly on large animal management, production and technology. The index contains information dating from 1973 forward.

Vet CD
Vet CD referred to as veterinary science database covers all aspect veterinary medicine, arthropod, helminthes, protozoa and fungal diseases of domestic and wild animals. Attention is given to zoo animals, wild animals, pets and farm animal.

It contains abstract citation published in Index Veterinarius and Veterinary Bulletin with bovver 750,000 records.

Internet Services
Access to Global On-line Research in Agriculture (AGORA)
AGORA is an internet portal with links to major scientific journals, bibliographic databases and other internet resources related to agriculture.

It is developed by Food and Agricultural Organization (FAO) in collaboration with World Health Organization (WHO), major scientific publishers and Mann library of Cornell University USA.

It also includes information on related sciences and social sciences such as environmental sciences, Food policy and agricultural economics.

It is one initiative that has given agricultural researcher and other users from this part of the world opportunity to have access to a wide range of sources of on-line resources at a low cost.

Health Internet- work Access to Research Initiative (HINARI)
This is another on-line initiative that provides free or very low cost on-line access to the major journals in biomedical and related social sciences to local, not –for profit institutions in developing countries.

Online Access to Research in the Environment (OARE)
This is an international public-private consortium coordinated by the United Nations Environment Programme (UNEP), Yale University, and leading science and technology publishers, enables developing countries to gain free or low cost access to one of the world's largest collections of proprietary environmental science literature.
Launched in October 2006, OARE has a mission to improve the quality and effectiveness of environmental research, education and training in low-income countries. In doing so, OARE will help achieve four primary development objectives

African Journals Online (AJOL)
AJOL is a database of journals published in Africa covering the full range of academic disciplines. The objective of AJOL is to give greater visibility to the participating journal and to the research it convey.
It aimed at promoting the awareness and the use of African journals in the sciences by providing access to Table of Contents (TOC) on the internet.

EBSCO
EBSCO is a worldwide leader in providing information access through print and electronic journal subscription services, research database development and production, online access to more than 150 databases and thousands of e-journals. EBSCO has been serving the library and business communities for more than 60 years. Additional information on EBSCO

Industries is available from http://www.ebscoind.com/

Benefits of E-Resources

The benefits researchers will derived from these sources of information are

1. Access to high quality and relevant literature
2. Increase in the quality and effectiveness of research
3. Increase in the awareness of research work in other part of the world

Jaques, Loeh, a famous biologist, and one generally acknowledged as the founder of general physiology in America, described the place of the library in research when he said: "*The library remains the greatest essential to discovery*"

…we imagine that it is the laboratory that men discover new truth and that if we can only provide well-equipped laboratories important truth will soon be discovered. That is not the case.

Real discoveries are actually made in the library and subsequently tested out in the laboratory. A new discovery is a new combination of old ideas , and those ideas are most likely to occur to the mind of the scientist, not when he is handling material things, but when he is brooding over the thoughts of other men and rethinking them himself. In those hours of profound reflection a new combination may occur to him and he goes to his laboratory to verify or disprove it.

References

Adedigba, Y.A. (1984). Research Institutes' libraries: function, problems and suggested solutions. Invited paper, Annual General Meeting of NLA, Oyo State Chapter, Ibadan 12th June.

Budd, John M. (1992). The library and its users: the communication process.

Harrod, Leonard M. (1987). The librarian glossary of terms used in librarianship, documentation, book craft and the reference book, England: Gower

Lamorde, A.G. (1992). The importance of library to research activities and problems of funding in Nigeria: A keynote address presented at the Conference of the NLA, NIPSS, Kuru, 7th September.

Mangla, P.B (1988) Agricultural Libraries in India: an overview. Journal of Library and Information science vol.13 No 2 pg 100

Tips on Protecting Computer and Data from Virus Threats

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A computer threat is anything causing economic damage to the computer industry as related to data or information storage, retrieval and its processing. Threats are grouped generally into viruses, Trojans, worms and other malware such as spyware. There is ambiguity about the terms *virus*. Antivirus vendors generalize it while other specifies the virus and in turn advertise their product(s) showing precisely what it/they can do.[°]

Definitions

A computer virus is a routine or a programme that can *infect* other programmes by modifying them or their environment such that a call to an infected programme implies a call to a possibly evolved, functionally similar, copy of the virus (Boudouin Le Charlier *et al.*, 1995).

A computer Trojan is a programme which the user thinks or believes will do one thing ('the perceived purpose'), and which may or may not do that thing, but which also does something else which is not necessary to accomplish the perceived purpose and of which the user would not approve (Ian Whalley, 1998).

A worm is a procedure that moves from a programme or session to another in the main memory (RAM) causing undesirable effects unknown to the user. A new virus release means that the author (owner/inventor) of the virus has spent some time to produce an effect that would take the same, if not longer, to counter.

Objectives

The objective of this seminar were to sensitize the Vom community about computer threats and identify virus infection on their personal computers; learn how to best protect their data from threats, avoid future computer virus infection and how to remove/heal or cure computer virus infection

Utilities: (Anti-Virus/Ant-Spyware and Firewall)

Utility Software (Antivirus) software automates protection against **threats** such as Viruses, Worms, Trojans and Spyware. Though one can manually protect himself against viruses by maneuvering the computer to counteract the symptoms of the viruses, however, this method is cumbersome and requires a very long learning curve and is rewarded with little impact on user's time. Antivirus software offers first hand protection within the scope of what the software is made for. Subscribing for protection against threats is not as easy as it seems. It is not just enough to install antivirus software and just walk away. The antivirus requires virus update and with time upgrade.

The difference between update and upgrade is that the update keeps the original version of the software installed but its virus definition database is updated while the upgrade is replacing the software with a more recent or higher version. Protection against threats is very specific.

Recommendations

When you install your antivirus software, update it as frequently as possible. There are some virus threats that are three months old. A fresh antivirus installation of software build dated or published before three months will

[°] Paper presented on 28th June, 2007 at NVRI auditorium

require update of that same period of time. A more recent update will not protect you against older viruses – only the latest threats.

Make sure your antivirus software is In-Good-Standing (I.G.S) and is recently upgraded and updated.

Scan flash drives for viruses with antivirus that is I.G.S when trying to exchange data with another PC.

e-mail Trojans: Be sure that your message sources are genuine and clean.

Try data files regularly on another clean system to check the integrity of your computer.

Avoid visiting crack and pornographic sites during browsing which may carry i-worms.

Publicize any of your findings.

References

Ian Whalley (1998): Testing times for Trojans; IBM TJ Watson Research Centre, Worktown Heights, NY 10598, USA.

Ian Whalley (1998): Talking Trojan; Virus Bulletin. June 1998 pp 9 – 10.

Boudouin Le Charlier, Abdlaziz Mounji and Morton Swimmer (1995): Dynamic detection and classification of computer viruses using general behaviour patterns. (windowsSecurity.com)

Sarah Gordon and Richard Ford, (1996): Real World Anti-Virus Product Reviews and Evaluations – the Current State of Affairs, Proceedings of the Nineteenth National Information Systems Security Conference, Volume 2, pp 526 – 538.

Modelling the Field Efficacy of Recombinant rDNA [Bm86] Anti -tick Vaccine Tickgard™ against *Boophilus* Ticks

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Ticks and tick–borne diseases affect animal and human health and are the cause of significant economic losses. Approximately 10% of the 867 tick species known to man act as vectors for a broad range of pathogens and are also responsible for damage directly due to their feeding behaviour. Jongejan and Uilenberg, (2004). Generally, tick – borne protozoan diseases (e.g. babesiosis and theileriosis) and rickettsial diseases (e.g. anaplasmosis and heartwater or cowdriosis) are pre- eminent health and management problems of cattle and small ruminants as well as buffaloes, affecting the livelihood of farming communities in Africa, Asia and Latin America (Perry et al, 2002; Minjauw and McLeod, 2003). Control of tropical ticks and tick-borne diseases, still depend mainly on intensive tick control using acaricides. However, these chemicals are toxic, leave residues in meat and milk and cause environmental pollution. The resistance of ticks to acaricides poses an increasing threat to livestock. Integrated tick control strategies are advocated and such strategies have been preferred for quite some time (Young *et al*, 1988). °

Vaccination using concealed antigen, was proposed by Galun (1978) and a protective antigen, Bm86 was subsequently identified and synthesized using recombinant DNA technology (Willades *et al*., 1989).

In this study a model to simulate field efficacy of recombinant DNA vaccine based on recombinant Bm86 gut antigen (TickGard™) from *Boophilus microplus* against *Boophilus microplus* and *Boophilus decoloratus* tick species was developed using the Markov chain model. The model follows population dynamics matrix exponential growth of the *Boophilus* ticks.

Materials and Method

Data collection

Data for the model were retrieved from the literature from various regions where the recombinant Bm86 vaccine trials were conducted against *Boophilus* ticks in cattle (Odongo, 2006 and Garcia – Garcia, 2000).

Programming Markov chain model

A Markov Chain model is a mathematical equation that has two major components: states and transitions. The model represents a system or process that moves between two or more states through transition. In the vaccination model, the transitional matrix has nine states based on the *Boophilus* ticks life cycle.

The Equation System in the model

$L1_{(i+1)} = s_2Q_{2(i)} + s_3Q_{3(i)}$ (Questing Larva),
$H_{(i+1)} = \theta \times L_{(i)}$ (Nymphs)
$X_{(i+1)} = \mu \times H_{(i)} + k_1 \times X_{(i)}$ (Adult males)
$Y_{(i+1)} = \mu \times H_{(i)}$ (Adult Females)
$P_{(i+1)} = f \times Y + k_1 \times P$ (Ovipositing Females)
$E_{(i+1)} = (n \times P_i) \times g + k_2 \times E_{(i)}$ (Eggs layed)
$Q_{1(i+1)} = h \times E_{(i)}$ (Larva with undeveloped cuticle)
$Q_{2(i+1)} = m_1 \times Q_{1(i)}$ (Larva with developed cuticle)
$Q_{3(i+1)} = m_2 \times Q_{2(i)} + k_3 \times Q_{3(i)}$ (Adult female Larva)

Modelling Method

The Markov chain Matrix modelling technique was employed to estimate the effect of vaccination on a naive population at different booster time of 10 days decadal period were predicted at various vaccine efficacies on *Boophilus decoloratus* and *Boophilus microplus*. The basic model was developed and used as control to compare with the immunized model. Recent evidence indicates that TickGard™ vaccine containing Bm86 antigen may also have effects in blocking pathogen transmission in cattle (Pipano *et al*. 2003). The components of

° Paper presented on 9th September, 2007 at NVRI auditorium

Markov chain Model (MCM) are (1) The equation system which involves nine states (2) Survival Rates on Host and (3) Egg-laying capability and Fertility. Simulations were made by calculating the number of individuals entering into next stage or interval (state) based on the probability of survival rates, mating probability of the adult females, and eggs hatchability in the present state. For consistency of the model a time step (decadal period) of 10 days is used in this model as well as vaccination booster intervals. Also in this model three approaches were adapted. Firstly, the population growth pattern was observed at 25^0C and relative humidity of 85% as control model. Secondly, the model was simulated for *B. decoloratus* and *B. microplus* until an equilibrium stage was seen and lastly, vaccination parameters were introduced in the model to evaluate the behaviour of the population for both ticks concurrently.

Results

Simulation results for immunized and non immunized population for both *Boophilus* ticks are presented in figures 1 and 2.

Discussion and Conclusion

Simulation of Immunized versus Control against the two tick species

Comparisons between simulations of the tick population density at 25^0C with relative humidity of 85% are shown in (Fig. 1) for vaccinated and unvaccinated Cattle for *Boophilus microplus*. There was an initial fluctuation in the model then equilibrium was stabilized at about day 46 for the control while equilibrium was gained at day 30 for the immunized cattle population which show a sharp drop in the slope of the graph as compared with simulation on *Boophilus decoloratus* (Fig. 2) while equilibrium was gained at about day 36 for the immunized under same the same control. These results and others given by Lodos Lodos *et al.,* (2000) who developed models that show the effect of vaccination on the tick population dynamics using Bm86 antigen proved that tick population can be predicted and control strategies designed targeted at effectively eliminating the *Boophilus* species in areas which harbour the vectors.

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References

Jongejan, F and Uilenberg, G. (2004). The global importance of ticks. Parasitology, 129 S3-S4, Cambridge University Press.

Labarta V, Rodriguez M, Penichet M, Lleonart R, Luaces Ll, De La Fuente J (1996). Simulation of control strategies for the cattle tick *Boophilus* employing vaccination with a recombinant bm86 antigen preparation. Veterinary Parasitology; 63(1-2): 131-60.

Beugnet, F., K Chalvet-monfray, ..P. Sabatier (1998). Use of a mathematical model to study control measures of the cattle tick *Boophilus microplus* population in New Caledonia Veterinary Parasitology. 77, 277-288.

Floyd, R. B., R. W. Sutherst and J. Hungerford (1995). Modelling the field efficacy of a genetically engineered vaccine against the cattle tick, *Boophilus microplus.* International Journal for Parasitology, vol. 25. No. 3, pp 285- 290.

Jorge Lodos, Oscar Boue, Jose De La Fuete, (2000). A model to simulate the effect of vaccination against *boophilus* ticks on cattle. Veterinary Parasitology 87, 315-326.

Minjauw B. and Mcleod A. (2003). Tick-borne diseases and poverty. The impact of ticks and tick borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburg, UK.

Caswell Hal (2001). Matrix population models: construction, analysis, and interpretation. 2nd ed. Sinauer associates, inc. Publishers, Sunderland, Massachusetts.

Table I: Baseline parameters constant used in the model as control

***Boophilus* state of development**	***B. decoloratus***	***B. microplus***
Larvo_nymph moult **θ**	0.91	0.91
Nymphal_ Adult moult (Male) **μ**	0.335	0.335
Nymphal_ Adult moult (Female) **μ**	0.335	0.335
Decadal survival probability of male $\mathbf{P_1}$	0.35	0.35
Survival prob. of Adult females	0.5	0.5

Table II: Parameters estimates in the immunization model.

Parameter	Survival Probability	Number of eggs Lay	Vaccine efficacy	Reference
Female ticks Control	0.65	2,500	0	Odongo , 2006
Female immunized	0.38	1,525	0.61	
Female ticks Control	0.50	4,500	0	Garcia - Garcia et al.
Female immunized	0.23	720	0.84	(2000

Mortality in eggs of 0.43 is constant in both species.

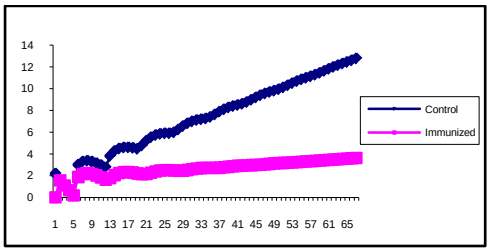
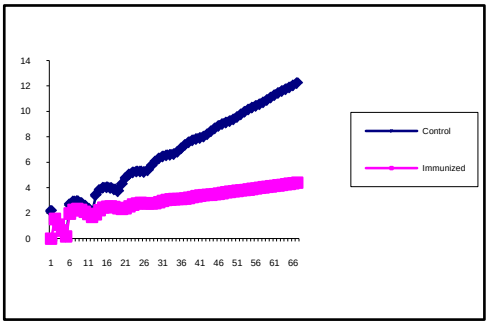


Fig.1. Immunization with Bm86 and control groups of *B. microphilus*

Fig.2. Immunization with Bm86 and control groups of *B. decoloratus*



Samples and Sampling

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A Sample can only be described with reference to a Population. In statistics the word Population has a much wider meaning than in everyday language. Any collection or assembly of people, items, things or numbers that ***is of interest in its own right,*** rather than because it may be representative of something larger, is a population. [°]

The population census

One well known statistical enquiry is the population census which is held in most countries. The census enquiry is addressed to every **household** and aims at getting information about **everybody.** The main purpose of censuses is to count the number of people as a basis for calculating potential military strength, tax revenue, housing, nutritional, medical and other needs, employment, accommodation, education, car ownership etc. The huge scale of the tasks of organizing, collecting, converting the raw data collected from a census into reliable statistical tables, which normally takes months if not years makes it difficult to conduct earlier than ten years interval in most countries. This led in 1966 to the introduction of the 10% sample census midway between censuses. The introduction of this sample in place of a full census saved money, and led to faster publication of information. Those are the main reasons for the use of samples. Another major reason is that sometimes making an inquiry changes, or even destroys, the item.

Relying on samples

It is not necessarily the size (whether small <30 or large >30) of items to be investigated that makes it a sample. If our aim in investigating a set of data is to make a single estimate with a view to illustrating a method or make particular scientific, or medical statements, and if we will not attempt to use our information as the basis for statements of wider applicability, the data we are working with is not a sample.

For the mean of a sample to be accepted as a reliable estimate of the mean of the population several factors will need to be considered carefully. Two that are within our control are the nature of the sample and its size. Other factors are beyond our control. If properly taken, a sample of 1000 from a population of 1,000,000 is as good as a sample of 1000 from 50, 000,000. What does matter is the size of the population variance.

The nature of the sample

If you derive your information from a sample, you can never be absolutely confident that your point estimate for the population is correct. But if you take the sample in the right way, you can be perhaps 95% confident about an interval estimate.

Another key point is that the sample should be representative of the population, which is not always easy to achieve. A sample that is not representative is biased. If many samples are taken and they are all biased in the same way, the bias is systematic.

One method of sampling is called random sampling. **A simple random sample** is a sample such that every item in the population has exactly the same chance of being included in it; and whether one item is included is in no way affected by whether another item is included. Unfortunately many people use the phrase "random sample" to refer to any convenient selection, which is likely to be far from

[°] Paper presented on 13th September, 2007 at NVRI auditorium

random. Obtaining (or "drawing") a random sample is not easy unless you know how. Random samples are not haphazard selections; they have to be carefully chosen. It is also important to note that the standard methods for taking random samples are strictly valid only if the sample is taken from an infinitely large population, in practice it is enough if the population is very large compared with the size of the sample. Use sampling with replacement for smaller populations. This has the effect of converting the population into one that is infinitely large. If the sample is random from a very large population, or has been taken with replacement, it is fairly easy to make correct statements about the reliability of results based on it. Random sampling is not guaranteed to produce a representative sample, but it is guaranteed not to produce a systematic bias.

The Sample Size

It can be shown that with a random sample, the reliability of a sample result as an indicator of a population result depends on the square root of the size of the sample, so a sample that is nine times as big will treble the precision. As the cost of collecting and processing the data increase with the size of the sample, this usually means that costs increase a great deal faster than precision. In choosing a sample size, the gain in precision has to be weighed against a greater gain in cost.

The population Variance

The reliability of a population estimate based on a sample also depends on the variance of the population data. A small variance means the data are bunched together so a sample is not likely to be seriously unrepresentative; but a large variance means the data are so spread out that an unrepresentative sample may easily arise.

Uses of Samples

1. To make estimates for population: One use of a sample is to allow the population parameters to be estimated. We say estimated because calculating them with certainty would be possible only if we had information about every member of the population. Every population parameter has its corresponding sample statistics. The first step in estimating any population parameter is to calculate the corresponding sample statistics, the second step is to ask whether the sample statistic (and especially the sample mean and the sample standard deviation) can be used immediately as an estimate of the population parameter, or whether some kind of modification of the sample statistic is necessary.

2. Another use of samples is to test ideas theories, beliefs or hypothesis about the population.

Sampling

In a simple random sample every item in the population has to have exactly the same chance of being chosen; and whether one item is included should be quite uninfluenced by whether some other item is included.

The essential stages in taking a random sample are :(a) Choose a sampling frame

(b) Decide on the sample size **n.** (c) Select **n** random numbers. (d) Use steps 3 and 1 to select the sample

A sampling frame is a list, numbered or capable of being numbered, that can be used as an acceptable substitute for a numbered list of all the items or people in the population under investigation.

T**he sample size** affects the precision and the cost. Although the precision of a population estimate based on a sample increases with the square root of the sample size, the cost of taking the sample and analyzing the data is likely to increase more rapidly. Determining a sample size involves balancing precision against cost and usually speed. **Random numbers** are best obtained from random number tables.

Controlled Sampling
The more common ones are:
Cluster sampling: Simple random samples are taken from one or more groups (or clusters) selected sometimes for convenience but preferably at random, rather than from the whole population. A "national" public opinion poll involving 1100 people may be organized to select from only 20 specified localities.

Quota sampling: This is often used in market research. Interviewers are told how many people of different characteristics (age, sex, appearance etc) they are to interview. Within this quota they usually choose at will, but may attempt some element of randomness.

Stratified Sampling: The sample is chosen so that it reflects important characteristics of the population. If 20% of the population consists of females living in rural areas, then 20% of the sample will consist of females living in rural areas. If this is achieved by selecting the rural females (and other strata) at random, it is a stratified random sample. Usually this is more reliable than a simple random sample.

Systematic sampling: The individuals in a sample are chosen systematically from what amounts to an ordered list, such as every 40th entry in a telephone directory. In some cases this quick procedure can give satisfactory results but there are always dangers arising out of possible patterns. As no two adjacent individual will be chosen, the chance of one individual being chosen is not independent of whether another is chosen. Others are **purposive** and **convenience** sampling.

Quota, Purposive and Convenience sampling are Non Probability sampling procedures. The aim of estimating population characteristics cannot be met through these procedures because we can obtain no valid estimate of our risks of error. In fact statistical inference using the estimates obtained from non probability sampling cannot be justified and should not be used.

References
Jim Fowler and Louis Cohen (1990) Practical Statistics for Field Biology: Open University Press, Milton, Keynes, Philadelphia.

John P. Lewis and Alasdair Traill (1999) Statistics Explained: Addison Wesley Longman, Essex, England.

Major pathogens of bovine mastitis in some parts of Plateau State, Nigeria

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Mastitis is defined as an inflammation of the mammary gland and affects lactating animals. It is one of the most important causes of economic losses in the dairy industry. Some of the related losses include decreased milk yield, increased cost of udder treatment, risks of antibiotic residue violation, culling and death (Boesch *et al*., 2007). Decreased milk production due to mastitis accounts for 75% of the total losses (Bennedsgaard *et al*., 2003). Udder pathogens affect food safety because they produce toxins that cause food poisoning (Umoh *et al*., 1990a). Currently there is dearth of information on mastitis in Nigeria. However, earlier studies reported a bulk milk mean SCC of 354,768 (Lombin and Esievo 1979). In Zaria the prevalence of the disease was 3.2% in traditional herds and 31.0% in settled herds (Umoh *et al*. 1990b). The objective of this was to assess management practices, current sub-clinical mastitis status and assay for agents of mastitis in some parts of Plateau state. The antimicrobial susceptibility of the agents of mastitis and antimicrobial drug-residues in bulk milk samples were also studied.[°]

Materials and Methods

Sampling area

Two herds each were selected from the 6 LGAs of Plateau state. In addition, one herd from a government farm and one from Bokkos Local Government were selected. Fourteen herds with a minimum of 10 milking cows were selected from each of the following villages: 14 villages.

Questionnaire survey

A standard questionnaire was administered to assess the management and milking practices in order to identify conditions that could lead to transmission of pathogens.

Sampling and Analysis

Composite milk (pooled milk from four quarters, CM) and bulk milk (pooled milk from one herd, BM) were collected aseptically. In all, 346 CM 45 BM and were collected and analysed. California mastitis test was used to screen each CM and BM samples for evidence of subclinical mastitis (Nierman, 2004). Samples classified as negative and trace were considered as negative and 1+, 2+, 3+ as positive (Umoh *et al*., 1990b).

All the samples were cultured for bacteria using standard procedures and the isolates confirmed using biochemical tests and serologically using latex agglutination test kits (Henning *et al*., 2004, Davidson *et al*., 2004). Total plate count (TPC) and total coliform count (TCC) of the BM samples were determined as described by Davidson *et al*. (2004). The pH, temperature, total solids of the milk samples was determined.

Antimicrobial sensitivity tests

The agar disc diffusion method was used and tested in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2002).

The isolates were culture standardized to a turbidity equivalent of 0.5 McFarland standards. Mueller –Hinton agar with disc on the surfaces were incubated at 37^{o}C for 24 hrs. Isolates with an inhibitory zone of ≥13mm was considered as susceptible and < 13mm as resistant. *S.aureus* (ATCC 25923) obtained from the Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria was used as control. Antimicrobial drug residue in bulk milk samples was performed using the Premi Test kit.

Data analysis

Chi-squared analysis was used to compare the frequencies of pH, CMT, growth of bacteria and resistant patterns from the different herds.

[°] Paper presented on 28th November, 2007 at NVRI auditorium

Data was also analyzed using Epi Info software (Dean *et al*. 2002). The strength of associations between CMT results and bacterial growth, between pathogens and CMT reaction and between susceptibility of
agents from CMT positive and negative samples were determined by calculating odds ratio (OR) and setting up 95% confidence intervals on them. Odd ratio values greater than unity denote association. Confidence intervals that include 1 are not statistically significant at P=0.05. Means of counts for each CMT reaction level was compared using Kruskal Wallis H test. Frequency of grades of bulk milk by herds was compared using chi-squared analysis. Correlation analysis was established between counts in bulk milk from each herd with CMT reaction, temperature and pH.

Results

Characteristics of herds

The number of cows in the 14 herds sampled ranged from 35 to 364 with a total of 1,843 and milking cows from 11 to 84 with a total of 556 (30.7%). Eleven (78.6%) of the herds reported having had cases of abortion/still birth in the last one year and 9 had cases of mastitis. All the herds relied on stream for watering and grazing as the main method of feeding. Only 2 herds offered supplementary feeding with grains offal.

Milking Practices

Hand milking was practised in the 14 herds sampled. In all cases the calves were introduced to the dam before milking. None of the farms practised pre-milking sanitation. In all cases, milkers had to performed many roles during milking. Milkers were always divided between milking and restraining the cows, controlling the calf, holding the milking bowl, lubrication of the teat and other distractions during milking. Milking was done out in the field with other cows and in a manure filled environment. Bulk milk was exposed to contamination between milking. Temperature of the milk ranged from 29 to 31^{o}C in 11 (78.6%) and 34-35^{o}C in 3 (21.4%) of the herds.

Treatment

Thirteen (93.9%) of the herds accepted treating the cows with antibiotics such as penicillin, tetracycline(LA) and tylosin in the last 6 months, 14(100%) with antihelminthic drugs and only one was treating the cows with an acaricide at the time of visit.

Prevalence of mastitis and bacterial growth

A total of 346 composite milk samples were collected and examined. The pH distribution, number with CMT positive results and growth of bacteria by herds were significantly different (P<0.05). On the whole 105 (30.3%) were CMT positive, 134 (38.7%) had bacterial growth and 72 (53.7%) of the cows had intra mammary infection IMI (CMT+ Growth +).

There was significant association between CMT reaction and growth of bacteria with odds ratio of 6.29 (3.80 < OR < 10.45). Similarly, 78 of the composite milk samples had *S. aureus*, 16 had CNS and 24 had *Streptococcus* spp. The three organisms also gave significant association with CMT results with an odds ratio of 3.2 (1.93 < OR < 5.56) for *S. aureus* 4.5 (1.51 < OR < 14.39) for CNS and 2.4 (1.05 < OR < 5.77) for *Streptococcus* spp. There was no association between *E. coli* growth and CMT. The major pathogens in association with mastitis in the study area were *S. aureus*, CNS and *Streptococcus* spp.

Quality of the bulk milk based on CMT

Out of the 45 bulk milk samples screened by CMT, 20 (44.4%) were negative (mean TPC $\log_{10}$ cfu ml^{-1} 2 to 6,TCC $\log_{10}$ cfu ml^{-1} 1 to 4), 9(20.0%) were intermediate (mean TPC $\log_{10}$ cfu ml^{-1} 3 to7, TCC $\log_{10}$ cfu ml^{-1} 1 to 4) and 16 (35.6%) were positive (mean TPC $\log_{10}$ cfu ml^{-1} 3 to 6, TCC $\log_{10}$ cfu ml^{-1} 1 to 5). The distribution of counts based on CMT reaction was not significant (P > 0.05).There was significant linear relationship between TPC and TCC of bulk milk from each source and CMT reaction (TPC: r^2 = 0.59; TCC: r^2 = 0.57; P < 0.05). Based on Pasteurized Milk Ordinance (PMO) grade A milk from farm should have a bacterial count and SCC of less than 10^5 cells/ml. In this study, only 11 (24.4%) of the bulk milk samples satisfied this requirement.

Antimicrobial drug residues

The 45 bulk and composite milk samples were pooled to obtain 14 samples (one pooled sample per herd). Residues test revealed that 7(50%) were positive for residues.

Antimicrobial susceptibility of the causative agents of mastitis

S. aureus and other Gram positive isolates were highly resistant to the following drugs used in

veterinary and human medical practice {range from 16 (20.0%) for streptomycin to 73 (91.3%) for cloxacillin) and 6 (4.4%) for gentamycin to 80 (59.3%) for augmentine} for veterinary and human drugs respectively. *E. coli* was more sensitive to both set of drugs. However, in both cases, there was no significant difference in resistant profile for isolates obtained from mastitis and non mastitis milk. The *S. aureus* isolates showed multiple resistance to many of the agents tested and particularly to oxacillin and methicillin.

Discussion

The study established high prevalence of mastitis with significant difference by herds. The major pathogens isolated had significant association with subclinical mastitis. The susceptibility patterns for isolates from subclinical and non clinical mastitis were not different. The study indicates the need for extension training on good dairy practices and the rational use of antibiotics in cattle in order to reduce the risk of resistance transfer and antibiotic residues.

Methicillin and oxacillin resistant *S. aureus* is a well known problem in human medicine. This may soon be an emerging problem in veterinary medicine in the study area.

In conclusion, the high mastitis level recorded had effect on milk quality and may also affect product safety. The frequent uses of antibiotics lead to antibiotic residues in raw milk and high number of resistant strains. There is need, for enlightenment on good dairy management, milking hygiene and prudent use of antibiotics by dairy producers.

Acknowledgement

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References

Bennedsgaard, T. W., C. Enevoldsen, S. M. Thamborg, and M. Vaarst, 2003: Effect of mastitis treatment and somatic cell counts on milk yield in Danish organic dairy cows. J. Dairy Sci. **86,** 3174-3183

Boesch, M., Doherr, G.M., Scharen, W., Schallibaum, M., Blum, J.W., 2007 Subclinical mastitis in dairy cows in swiss organic and conventional production system. *Journal of Dairy Science* **74** 86-92.

Clinical Laboratory Standard Institute CLSI, 2002: Performance standard for antimicrobial disk susceptibility tests. 7th edn. Approved Standard. Document N2-A7 NCCLS, Wayne, PA

Davidson, P.M., L. A. Roth, and S. A. Gambrel-Lenarz, 2004: Coliform and other indicator bacteria. In Wehr, H.M. and Frank, J.F. eds. Standard and Methods for the examination of dairy products. 17th edn. APHA, Washington DC 20001 USA 187-226.

Dean, A.G., T. G. Arner, G. G. Sunki, R. Friedman, M. Lantinga, S. Sangam, J. C. Zubieta, K. M. Sullivan, K.M. Brendel, Z. Gao, N. Fontaine, M. Shu, and G. Fuller, 2002: Epi Info™, a database and statistics program for public health professionals. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Henning, D.R., R. Flowers, R. Reiser, and E. T. Ryser, 2004: Pathogens in milk and milk products. In Wehr, H.M. and Frank, J.F. eds. Standard and Methods for the examination of dairy products. 17th edn. APHA, Washington DC 20001 USA 103-151.

Lombin, L.H., Esievo, K.A.N., 1979 Cell counts in bulked milk supplies from dairy farms of Northern Nigeria. *Tropical Animal Health & Production* 11 171-174

Nierman, P. 2004: Methods to detect abnormal milk in Standard Methods for the examination of dairy products, 17th edn. American Public Health Association, Washington DC 20001 USA 281-292.

Umoh, V.J., A. A. Adesiyun, and N. E. Gomwalk, 1990a: Enterotoxigenicity of staphylococci isolated from raw milk obtained from settled and nomadic herds around Zaria, Nigeria. Revue. Elev. Med. Vet. Pays. Trop. 43 43-47.

Umoh, V.J., A. A. Adesiyun, and N. E. Gomwalk, 1990b: Antibiogram of staphylococci strains isolated from milk and milk products. J. Vet. Med. B. 37, 701-706.

Table 1: Antimicrobial agents and concentrations

Antimicrobial agent	**Concentrations (µg)**
Augmentin	30
Amoxicillin	25
Erythromycin	5
Tetracycline	10
Cloxacillin	5
Gentamycin	10
Cotrimoxazole	25
Chloramphenicol	30
Methicillin	5
Oxacillin	1
Vancomycin	5
Ampicillin	33
Streptomycin	100
Clindamycin	2
Lincomycin	19
Penicillin L	5
Tylosin	150
Trimethoprim-sulpha	5.2 + 240

Molecular and Spatio-Temporal Analyses of the Spread of Avian Influenza H5N1 in Nigeria

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Several countries including Nigeria have been affected by highly pathogenic notifiable avian influenza (HPNAI) H5N1 strains between 2003 and now (1, 2). The currently circulating HPNAI H5N1 originated in Guangdong, China and since 1996, it has devastated the economies of countries affected. The poultry sector is especially important in Nigeria because it contributed approximately 4.45% of the total animal contribution to agricultural GDP in 2004 (6). The nearly 160 million birds are composed of about 60% backyard poultry stock and about 40% commercial or semi-commercial birds (7). HPNAI, has caused high levels of mortality, restriction in international trade, infection of various animal species, endangered food security and carries potential for a human pandemic (1, 5, 8-12,). [°]

Increasingly, diseases with emergency potential like HPNAI are becoming more connected with higher densities of livestock, increasing trade resulting in the movement of people and products and breaches in biosecurity at various levels (national, regional and farm) despite advancements in information dissemination and management practices.

Nigeria first reported HPNAI H5N1 in January, 2006, but has since recorded multiple infections in many states of the federation. Factors thought to be responsible include illegal trade, poultry movements, poor border controls, human activity and migratory birds.

In this study, the 2006 avian influenza H5N1 infection in Nigeria was analyzed using molecular biology, geographic information systems (GIS)/remote sensing and ecologic niche modeling.

Materials and methods

Molecular Characterization

Tissue samples (lung, liver, spleen, heart, trachea and intestine) and swabs (cloacal and tracheal) were collected at outbreak locations or received by the National Veterinary Research Institute. Other samples were received through submission from the national active surveillance programmes. Samples were processed at the NVRI's Viral Research laboratory for virus isolation and biological characterization. All analyzed viruses were dispatched to Biotechnology Division, Onderstepoort Veterinary Institute, South Africa for molecular characterization. Sample were characterized through RT-PCR and sequencing of the full length HA gene. Blast homology searches (http://www.ncbi.nlm.nih.gov/blast) were used to identify 76 closely-related sequences representing wide species, geographical and spatial distributions and including all HPAI H5N1 sequences available from Africa. Multiple alignments were performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/index.html, 13). Pair-wise nucleotide sequence identities were calculated using Bioedit. The region of the HA genes of Nigerian viruses phylogenetically analyzed corresponds to nucleotides 92 to 1633 of the complete 1730 nucleotide protein encoding region of the HA gene of HPAI H5N1 viruses.

Phylogenies were reconstructed using the Neighbour-joining method in MEGA 3.1 software (14), the Kimura 2-parameter sequence evolution model, and 1000

[°] Paper presented on 14th February, 2008 at NVRI auditorium.

bootstrap replicates to assign confidence levels to branches.

The genetic sequences were published with the GenBank under the accession numbers EF631164-EF631187.

Spatial-Temporal Analyses

Outbreak locations were geo-referenced using a global positioning satellite system (GPS) (Garmin nuvi 370® GPS, Garmin, Olathe, KS, USA). Locations difficult to access were geo-referenced using TADinfo® version 1.101 software (a pre-georeferenced package customised for Nigeria) (15). Full epidemiological data were taken and confirmed with the data deposited in NVRI, Vom.

The collated geo-referenced data were confirmed using available databases (http://middleware.alexandria.ucsb.edu/client/gaz/adl/index.jsp), (http://gnswww.nga.mil/geonames/GNS/index.jsp) and (www.randmcnally.com).

The confirmed data were stored electronically and exported into ArcGIS 3.3® (ESRI, Redlands, CA, USA) and ArcView 8.0® (ESRI, Redlands, CA, USA) for epidemiological analyses [Ecologic Niche Modelling (ENM) and Spatial and temporal analyses (single epidemic front, neighbour to neighbour spread, local/long diffusion, spatial clusters and autocorrelations and reproductive numbers amongst others)].

In ENM, the method most frequently applied to questions of disease transmission has been the Genetic Algorithm for Rule-set Prediction (GARP), an evolutionary-computing approach (16, 17). *The GARP algorithm* has been widely applied to questions of disease transmission (18, 19), and its predictive ability has been tested under diverse circumstances (20-22).

Questionnaire Survey

The survey was carried out between November 2006 and January 2007. A set of structured questionnaires was designed, pre-tested and further evaluated through experts' opinions. Eight of the Nigerian HPAI H5N1-affected states (Kaduna and Kano [north]; Plateau Bauchi, Nasarawa and Abuja [central]; and Ogun and Lagos [south]) were selected, and randomly selected farmers were allocated questionnaires to test their knowledge of AI. Telephonic and personal interviews were used to confirm the collected data before evaluation. Positive and negative responses were evaluated according to published guidelines of OIE, CDC, WHO, OFFLU, and FAO. The response rate to the questionnaires was 67.5% (135) comprising farmers with infected and uninfected flocks. The size of the farms owned by farmers evaluated ranged from a few hundred birds to over 70,000.

Results

Molecular analyses using the haemagglutinin gene indicated that Nigeria was infected by multiple strains of the virus. There appeared to be shared epizootics between Nigeria and Egypt and there was a cluster of African viruses (Nigeria, Cote d'ivoire, Burkina Faso and Sudan); it also appeared that the spread within the country was not by wild bird but rather market, road and farm-farm related. Molecular characterization is published in two articles (*Molecular characterization and epidemiology of highly pathogenic avian influenza H5N1 in Nigeria.* Epidemiology and Infection. July 2008, 17:1-8.) (*Predictable ecology and geography of avian influenza (H5N1) transmission in Nigeria and West Africa.* Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008, 102:471-479)

Spatio-temporal analyses indicated that between January 16 and June 26 of 2006, 113 poultry farms reported AI infections. A rapid growth in epidemic size was observed, which peaked at the fifth epidemic week. At epidemic weeks 4 and 5, the effective reproduction number R was between 1.5 and 2. State-level human population, poultry population, and road

densities were positively but marginally associated with prevalence (cases/sq km): the highest (adjusted) *R2* (prevalence regressed on road density) was equal to 0.312 ($P<0.01$, figures not shown). Most infected farms were located at less than 10 km from the major national highway network. Only at epidemic week 4 and later were cases observed at farms located 100 km or more from the nearest road intersection The median distance between the first 5 farms reporting infections (by epidemic day 4) and the nearest road intersection was 24.2 km.

To facilitate early decisions on control policy (based on spatially explicit data), a simulated (although conservative) scenario was built. A decision-oriented test, based exclusively on information available by epidemic day 4, examined whether a policy could be chosen and implemented before the end of the first infectious period (estimated to be 10 days).

All of the tests supported the same inference: epidemic dispersal was promoted by proximity to major roads. A principle of successive contact was also established. The ENM strongly suggests that the virus has a potential for further spread in Nigeria and other West African countries, since at the time of these analyses, several other West African countries had reported infection.

Discussion and Conclusions

This study suggests that the factors that may have aided the spread of the infection in Nigeria included:

1. Inexperience and associated time loss (infection →latent period → evidence and clinical signs → reporting → confirmation → culling/control → clean up/disinfection)
2. Continued hurried sales
3. Inappropriate disposal
4. Poor/delayed reporting structure
5. Poor marketing structures and unmanned road networks
6. Weak implementation of control measures and continued shared services
7. Un-reviewed preparedness

This work concludes that successive contact was the primary mode of spread of HPAI H5N1 in Nigeria and the road network without effective interstate border control assisted spread. There is no concrete evidence (molecular or spatial) to suggest wild bird as being primarily responsible for outbreak spread and effective containment in the first 2 weeks would have limited the spread to only 4 states. Further work is therefore encouraged.

Acknowledgement

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References

World Organisation for Animal Health. Update on Highly Pathogenic Avian Influenza in Animals (Type H5 and H7). Available from http://www.oie.int/downld/avian%20influenza/A_AI-Asia.htm . Accessed on January 18, 2008.

World Health Organisation (WHO). Cumulative number of confirmed human cases of avian influenza A/ (H5N1) reported to WHO by 18th January, 2008. Available at http://www.who.int/csr/disease/avian_influenza/country/cases_table_2008_01_18/en/index.html accessed on 18th January, 2008.

Sonaiya, E.B., Branckaert, R.D.S. & Gueye, E.F. 1999. Research and development options for family poultry, in *First INFPD/FAO Electronic Conference on Family Poultry,* edited by E.F. Gueye. Rome: FAO.

McDermott, J.P., Coleman, P.G. & Randolph, T. 2000. Methods for assessing the impact of infectious diseases of livestock—their role in improving the control of Newcastle disease in Southern Africa. *SADC planning workshop on Newcastle disease in village chickens*, Maputo, Mozambique, edited by R.G. Alders & P.B. Spradbrow.

FAO 2002. Manual on the preparation of national animal disease emergency preparedness plan: animal disease emergency —their nature and potential consequences. http://www.fao.org/ag/agah

Central Bank of Nigeria 2004. Annual report and statement of account. Abuja: Central Bank of Nigeria.

Adene, D.F. & Oguntade, A.E. 2006. *The structure and importance of the commercial and village based poultry industry in Nigeria*. Rome: FAO.

Otte, M.J., Nugent, R. & Mcleod, A. 2004. Assessment of socio-economic impacts and institutional responses. FAO: Rome (Livestock Policy Discussion Paper, No. 9).

European Commission 2004. Council Directive 92/40/EEC of 19 May 1992. Introducing Community measures for the control of avian influenza. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31992L0040&model=guichett

Rushton, J., Viscarra, R.E., Bleich, E.G. & Mcleod, A. 2005. Impact of avian influenza outbreaks in the poultry sectors of five South East Asian countries (Cambodia, Indonesia, Lao PDR, Thailand, Vietnam). Outbreak costs, responses and potential long term control. http://www.hewsweb.org/downloads/avian_flu/docs/pdf/impacts.pdf

Holtz-Eakin, D. 2005. A potential influenza pandemic: Possible macroeconomic effects and policy issues. Congressional Budget Office Submission to the US Congress, Washington DC.

Ducatez, M.F., Olinger, C.M., Owoade, A.A., De Landtsheer, S., Ameerlaan, W., Niesters, H.G.M., Osterhaus, A.D.M.E., Fouchier, R.A.M. & Muller, C.P. 2006. Multiple introduction of H5N1 in Nigeria. *Nature,* 442: 37.

Thomson JD, Higgings DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 1994; 22: 4673-4680.

Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Briefings in Bioinformatics* 2004; 5: 150-163.

Emergency Prevention Systems-FAO (2006). TADinfo® version 1.101 software (a pre-georeferenced package customised for Nigeria).

Stockwell, D.R.B., Noble, I.R., 1992. Induction of sets of rules from animal distribution data: A robust and informative method of analysis. Math. Comput. Simul. 33, 385-390.

Stockwell, D.R.B., Peters, D.P., 1999. The GARP modelling system: Problems and solutions to automated spatial prediction. Int. J. Geogr. Inf. Sci. 13, 143-158.

Costa, J., Peterson, A.T., Beard, C.B., 2002. Ecologic niche modeling and differentiation of populations of *Triatoma brasiliensis neiva*, 1911, the most important Chagas' disease vector in northeastern Brazil (Hemiptera, Reduviidae, Triatominae). Am. J. Trop. Med. Hyg. 67, 516-520.

Peterson, A.T., 2007. Ecological niche modeling and spatial patterns of disease transmission. Emerg. Infect. Dis. 12, 1822-1826.

Anderson, R.P., Lew, D., Peterson, A.T., 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. Ecol. Modell. 162, 211-232.

Elith, J., Graham, C.H., Anderson, R.P., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J.M., Peterson, A.T., Phillips, S.J., Richardson, K., Scachetti-Pereira, R., Schapire, R.E., Soberon, J., Williams, S., Wisz, M.S., Zimmermann, N.E., 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29, 129-151.

Peterson, A.T., 2001. Predicting species' geographic distributions based on ecological niche modeling. Condor 103, 599-605.

Anti-inflammatory Activity of *Dichrostachys glomerata* Leaf Ethanolic Extract

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The potential of medicinal plant research in animal and human health care is no longer in doubt having gained recognition in several nations of the world and WHO (Githiori *et al,* 2003). One of the frequently used medicinal plants is *Dichrostachys glomerata* Forsskal (family; *Leguminosae*) popularly known as Sickle pod (English), '*dundu*' in Hausa, '*kara*' in Yoruba and *ami ogwu* in Igbo. The stem-bark, roots and leafs of *D. glomerate* are used for diseases including pneumonia, dysentery and diarrhea (Dalziel, 1937). This study was carried out to evaluate the potential antidiarrhoeal effect of *D. glomerata* leaf extracts on experimental models.[°]

Materials and Methods

Plant Materials

Fresh leaves of *D. glomerata* were collected from Vom, Plateau State between March and April, 2006. The plant was identified and authenticated by the Biological Sciences Herbarium, Ahmadu Bello University, Zaria.

Preparation of the Extract

The ethanolic leaf extract was prepared using standard protocol (Sofowora, 1983) and the freshly prepared leaf extract subjected to standard photochemical screening for various constituents according to the methods of Trease and Evans, (1996). The median lethal dose (LD_{50}), of the *D. glomerata* leaf extract was determined in mice according to the methods of Locke (1983).

For antinflammatory studies rats were used. The rats were divided into five groups (I-V) of eight rats per group and the acute inflammation of the right hind paw induced using fresh chicken egg albumen (0.5ml/kg s.p) (Oyewole, 2004). Rats in group I were given 5ml/kg of distilled water orally as control, while those in groups (II, III and IV) received graded doses of the extract (200, 400 and 800mg/kg per os) respectively. Rats in the 5th group received indomethacin (10mg/kg per os). Inflammation was evident 5-10minutes following the sub planter injection of egg albumen. The measurement of paw size was carried out for 3hrs at 30min interval (30, 60, 90, 120, 150,180min). The inhibitory activity was calculated using the formula

$$C_o - \frac{C_t}{C_o} \times 100$$ (Oyewole, 2004).

Where Co = is the average inflammation (right hind paw oedema) of control (group I) rats at a given time and Ct = is the average inflammation of (groups (II, III, IV and V) rats treated with plant extract and indomethacin respectively at the same time.

Results were evaluated using one-way analysis of variance (ANOVA) (Snedocor and Cochran, 1980). Values of $P<0.05$ were considered significant.

Results and Discussion

Photochemical analysis of the extract gave positive results for flavonoids, tannins, saponnins, resins, triterpenoids, reducing sugars and carbohydrate. Flavonoids and tannins were highly positive. Flavonoids abundant in this plant have been shown to inhibit lipooxygenase, phospholipase A_2 and cyclooxygenase the key enzymes involved in prostaglandin biosynthesis (Manthey *et al,* 2004). The orally

[°] Paper presented on 8th May 2008 at NVRI auditorium

determined LD_{50} value was established as 3,500+310 mg/kg the relatively high oral median lethal dose suggested that the leaf extract was relatively safe in mice.

The extract produced a significant ($P< 0.05$) dose and time related reduction in fresh chicken egg albumen-induced acute inflammation. The maximal effect was similar to indomethacin-a non-steroidal anti-inflammatory drug. Indomethacin is commonly employed in the treatment of rheumatoid arthritis and osteoarthritis due to its anti-inflammatory and analgesic effects (Viana *et al,* 2003). This result suggests that like indomethacin the leaf extract of *D. glomerata* produces antinflammaory effects. This finding validates the use of *D. glomerata* leaf extract as natural remedy for inflammatory diseases in many communities in Nigeria.

References

Dalziel, J.M (1937). *The useful plants of West Tropical Africa*. Vol. 10: Crown Agents for the Colony, London, 612pp.

Locke, D. (1983). A new approach to practical acute toxicity testing. *Arch. Of Toxicol;* 54: 275 – 287.

Githiori, J.B; Hogland, J; Waller, P.J. and Baker, R.L. (2003). Evaluation of Anthelmintic properties of extracts of some plants used in livestock deworming by pastoralists in Kenya. *Veterinary Parasitology*, 118: 125-226.

Manthey, J.A; Grohmaun, K. and Guthric, N. (2002). Biological properties of citrus flavonoids pertaining to caner and inflammation *Current Med. Chem*.; 9:1999 – 2013.

Nwude, N. (1997). Ethnoveterinary pharmacology and ethnoveterinary practices in Nigeria: an over view. *Tropical Vet*. 15: 117 – 123.

Oyewole, J.A.O. (2004). Evaluation of analgesic, anti-inflammatory and antidiabetic properties of *Sclerocarya Bierrea. Phytotherapy Res.* 18: 601 – 608.

Snedocor, O.W. and Cochran, W.G. (1980). *Statistical methods*. IBH publishing company Ltd; New Delhi, India, 201pp.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa* 2nd Edition. Spectrum Books Ltd; Nig. Pp 135 – 153.

Trease, G.E. and Evans, W.C. (1996). A *Textbook of Phramocology*, 6th Edition, Bailliere Tindall, London, 795pp.

Viana, G.S.B; Bandaira, M.A.M. and Matos, F.J.C. (2003). Analgesic and anti-inflammatory effects of chalcones isolated from *Myracrodrum* urundenva. *Phytomedicine,* 10: 189 – 195).

In Vitro Screening for Antibacterial, Antimycoplasmal and Toxicity of Acetone Extracts of Selected Plants from Northern Nigeria.

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The earth is estimated to contain up to half a million plant species of which nearly 10% are used for food and 10-15% as drugs (Borris, 1996). Currently, about 119 drugs of modern medicines are derived from 90 plants, of which 74% are of ethno-medical origin.

Plants are important for pharmacological research and drug development, not only when bioactive phytocompounds are used directly as therapeutic agents, but also as starting materials for synthesis of drugs or as models for pharmacologically active compounds (Solecki, 1975).

Currently, plants which have been documented as traditional medicines are being examined in the hope of finding new or improved medication. This includes research on the antimicrobial, antihelminthic, antifungal, antiviral, anti-inflammatory and anti-oxidant activity of plant extracts, as well as on other aspects of systemic pharmacology (McGaw *et al,* 2000).

Opinion about the safety, efficacy and the appropriateness of medicinal herbs varies widely among medical and health professionals in countries where herbal remedies are used. The general perception that herbal drugs are safe and free from side effects is not true. Herbs can produce undesirable side effects and can be toxic. However, it may take more to cause toxicity, because herbs usually are not as potent as manufactured drugs, and compared with synthetic drugs the adverse effects of most herbal drugs are relatively infrequent (Shukla, 2003).

Materials and Methods

The leaves of twenty (20) and the seeds of one (1) medicinal plants traditionally known for their antibacterial and or anti-respiratory tract activities were collected around Zaria in Kaduna State of Nigeria between February and March, 2007. The plants were identified and confirmed at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where voucher specimens were deposited.

The plant materials were dried under the sun, pulverised and extracted with acetone at 1:15 w/v. The minimum inhibitory concentrations for each plant were carried out using the microplate method of Eloff (1998) against *S. aureus* and *E. coli* and metabolic inhibition method (Muraina *et al.,* 2008a) against *M. mycoides* subsp *mycoides*. The qualitative phytochemical analysis of all the extracts was carried out using the TLC fingerprinting method as described by Kotze and Eloff (2002) and the analysis of the best antimycoplasmal extract was done by the method of Trease and Evans (1989).

Antioxidant properties of the extracts were also assessed using the qualitative DPPH method and the quantitative MTT method (Muraina *et al*., 2008b). The extracts were also assessed for their cytotoxicity using the colorimetric methods of Mosmann (1983).

Results

The phytochemicals of the extract of *C. procera* (the best antimycoplasmal extract) reveals the presence of alkaloid, tannins, saponins, cardiac glycosides, steroidal ring and flavonoids. There was no anthraquinone.

The number of plant extracts and their MIC values on different organisms are shown in Figure 1. Only two extracts had best activities on *S. aureus* (i.e. *A. leiocarpus* and *G. senegalensis*) and one extract each on *E. coli* and *M. mycoides mycoides* (i.e. *A. leiocarpus* and *C. procera*) respectively with MIC value of 0.08mg/ml. On the other hand, only the

extract of *A. sisalana* had the poorest activities on both *S. aureus* and *E. coli* with MIC value greater than 2.5mg/ml.

Ten of the extracts had good antioxidant properties with antioxidant compounds detected at a concentration lower than 0.04mg/ml. Four extracts had poor properties with antioxidation not detected at a concentration greater than 5mg/ml. The extracts with good properties were *A. leiocarpus, T. laxiflora, T. macroptera,* and *G. senegalensis* whereas those with poor properties were *A. sisalana,* and *C. alata.* Cytotoxicity on vero cells showed that the extract of *V. amygdalina* was the most cytotoxic while that of *A. occidentale* was the least cytotoxic among the plant tested.

Discussion

The plant extracts with MIC equal or less than 0.1mg/ml are promising plants for antimicrobial compound and according to phytomedicine journal; this can be explored further to isolate the active ingredient(s). The extract of *A. leiocarpus, G. senegalensis* and *C. procera* are potential plant extracts for antimicrobial compounds. The presence of phytochemical and antioxidant compounds may be attributed to antibacterial and antimycoplasmal activities. It was interesting to observe that plant extract with poor antioxidant properties such as *A. sisalana* had the weakest antimicrobial activities, linking antioxidant compound with antimicrobial activities. It was also interesting to note that the extract of *V. amygalina* (which is a plant used as vegetable by people) was the most cytotoxic. The processing of the plant before consumption where it is soaked for a long period in water and then dried before cooking may explain the detoxification of the plant.

Conclusion

It was concluded that the extracts of *A. leiocarpus/G. senegalensis* and *C. procera* had the greatest antibacterial and antimycoplasmal activity whereas the extract of *V. amygdalina* was the most cytotoxic among the extracts tested. It's recommended that the extract of *C. procera* be studied further so as to isolate the active antimycoplamal compound(s) which can be developed into medicinal product against Contagious Bovine Pleuropneumonia (CBPP).

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References

Borris, R.P. (1996). Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology,* 51: 29-38.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12 (4): 564-582.

Eloff, J.N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica,* 64: 711-713.

Kotze, M., Eloff, J.N. (2002). Extraction of antibacterial compounds from Combretum microphyllum (Combretaceae). *South African Journal of Botany,* 68: 62–67.

McGaw, L.J., Jager, A.K. and Vanstaden, J (2000). Antibacterial, anthelminthic and antiamoebic activity in South African medicinal plants. *Journal of Ethnopharmacology,* 72: 247-263.

Mosmann, T.J. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65: 55–63.

Muraina I.A., Picard, J. and Eloff, J.N. (2008a): Development of a reproducible method to determine minimum inhibitory concentration of plant extract against a slow-growing mycoplasma organism (*Phytomedicine Journal*-In press).

Muraina I.A., Suleiman, M.M. and Eloff, J.N. (2008): Can MTT be used to quantify the antioxidant activity of plant extracts? (*Phytomedicine Journal*- In Press).

Shukla, Y. and Pal, S.K. (2004). Complementary and alternative cancer therapies: Past, present and future scenario. *Asian Journal of Cancer Prev*ention, 5(1): 3-14.

Solecki, R.S. (1975). Shanidar VI: A Neanderthal flower burial in Northern Iraq. *Science,* 190: 880-881.

Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy*. English Language Book Soceity. Bailliere Tindall, 13th edn. London, pp. 612.

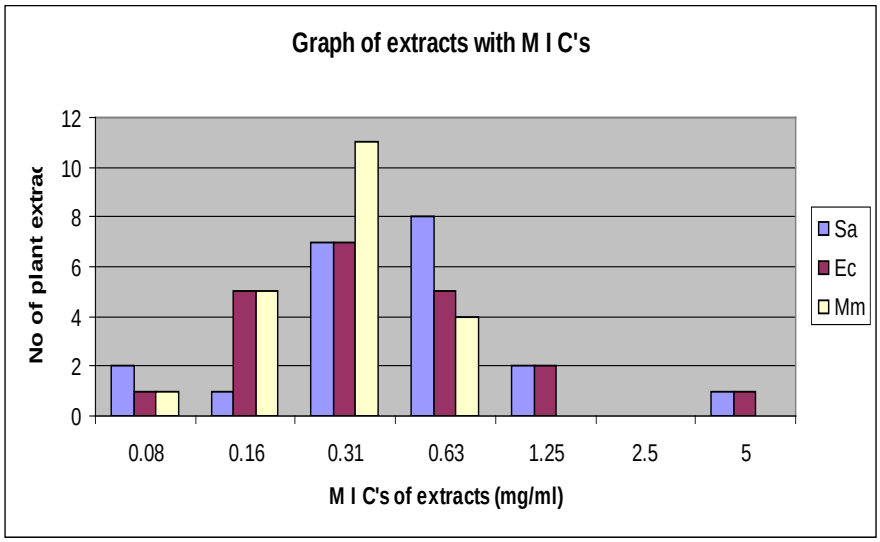

Fig. 1: keys: Sa (*S. aureus*), Ec (*E. coli),* Mm (*M. mycoides*)

African Horse Sickness Serotype 2 Extends to the Northern Hemisphere

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Infectious and emerging diseases are becoming widespread and are particularly important in developing economies. These diseases pose grave dangers to humans and animals as they could result in ecological changes which may force animal vectors to seek human hosts. The increasing intimacy between man and animal may encourage the sharing of infections while human encroachments into normally uninhabited areas are encouraging vectors to seek new hosts. Also, the unusually high exposure to animal pathogens may intensify diseases such as African Horse Sickness.[°]

The African horse sickness virus (AHSV) which causes African horse sickness belongs to the family *Reoviridae.* It is a double stranded RNA virus of the genus *Orbivirus* whose vector is principally the *Culicoides* (biting midges) "Kotonkan". It manifests in different serotypes including serotypes 1-9. In Nigeria, the disease was first reported in 1971 and since then it has been reported sporadically.

The disease is considered endemic in Sub Saharan Africa (East and West Africa) and parts of North and South African territories, the Middle East and Southern Asia. Before now, the serotype 2 of the virus had never been reported in the Northern hemisphere. The disease is a non-contagious, infectious arthropod-borne disease of equine that may be diagnosed by clinical and laboratory methods.

Case Report

In late 2006, an unknown rapidly fatal disease which affected both sexes of indigenous and exotic breeds of horses, aged between 5 and 10 years was reported in the Lagos Polo club, Ikoyi (6D 27'N, 3D 26'E) and spread rapidly to other locations including; Kano (12D 02'N, 8D 36'E), Kaduna(10D 29'N, 7D 25'E), Yola (9D 16'N, 12D 26'E), Katsina (13D 00'N, 7D 36'E) and Ibadan (7D 26'N, 3D 55'E). It severely affected the equine community and compromised sport utility of equines, caused catastrophic and psychological trauma to horse owners and led to financial losses.

Materials and methods

A combined team of staff of National Veterinary Research Institute, Vom, Faculty of Veterinary Medicine, University of Ibadan and the Lagos Polo Veterinary Team conducted field investigations. Tissue, blood and serum samples were collected and transported to the laboratory under cold chain. Blood and serum samples were packaged and sent to the Onderstepoort Veterinary Institute (OVI), South Africa for further laboratory analyses.

Viruses and diagnostic samples

Serum and blood-clot of a horse that died during the outbreak in Lagos were provided to the reference centre at OVI. The South African field isolates of AHSV1 and AHSV2 used in this study was kindly provided by the OIE reference centre for African horse sickness virus, South Africa. Prior to RNA extraction, all field viruses except that from Lagos were propagated in 75cm^2 flasks of BHK21 cells. The origins of the viruses used in this study are given in Table 1.

Virus Neutralisation and ELISA

AHSV specific antibodies were detected by the AHSV indirect ELISA described by Maree and Paweska (2004). The serotype of the viruses was determined using the Virus Neutralisation Test (OIE, 2004).

AHSV RT-PCR

AHSV viral RNA was detected using the AHSV RT-PCR as described by Bremer et al. (1998). The diagnostic PCR was done in accredited facilities at ARC-OVI .

[°] Paper presented on 19th June, 2008 at NVRI auditorium

Table 1: Passage History, Serotypes and Genotypes of the AHSVs used in the study

Virus strain	Origin	Passage history	Serotype (VNT)	Genotype (sequence)
19/06	Free State	Equine blood 1S-2 Vero	2	**2**
20/06	Gauteng	Equine lung-1S 2Vero	1	**1**
37/06	Kwazulu-Natal	Equine spleen/lung1S-2 Vero	2	**2**
54/06	Gauteng	Equine spleen-3 Vero	2	**2**
62/06	Northern Cape	Equine spleen-2 Vero	2	**2**
66/06	North West province	Equine lymph node-2 Vero	2	**2**
92/06	Botswana	Equine spleen-3 Vero	2	**2**
115/06	Eastern Cape	Equine spleen-2 Vero	2	**2**
132/06	Western Cape	Equine blood clot-2 Vero	2	**2**
147/06	Free State	Equine spleen-2 Vero	2	**2**
169/06	Eastern Cape	Equine spleen-2 Vero	2	**2**
170/06	Western Cape	Equine spleen-6 Vero	1	**1**
216/06	Kwazulu-Natal	Equine spleen-2 Vero	2	**2**
217/06	**Eastern Cape**	**Equine spleen-2 Vero**	**2**	**2**

dsRNA extraction and purification

Total RNA was extracted from infected cells using Tri-Reagent (Molecular Research Centre) as described by the manufacturer. The blood clot (approx 2ml) was dislodged by homogenization in 2ml 0.5% Triton X-100 with a 5ml syringe and a 25G needle. Total RNA was extracted from the homogenate using the method described above.

Genome amplification, partial S2 sequencing and phylogenetic analysis

The complete genomes of all isolates described here were amplified using the method as described by Potgieter *et al*. (2002) with some major modifications. The segment encoding VP2 (S2) was purified from the amplified genome after separation of the amplified segments on a 1% agarose gel. The terminal end of each VP2 segment was partially sequenced using a "phased primer" similar to that described by Maan et al (2007) with some modifications. Partial VP2 sequences were aligned with those of the reference strains of AHSV1 and 2 (Potgieter *et al*, 2003) using the clustalW in MEGA (Tamura et al., 2007). Sequence of the closest relative of AHSV2 (AHSV1) was included as well as those of the vaccine strains of AHSV1 and 2.

Results

Type and serotype determination

Results from the AHSV ELISA and RT-PCR showed that the blood in the sample from Lagos contained AHSV RNA and that the serum contained AHSV specific antibodies (results not shown). VNT results showed that the serum contained AHSV serotype 2 specific antibodies.

Genome amplification

The successful genome amplification of the virus from Lagos is the first report of the complete genome amplification of AHSV from blood. The results are not shown here but will be published as part of a more comprehensive paper on the amplification and sequencing of complete viral dsRNA genomes.

Phylogenetic analysis

Initial alignments of the partial S2 sequence from the Lagos AHSV isolate showed that it was closely related to AHSV serotype 2 (results not shown). A phylogenetic tree compiled from the alignments of partial S2 sequences of viruses from Lagos, South Africa and Botswana is shown in Figure 1. It is clear from the results that the S2 sequence of the AHSV2 isolate from Lagos is almost identical to AHSV2 viruses from South Africa and Botswana. This group of viruses was not identical to the vaccine virus of AHSV2 and also not to AHSV2 isolates from the Kwazulu-

Natal province in South Africa. There is also a clear distinction between AHSV1 and AHSV2 isolates.

Discussion

The clinocopathological findings as well as the laboratory analyses (serologic, virologic and molecular) confirmed outbreak of AHS serotype 2. The situation was possibly made worse with the fact that the affected populations were naïve to infection with AHS serotype 2 and the continuous spread of infection was occasioned by on-going polo tournaments. There were unconfirmed reports of importation of horses before the tournaments and these in addition to the abundance of *Culicoides* may have precipitated these outbreaks; but no link was established between the vaccine strains from South Africa and the outbreaks in Nigeria.

Biting midges are the only proven vector of AHS and these were found in abundance in the warm damp soil in and around the stables within the Polo club. Their roles in the spread of infection are therefore suspected in the presence of diseased animals.

Strict vector control around the stables, coordinated horse gaming, updated vaccination of horses as well as their routine tests and good management practices are encouraged to prevent future outbreaks of AHS.

Biosecurity (biocontainment and bioexclusion), good peri-stable hygiene practices, and further research into culicoides identification and their actual role in the Nigerian ecological system and African horse sickness are encouraged. Most importantly, the research community should carefully consider the possibility of other infectious diseases already living with us or expanding their territories.

Reference

Fasina, FO; Potgieter, AC; Ibironke, AA; Bako, B; Bwala, DG; Kumbish, PR. (2008). First Report of an Outbreak of African Horse sickness Virus Serotype 2 in the Northern Hemisphere. *Journal of Equine Veterinary Science*, 28(3): 167-170.

Maree S, Paweska JT. (2005). Preparation of recombinant African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. *Journal of Virological Methods*. 125: 55–65.

World Organization for Animal Health (OIE). African horse sickness. In: Manual of diagnostic tests and vaccines for terrestrial animals. Paris, France; OIE; 2004. Also available at: http://www.oie.int/eng/normes/mmanual/A_00034.htm.

Bremer CW, Viljoen GJ. (1998). Detection of African horse sickness virus and discrimination between two equine orbivirus serogroups by reverse transcription polymerase chain reaction. *Onderstepoort Journal of Veterinary Research*. 65:1–8.

Potgieter AC, Steele AD, Van Dijk AA. (2002). Cloning of complete genome sets of six dsRNA viruses using an improved cloning method for large dsRNA genes. *Journal of General Virology*. 83:2215–2223.

Maan S, Rao S, Maan NS, Anthony SJ, Attoui H, Samuel AR, et al. (2007). Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. *Journal of Virological Methods*. 143:132–139.

Potgieter AC, Cloete M, Pretorius PJ, van Dijk AA. (2003). A first full outer capsid protein sequence data-set in the Orbivirus genus (family Reoviridae): cloning, sequencing, expression and analysis of a complete set of full-length outer capsid VP2 genes of the nine African horse sickness virus serotypes. *Journal of General Virology*. 84:1317–1326.

Tamura K, Dudley J, Neil M, Kumar S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 10. 1093/molbev/msm092.

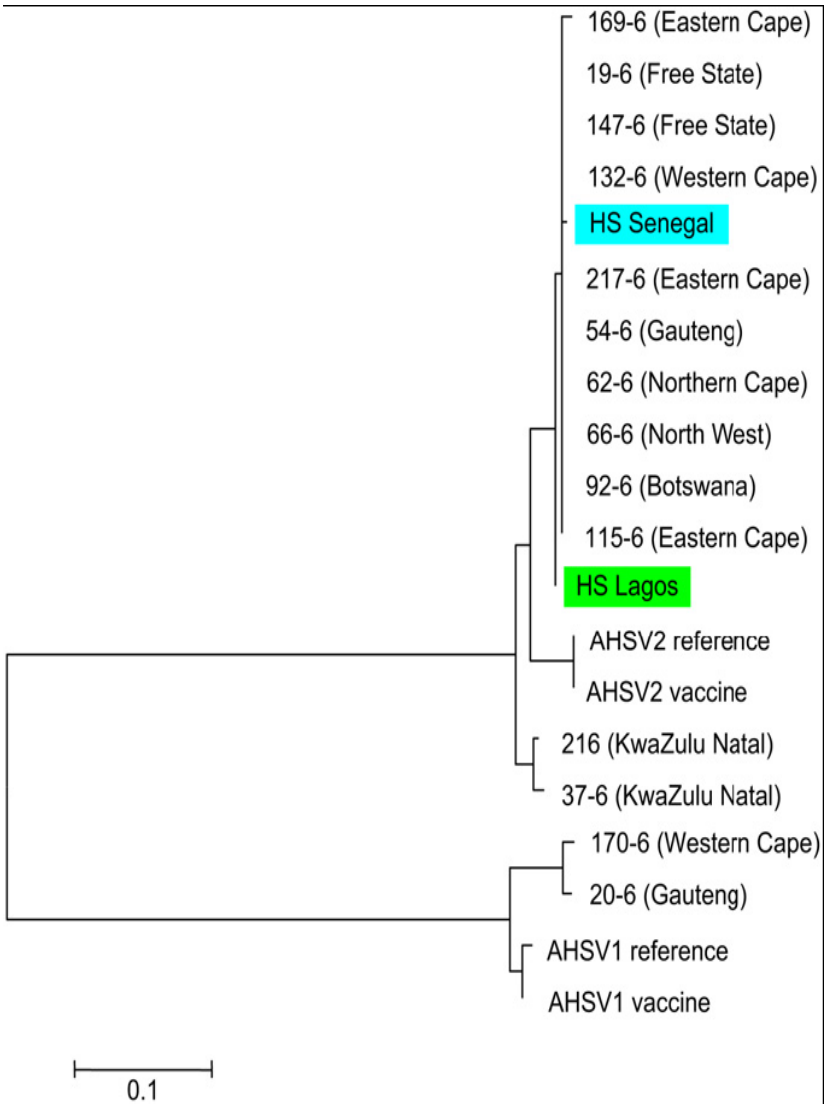

Figure 1: Lagos and Senegalese isolates phylogenically grouped with the AHSV serotype 2 from different parts of South Africa

Retirement and Planning for Retirement

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Most Nigerians working in the public service are familiar with the word "RETIREMENT" which was popularized by the successive Military administrations with their practice of retiring public officers "with immediate effect" Retirement when unexpected can be devastating, frustrating, can lead to mental problem or even death. To avoid the attendant problems associated with retirement, it is very important for every "active" employee to know that there is time for everything under heaven; a time to take up a job and a time to leave it. Then it is important to start preparing one's mind towards that time so that when it comes sooner than expected, there are no shocks. [°]

Workers in the Public and Private Sectors should be as wise as the ant. The ant, a very tiny creation without guide or leaders to give directives knows that a time of hardship is coming and so it prepares itself to face it by building and re-building its house and gathering food in it. There is therefore an urgent need for us the "active" employee of today to prepare to become the retirees of tomorrow.

Reasons for Retirement

Physical and mental pressures may arise with ageing and this is the reason for administratively setting retirement age limits. The need for employers to open up higher positions to staff who are more active mentally and physically is another reason. Financial solvency i.e. the ability of the individual to afford to be on his or her own or disenchantment with the work environment and work ethics, failing health or a desire to have greater control over the ordering of one's life are all reasons for retiring from service. Retirement is compulsory after 35 years of service or when an officer reaches 60 years. Retirement may be voluntary or it may also occur in the public interest.

Facets of Retirement

Retirement may be seen in different ways
E.B. Flippo sees retirement as a "role less role" leading to mental and physical illness and sometimes to pre-mature death. He describes it as "idleness and a living death"

K. Mossman describes retirement as "Unoccupied persons like the empty container collapse most readily under external pressure". "To become miserable one should have leisure to bother about whether one is happy or not" and "A perpetual holiday is a good working definition of Hell"

J.A. Akani describes retirement as "A time to sleep late, vacation, visit friends e.t.c. "A period of uselessness filled with empty make-work projects"

J.W. Bettman: "One spends years in preparing for an occupation but often retires from it with little or no preparation.

Need to Plan for Retirement

Planning for retirement enables individuals to differentiate between dream and reality well in advance. It enables one take a close look at all the factors which have a bearing on life in retirement and to pursue the realities rather than mere assumptions, one reality is that in most cases, post retirement benefits fall short of earnings in prime days.

Another reality is that several regrets come into focus at the point of retirement; regrets of actions not taken, those taken but with unpleasant consequences and regrets of actions wrongly taken. Planning for retirement helps bring these types of regrets to a minimum and assists in focusing on post retirement. It also helps ascertain how we should make ourselves comfortable in

[°] Paper presented on 4th August, 2008 at NVRI auditorium

retirement and minimise discomfort to the family.

Factors to Consider in a Retirement Plan
Target saving for retirement (how much would one need in retirement?)

Accommodation
1. Will I start a small business after retirement? (SWOT) analysis.
2. Investment options
3. Dependants and their expenses
4. Inflation
5. Health

Suggestions for Planning for Retirement
In order to ensure satisfaction in retirement, the following specific lines of action are suggested
1. There should be gradual disengagement from work.
2. Counseling sessions should be held for officers whose retirement is impending so as to prepare them psychologically.
3. Employees preparing for retirement should start developing new interests outside the employer's circle.

Above all, retiring officers should identify and start pursuing post-retirement occupations suitable for their life styles. With regard to the last strategy, a choice can be made between the following
1. Getting another paid job
2. Pursuit of hobbies
3. Starting one's own business.
4. Investing, training, physical development, spiritual development, health monitoring.
5. Time management strategy
6. Managing change in retirement.
7. Contingency issues at retirement.

Steps to Self Employment
Have a dream, do away with fear of the unknown, failure and believe that you have what it takes to convert any dream into reality. Approach the dream with a passion.

Getting started
1. Acquire the skill
2. Source funds
3. Obtain a strategic location
4. Recruit the right people
5. Be a person of high integrity.
6. Be Customer- focused
7. Keep proper financial records.
8. Separate business finances from domestic finances.

Conclusion
Planning for retirement makes the future better and it helps to increase the chances of longevity of the retiring officer. It allows the individual to prepare for the future by developing a series of ideas. It allows the individual to examine his/her strengths and weaknesses in terms of what to do after retiring. It prepares the mind for the challenging task of managing one's initiatives to determine the best option taken. It removes the psychological effect of the fear of the unknown after retiring. After retirement, retirees are by being effective and efficient time managers, managing change, and ensuring a balanced life.

References
E.B. Plippo, Personnel Management. McCraw Hill Series in Management. 1980.

S.M. Ngu. Personnel Management in Nigeria: Principles and Practice. Gaskiya Corporation Limited, Zaria. 1994.

K. Mossman, Looking forward to Retirement. Ward Lock Limited, London, 1971.

J.A. Akanni, Management: Concepts, Techniques and Cases. Julab Publishers Ltd, Ibadan, 1987.

Stahl O.G. Public Personnel Administration. Harper and Row Publishers, London 5th Ed. 1971

C. Booth, "Old age Pensions and the aged poor" in pension scheme practices Pitch M. and Victor W. Hutchinsons, and Co. Ltd, London, 1967.

Harold Bieby, Today's Pensions Livingstone and Co, London, 1956. Macre and Strieb, Pension: The due of Retirees. Mento Press Ireland, 1959.

Multiplex PCR using Sequence Characterized Amplified Regions (SCAR) Makers for Identification of *Eimeria Species* in Chicken.

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Coccidiosis is caused by intracellular parasitic protozoa of genus *Eimeria* with infections frequently presented as simultaneous infections of multiple species (Barta 2001).In the chicken, seven species exhibiting different pathogenicity include; *E. acervulina, E. brunetti, E. maxima, E. tenella, E. mitis, E. praecox and E. necatrix.* These obligatory parasites affect the enteric and cause lesions of variable extents and severity (McDougald & Reid, 1997).°

Coccidiosis in Nigeria remains a limiting health problem in intensive poultry husbandry with moderate to high prevalence rates of 36-43%. Control measures are based on the use of anticoccidials in feeds. The increasing problem of drug resistance to anticoccidials (Chapman 1999), vaccinations with virulent or attenuated parasites and attempts to develop nonviable vaccines has met with mixed results. Species differentiations based on biological features alone has not possible (Barta, 2001)

Molecular methods used for Coccidia identification included isoenzyme analysis, DNA hybridization and RAPD-PCR. Previous studies by Johnston and Fernando, 1995 and 1997; described the extensive use of isoenzymes' electrophoretic profiles for *Eimeria* speciation and detection of differences between *Eimeria* species and RAPD-PCR for strain differences and characterisation at molecular level. Although, results were promising, isoenzyme methods required way too many oocysts, yielded limited number of variable enzymes, low levels of polymorphism and considered too cumbersome and time-consuming. RAPD- PCR was difficult to standardize and adopt universally coupled with the fact that it could not deal with mixed samples. Shirley (1994) had earlier proposed the use of DNA hybridization for species and strains discrimination but for its complexity, its use was also impaired across different laboratories (Procunier et *al* 1993; Shirley 1994; Johnston & Fernando 1995& 1997; Fernandez et al 2003). Thus molecular techniques using PCR of DNA extracted from the parasite is the current method recommended for specie differentiations.

Fernandez et al (2003) reported the use of a novel sets of SCAR Makers isolated from RAPD fragments incorporated into a cost effective simple multiplex PCR that allowed simultaneous differentiation of all seven species of Eimeria affecting chicken and propose its effective universal usage where strains from different distinct geographical regions were tested.

This study evaluated the usefulness of Multiplex PCR of the small subunit r RNA developed from SCAR Makers in the simultaneous discrimination of *Eimeria* species of chicken from Nigeria and two different geographical sources.

Materials and Methods

Oocysts were obtained from samples submitted to the National Veterinary Research Institute's Central Diagnostic

° Paper presented on 11th November, 2008 at NVRI auditorium

Laboratories, and the Parasitology Division, Vom. The Houghton (H) strain of *E. tenella* was obtained as pure lines from the Institute for Animal Health (IAH), Compton UK.

Faecal material were homogenized and filtered. Filtrate was pelleted by centrifugation at 900g for 2 min. The supernatant was discarded and pellets were re-suspended in distilled water.

Oocysts were finally recovered by flotation method in a saturated solution of sodium chloride. The mixture was centrifuged at 400g for 10–15 min and the oocysts were removed from the surface of the supernatant, by decantation and re-centrifugation. The oocysts were re-suspended in water, and washed with PBS and centrifuged four times to remove the salt solution. The salt-free oocysts suspension was then stored in 2.5% potassium dichromate solution at 4^{o}C.

DNA Extraction

Extraction method was based on the use of DNAzol Method (GIBCO BRL Life Technology).

Multiplex PCR and Identification of the PCR Products

Multiplex PCR reaction amplification was performed in 25µl total volumes and included 12.5µl of premix, 10.5µl Nuclease Free Water, and 2µl Template DNA. Typically the premix consisted of the following: 100 µM dNTP, 50mM MgCl2, 10x $MgCl_2$ Buffer, 5U of Invitrogen DNA Taq Polymerase; primer concentrations 10µM for each primer pair of Ac-01- F,Ac-01-R bp 811; Br-01-F,Br-01-R,bp 626; Tn-01-F ,Tn-01-Rbp 549; Mt-01-F,

Mt-01-R bp460; Pr-01-F, Pr-01-R bp354; Mx-01-F, Mx-01-R bp 272; and Nc-01-F,Nc-01-R bp 200 was used.

Cycling conditions consisted of an initial Denaturation at 96 ^{O}C for 6min and 40cycles of 1 min at 94 ^{O}C and 2min at 55 ^{O}C, with a final extension step at 72 ^{O}C for 5min. Amplification reactions were performed with a PTC 1148(100-240 V) MJ Mini Gradient Thermal Cycler (BIO RAD California, USA).

Amplicons produced were analysed by separation on 2% agarose gel stained with ethidium bromide and visualized under UV light.

Results and Discussion

Seven species, *E. acervulina* ,bp 811*; E. brunetti,* bp 626; *E. tenella* bp539*; E. mitis*, bp 460; *E. praecox* bp 354*; E. maxima*, bp 272 *and E. necatrix* bp200, were identified by multiplex PCR. Most samples revealed multiple species of *Eimeria*, infections. The result showed that it is possible to detect simultaneously, multiple infections in a single - tube reaction . The Multiplex PCR using SCAR markers worked successfully and this study confirms the possibility of differentiating coccidia oocysts in a sample containing one or more species using RAPD-SCAR markers and demonstrates the simultaneous detection of the 7 *Eimeria species* affecting chicken in a single-tube reaction.

The development of Sequence-Characterised Amplified Regions (SCAR) Makers, was first described by Paran and Michelmore (1993). It derived from Randomly Amplified Polymorphic DNA (RAPD) in which specific fingerprints were generated and re-amplified under strictly controlled conditions by pairs of specific primers worked effectively in detecting and discriminating all *Eimeria species* of chicken.

Figure 2: Multiplex PCR for Vom, Guelph & Houghton samples.

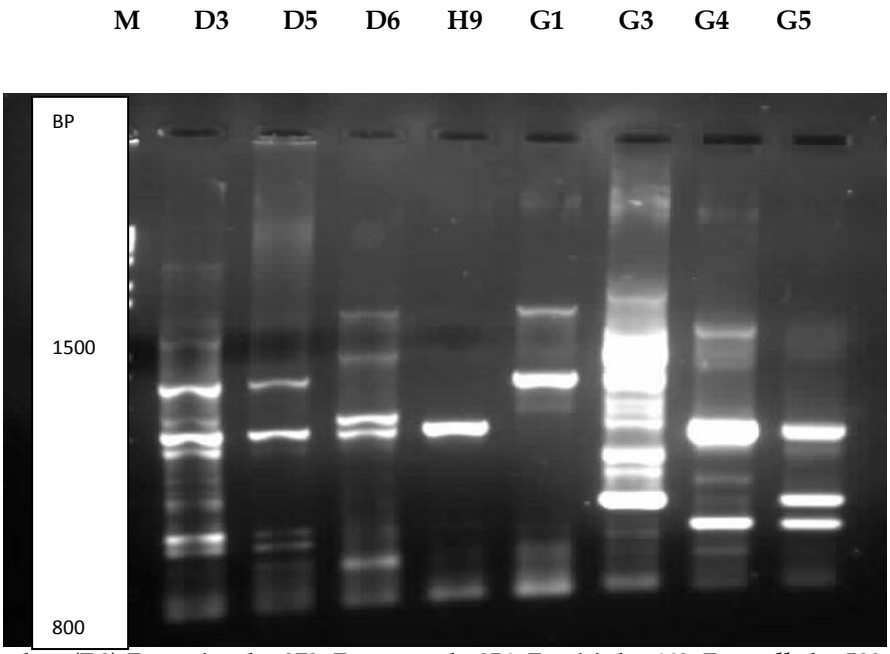

Fig.2: M 100 marker, (D3) *E .maxima* bp 272; *E. praecox* bp354; *E .mitis* bp 460; *E .tenella* bp 539; *E. brunetti* bp 626; *E. acervulina* 811; (D5) *E. maxima* bp272; *E. tenella* bp539; *E .brunetti* bp626; (D6) *E. tenella* bp 539;*E. necatrix* bp200; (H9) *E. tenella* bp539; (G1) *E. brunetti* bp626; *E. acervulina* bp811; (G3) *E. praecox* bp354; *E .mitis* bp 460; *E. tenella* bp 539; *E. brunetti* bp 626; *E. acervulina* bp811; (G4) *E. necatrix* bp200; *E. tenella* bp539; *E .acervulina* bp 811 (G5) *E. necatrix; E .praecox* bp354; *E. tenella* bp539

Fernandez *et al* (2003) isolated RAPD -SCAR Makers and generated an integrated economical simple multiplex PCR that allowed simultaneous differentiation of all seven species of *Eimeria* affecting chicken and reported its effective universal application for species/strains discrimination from two different continents.

The SCAR, M-PCR was able to differentiate all the *Eimeria spp* from diff regions used in this study. We tested samples from 3 different continents and thus concluded that Multiplex PCR, using SCAR Markers offer a potential universal tool for the epidemiological study of coccidiosis in poultry in general. Our trials confirmed the observations of Fernandez *et al* (2003) where use of M-PCR, SCAR markers was not affected by internal variations within species and is applicable worldwide. It may however be limited in terms of differentiating strains of eimeriid oocysts. We observed some differences in SCAR generated sequences in *E. maxima* [data not shown] and recommend that more work be done in this area where SCAR-RAPD generated sequences could be used for strain differentiation.

Other attempts at detection and discrimination of *Eimeria species* using the internal transcribed spacer1 (ITS1) regions of ribosomal DNA (rDNA), RAPD-PCR and isoenzymes analysis have been described and demonstrated to be effective in *Eimeria species* detection and differentiation and in some cases used to characterize strain variability. However, the various setbacks involved in the use of these methods caused by several factors of enzyme sources, primers, DNA concentrations, buffer composition, thermocycler models restricted and impaired their wide use across laboratories.

The added advantage of being able to detect very low infections confirmed in our study where isolates containing less than 1000 oocysts per ml was used.

With the encouraging results obtained in this study, similar Multiplex PCR methods for detecting Coccidiosis in other intensively reared birds like turkeys are suggested. The usefulness of SCAR Maker, M-PCR can be assessed in maintaining vaccine integrity, monitoring of infection in a poultry establishment especially with focus on drug administration and efficacy.

To the best of our knowledge, this report is the first on the use of SCAR Markers in the multiplex -PCR diagnostic assay of *Eimeria species* in chicken to include samples from Nigeria.

Acknowledgements
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References

Barta R. John (2001) Coccidiosis. Encyclopaedia of Life Sciences Nature Publishing Group /www.els.net.

Chapman, H.D. (1999). Anticoccidial drugs and their effects upon development to immunity to Eimeria infection in poultry. Avian Path; 28: 521-535

Fernandez S., A.H. Pagotto, M.M. Furtado, A. M. Katsuyama, A. M. B. M. Madeira and A. Gruber (2003). A Multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl. Parasitology 127, 317 – 325.

Johnston, D. A. & Fernando, M. A; (1995). Eimeria spp. of the domestic fowl: analysis of genetic variability between species and strains using DNA polymorphisms amplified by arbitrary primers and denaturing gradient- gel electrophoresis. Parasitology Research 81, 91-97.

Johnston, D. A. & Fernando, M.A; (1997).Isoenzymes of Eimeria from the domestic fowl: Electrophoretic variants among species, strains and clones. Parasitology Research 83, 464-470.

Mc Dougald, L. R. and Reid, W.M; (1997). Coccidiosis. In Diseases of Poultry, 10th Edition (Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R. &Saif, Y.M.), pp.865-883 Iowa State University Press, Amiens, USA.

Paran, I. & Michelmore, R.W; (1993). Development of reliable PCR –based markers to downy mildew resistance genes in lettuce. Theoretical and Applied Genetics 85, 985-993.

Procunier, J.D; Fernando, M. A. & Barta, J. R; (1993). Species and strain differentiation of *Eimeria* spp. of the domestic fowl using DNA polymorphisms amplified by arbitrary primers. Parasitology Research 79, 98-102.

Shirley, M. W. (1994). Coccidial parasites from the chickens: discrimination of different populations of Eimeria tenella by DNA hybridisation. Research in Veterinary Sciences 57, 10-14.

Direct Rapid Immuno histochemistry Test (DRIT): An Alternative Tool for Rabies Diagnosis in Nigeria

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The first diagnosis of rabies in humans and animals were in '1912 and 1925 respectively (Boulgher and Hardy, 1960). Between 1928 and 1990 a total of 3770 confirmed rabies cases (Oboegbulem, 1994) while between 1991and 2005 a total of 1039 confirmed rabies cases (Garba, 2007) that is a total of 4809 specimens were diagnosed positive for rabies by the NVRI rabies laboratory between 1928 and 2005 in Nigeria . The diagnostic technique employed by NVRI, Vom is Sellers staining for demonstration of Negri body. [°]

Direct Rapid Immunohistochemistry Test (DRIT)

The Direct Rapid Immunohistochemistry test (DRIT) is an unlicensed procedure designed for consideration as a potential confirmatory measure of the direct fluorescent antibody test. (CDC, 2008). RIT may also be used to enhance field surveillance among suspect wildlife or stray dogs, particularly in support of national, regional, state, or local oral vaccination programs.

A Need for DRIT as Alternative Tool for Rabies Diagnosis in Nigeria

Apart from the national anti rabies campaign held in 1982 (Oboegbulem, 1994), there has not been a major systematic and well co-ordinated vaccination campaign against rabies in Nigeria. The key to any successful strategy for the control of rabies lies in the ability to identify the disease promptly. To date, there have been only six human survivals from clinical rabies worldwide (Willoughby *et al.*, 2005). The successful recovery of these patients was partly due to early detection of the disease. The main problems of rabies control or eradication in the reservoir host (dogs especially) is the occurrence of asymptomatic or carrier state of rabies as been reported in Africa and elsewhere in the world (Bell, 1975; Fekadu and Baer, 1980; Fekadu, 1988). Reports from Northeast (Ajayi *et al.*, 2006) and Northwestern Nigeria (Garba, 2007) suggest the occurrence of the rabies viral antigen in the brains of apparently healthy slaughtered dogs. The Direct rapid immunohistochemistry test, a sensitive and relatively inexpensive technique can be an alternative and prompt diagnostic tool for rabies in Nigeria; especially its use with light microscope which is available in every laboratory across the country.

Conclusion

DRIT if accepted and implemented as a diagnostic tool will no doubt enhance the surveillance and diagnosis towards improved prevention and control of rabies in Nigeria. It is therefore suggested that the stakeholders especially the University teaching hospitals and National Veterinary Research Institute, Vom initiate some work using DRIT for rabies diagnosis with a view to adopting it as a diagnostic method for rabies diagnosis.

References

Ajayi, B.B; Rabo, J.S; Baba, S.S (2006). Rabies in apparently healthy dogs: histological and immunohistochemical studies. The Nigerian Postgraduate Medical Journal.13 (2), 128-134.

Centers for Disease prevention and Control (2008). Direct rapid immunohistochemistry test (DRIT) for the detection of rabies virus

[°] Paper presented on 9th October, 2008 at the conference room NVRI quality control laboratory

antigen. National conference workshop on rabies organized by the Department of Veterinary Surgery and Medicine, Ahmadu Bello University- Zaria, Nigeria in collaboration with FMoH, Nigeria and Centers for Disease controls, Atlanta-USA. Held at the Institute for Development Research, ABU-Zaria 17th -21st June 2008. Pp1-13

Bell, J.F. (1975). Latency and abortive rabies. In: G.M Baer (Ed.).The Natural History of Rabies Vol.1pp.331-350.

Boulger, L.R. and J. Hardy. (1960). Rabies in Nigeria. West Africa .Med.J. 9(6), 223-234.

Fekadu, M. and G.M. Baer. (1980). Recovery from rabies of two dogs inoculated with rabies virus strain from Ethiopia. American J. of Veterinary Research. 40(10). Pp1632-1634.

Fekadu, M. (1988).Pathogenesis of rabies virus infection in dogs. Rev.Infect. Dis. 10(4). Pp678-83.

Garba, A (2007). Studies of rabies virus antigen in the brain of apparently healthy slaughtered dogs from Sokoto and Katsina States Nigeria. A Masters Dissertation. Department of Veterinary Public Health, Faculty of Veterinary Medicine Usmanu Danfodiyo-Sokoto, Nigeria. January '2007.

Oboegbulem, S.I (1994). Rabies in Man and Animals. 1st ed. Fidelity publishers, Enugu, Nigeria. Pp 1-23

Willoughby RE, Tieves KS, Hoffman GM, Nancy, S; Ghanaycu, MD; Catherine, M ; Amlie-Leford, MD; Micheal, J; Chusid, M.D; AND Rupprecht, C.E (2005). Survival after treatment of rabies with induction of coma. *N Engl J Med; 352: 2508--14.*

The Logframe for Monitoring and Evaluation System Design Objective

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The Logic Framework or logframe is essentially a planning tool which subject project design to a systematic approach providing detailed information, invaluable for monitoring and evaluation. It is therefore considered an invaluable aid to project planners and implementers. Project management problems arise because, expected outcomes are too vaguely stated, important assumptions that may affect project success are not explicitly spelt out, responsibilities are not clear and monitoring and on-going evaluation activities are inadequately conducted.[3]

The logframe provides a format for organizing information in order to highlight the relation between means and ends in project design. It specifically

1. Forces the planner to fit the main component of project into a single framework.
2. Spells out in more definitive terms the results expected.
3. Distinguishes between what needs to be done, what we can do, the resources required and the effects we hope to achieve.
4. Promotes the establishment of more realistic targets.
5. Provides measures to monitor the progress of projects.
6. States assumptions required to make casual linkages possible.

The logframe matrix has both vertical and horizontal consistencies which link together the different squares. The hierarchy of project objective (i.e. inputs, outputs, purpose and goal) occupies the vertical axis while the goal statements, assumptions, indicators and means of verifying them take up the horizontal axis so as to create a series of cells which contain the basic ingredients of the project design.

For project implementers, the logical framework is a means of clarifying the project design. It also brings into focus the indicators of success and the specific targets that must be achieved and when. This information becomes the data for designing, monitoring and evaluation systems.

There are various versions of the logframe. The original model a 4 x 4 matrix developed by practical concepts incorporated was popularized by the United States Agency for International Development (USAID). There are also the 5 x 5 and 3 x 3 matrix design models. The implementation logframe differs from the project Design Logical Framework in that it emphasizes the activities and areas of responsibility of the project team and management interest during the implementation phase of the project.

THE STRUCTURE OF THE LOGFRAME

The logframe Matrix/Format, as shown in Figure 1 is the key element in the logframe methodology. A properly completed matrix brings into focus the main components of a project plan and the important relationships.

The format proposed for monitoring and evaluation system designing elaborates on this basic format by specifying the statement of assumption by attaching indicators, and means of verification of performance. This will clarify external factors for ease of monitoring (see Figure 2) **The VERTICAL LOGIC** (or project

[3] Paper presented on 28th October, 2008

logic) is a set of means and ends interrelation in a logical fashion and intended to define the way the project inputs are transformed into development goals. It is based on the assumption that the achievement of ultimate project objectives proceeds through a hierarchy of sub-objectives linked by a set of hypothesis

1. If we provide the following inputs, then we produce the requisite output.
2. If we produce those outputs, then the purpose will be achieved.
3. If the purpose is achieved, then the goal will be realized.

The HORIZONTAL LOGIC helps to clarify the statements of objectives, by identifying what is to be produced and the evidence that will signal the success of each level of the project objective hierarchy and necessary assumptions it list.

* Measures indicators of progress or performance

* Means of verification

With the addition of these elements to the vertical logic, a logframe is completed. At this stage it displays both vertical and horizontal consistency that should guarantee successful implementation. In order to complete the horizontal logic at this level, we need to answer the following questions.

1. How can we measure if the project has achieved the objective? This is the purpose of INDICATORS
2. How shall we get proof that we have achieved the objective set? By what means can the indicator be measured or verified? (MEANS OF VERIFICATION?)

The preparation and implementation of the logframe design for M & E requires the participation of all stakeholders/beneficiaries.

The need to generate information on input deliveries, work schedules, targeted outputs and other required actions accompanying projects requires proper tracking and checking on-station and field project implementation. This is the aim of a well designed logframe for monitoring and evaluation.

REFERENCES

Olive Publications Planning for Monitoring and Evaluation, Olive 2002

Shapiro, Janet Evaluation: Judgement Day or Management Tool? Olive 1996

ARMTI Course Manual: Agricultural Project Monitoring and Evaluation, (ARM 223)

Concepts, Issues and Problems in Monitoring and Evaluation of Rural Development Projects: United Nations Economic and Social Council Publication, 1987.

FIGURE 1: **The "Logical framework"**
Life of project:
From FY_____ To FY_____
Total funding_____
Project title_____

Narrative summary	Objectively verifiable indicators	Means of verification	Important assumptions
Programme or sector goal: the boarder objectives to which this project contributes	Measures of goal achievement		**Assumption of achieving goal targets**
Project purpose	Condition that will indicate purpose has been achieved: end of project status		**Assumptions of achieving purpose**
Output	Magnitude of output		**Assumptions for providing output**
Inputs	**Implementation target (type and quantity)**		**Assumption for providing input**

FIGURE 2: "**Logframe for Designing Monitoring and Evaluation system**"

Life of project _____
Total funding _____
Project title _____

PROJECT OBJECTIVES			ASSUPMTIONS		
Narrative summary	**Performance indicators**	**Means of Verification**		**Performance indicators**	Means of Verification
Goal **Purpose**			Purpose to Goal Output to purpose		
Inputs			**Inputs to Output**		

USAID Programme on Control of Highly Pathogenic Avian Influenza in Nigeria: Lagos Zone

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Nigeria recorded its first case of Avian Influenza in February 2006, near Kaduna, since then the disease has spread throughout the country across several Local Government Areas (LGAs) and States.°

In response to the Avian Influenza outbreak in Nigeria, the Federal Government and International Agencies such as FAO in partnership with WHO/OIE implemented emergency technical programmes to prevent, control and if possible eradicate the dreaded disease. To effectively achieve this goal, there is a need for robust veterinary services and logistics, which is currently inadequate in Nigeria. Thus the need for assistance from development partners, such as the USAID to assist in provision of technical needs for the control.

The USAID multi-donor project, programming/technical and administrative supports were provided and coordinated through the UNDP/UNV /FAO while Federal Livestock Department and Pest Control Services (FLD&PCS) played host with the primary areas of assignments in designated states under the country's epidemiological zones. This paper presents reports on HPAI status and control efforts in the Lagos zone.

Materials and Methods

Eight recruited participants served as UN volunteers during the project period. Volunteer facilitated project implementation and capacity building of surveillance officers in their zones and also produced monthly reports in consultation state Directors of Veterinary services.

The project goals of this project were

1. Laboratory confirmation of suspected and reported cases of avian influenza outbreak.
2. Epidemiological investigation of all outbreaks by the Lagos-Ogun special diagnostic team in accordance with agreed terms.
3. Passive surveillance of farms by Veterinary Officers through the identification and registration of farms, provision of farm-health logbooks and on-farm briefing on the importance of biosecurity.
4. Active surveillance of the disease in live bird markets over a period of six weeks. Sera, tracheal and cloacal swabs were to be collected and sent to NVRI Vom for laboratory analysis.
5. Weekly fumigation and decontamination of live bird markets
6. Training of personnel in A.I disease recognition and actions required in possible outbreak.
7. Public enlightenment through workshop/seminars and media publicity for the farmers and other stakeholders on the importance of biosecurity.

Results

In 2006, Lagos recorded 69 outbreaks in 19 locations spread over 8 LGAs (Agege, Alimosho, Amuwo-dofin, Epe, Ojo, Badagry, Ikorodu, Ifako-jaiye), this continued until 2007 with additional 8 outbreaks in 9 locations, while Ogun state recorded it initial outbreak in June 2007 spreading across 4 LGAs (Obafemi-owode, Ewekoro, Ifo, Ado-odo/Ota) in 19 locations. A total of 679,632 birds were at risk, while 419,922 died of the disease and 109,455 birds were depopulated in both states (Table I). The virus affected all types of commercial birds including ostrich and pigeons.

° Paper presented on 30th October, 2008 at NVRI auditorium

Discussion

There were 21 confirmed cases of AI with 19 in Ogun State and 2 in Lagos State. The epidemiological investigation revealed that A.I cases peaked in September and ceased completely in October 2007. Out of the 21 cases recorded, 10 (47.5%) farms were managed on deep litter while 6 (28.5%) were managed on battery cages. Outbreaks were also recorded in 5 farms (23.8%) with both husbandry management practices. The duration of infection in all cases ranged from between 4 and 21days. There was a history of poor bio security practices complicated by doubtful AI vaccination information.

There was also a high number of outbreaks amongst settlements where poultry farms were in close proximity of <1km apart and a few cases where poultry markets were situated in within a 6kms radius to infected farms. This close circuit facilitated the common sharing of service providers; animal health officers, feed transporting system, egg and live poultry merchants/retailers. Illegal poultry movement, human interactions and movements within and out of previous outbreak sites and exchange of infected paper egg crates between farms were observed. There was a high number which appeared to have survived the outbreaks. This may be of epidemiological significance as these birds could serve as potential immune carriers capable of re-contaminating the environment. The standard control and containment strategies adopted were quarantine, depopulation and decontamination of equipment and premises, while the dead birds were buried on farm dumping sites. Feacal droppings were burnt, buried, dumped or used as organic manure by vegetable farmers. This has short comings as a result of distortion in the ecosystem with a resultant great public health consequence.

Conclusion

From this trend of events, at the end of October 2007, it can safely be concluded that the Avian Influenza outbreaks have subsided possibly due to under or none reporting attitude of the farmers for a fear its negative impact on poultry trade, and or a possible hibernation of the virus in some suitable foci. This could, however, cause localized, regional and or global pandemic on re-emergence and thus requires continuous evaluation of the infection status and control efforts particularly in animals to avoid possible human infection.

Acknowledgement

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	Lagos State	Ogun State	Total
Number of farms affected	34	19	53
Number of LGAs affected	8	4	12
Number of birds at risk	620,199	59,433	679,632
Number of birds death	414,910	5,012	419,922
Number of birds depopulated	64,325	45,130	109,455

Table I: Distribution of HPAI outbreaks in Lagos Epidemiological zone

Utilization of Differently Processed Pigeon Pea (*Cajanus cajan* L. Millsp) Seed Meal by Broilers and Cockerels

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The pigeon pea (*Cajanus cajan* L. Millsp) is perhaps the most widely grown agricultural legume in tropical and sub-tropical countries. The protein content of the seed ranges from 19 – 29.3% (Purseglove, 1984; Amaefule and Nwagbara, 2004) and the protein quality is reasonably good except like most leguminous grains; it is deficient in sulphur amino acids in comparison to animal protein. [°]

The pigeon pea's wide availability, nutritive value and low human food and industrial preference place it as a suitable alternative to maize and soyabeans in the diets of monogastrics. This will not only reduce cost of monogastric feeding, but also enhance the productive base of livestock farmers. However, like most leguminous crops, the seed contains antinutritional factors like trypsin and amylase inhibitors that limit its use by monogastric animals (Amaefule and Obioha, 2001). The seeds therefore, require processing to deactivate such antinutritional factors before they are efficiently utilized by the animals. The aim of this study was to determine the potential of pigeon pea as an alternative source of dietary energy and protein for poultry with the specific objective of studying the effect of different methods of processing of pigeon pea seed meal on performance of broilers.

Materials and Methods

Source and processing of pigeon pea

The pigeon pea seeds in this study were the brown variety type obtained from Angwar Mailafiya market in Jama`a Local Government Area of Kaduna State. The seeds were weighed and processed in four different ways: The first (T_1) was crushed and included raw in the diet labelled Raw Pigeon Pea (RPP). The second (T_2) roasted for 25 mins, crushed and included in the diet. The third (T_3), was boiled in water (100^{o}C) for 30mins, sun dried for 3 days, crushed and included in the diet. The fourth (T_4), was crushed and passed through an extruding machine (steam heated) at 121^{o}C for 25 seconds. The extruded flakes were sun dried for a day, crushed and included in the diet.

Experimental birds and management

In a completely randomized design, two hundred (200) seven - day old unsexed broiler chickens (Anak, 2000 strain) were divided into 5 groups of 40 birds each. Each group was further divided into 4 replicates of 10 birds each. Each replicate was housed in a floor pen measuring 2.4m^2 equipped with feeders and drinkers and the floor covered with wood shavings as litter material. The birds were vaccinated at 12 and 26 days against Gumboro and at 20 days against Newcastle disease.

Experimental diets

The birds were fed five different isonitrogenous diets at starter (23% CP) and finisher (21% CP) phase. The diets labeled T_1 (control), T_2, T_3, T_4 and T_5 contained 0.00% (control) and 30.00% levels of inclusion of pigeon pea seed meal processed by different methods respectively. The diets along with clean drinking water were provided *ad libitum* throughout the 8 weeks of the experiment. The broiler starter mash was fed for 3 weeks and the finisher mash fed for the remaining 5 weeks.

Chemical analysis

The experimental diets and differently processed pigeon pea seeds were analyzed for proximate composition (AOAC, 2006). The processed pigeon pea seeds were also analyzed for amino acid profile as described

[°] Paper presented on 28th November, 2008 at NVRI auditorium

by Spackman *et al*. (1958) and antinutritional factors (trypsin inhibitor, haemagglutinins, cyanide, oxalates, phytic acid and tannins).

Statistical analysis

Data collected on growth performance indices were subjected to statistical analysis using SPSS (2006).

Chemical analysis

The levels of antinutritional factors (Table 1) in the raw seeds are comparable with what was reported by Salunkhe, *et al*., (1985), D'Mello, (1995), Udedibie and Carlini, (2002) and Onwuka, (2006). The phytic acid content of the raw seeds in this study is however higher than 12.0mg/100g reported by Onimawo and Akpojovwo (2006). Also, trypsin inhibitor level of the seed in this study is higher than 4.8mg g^{-1} reported by Purdue, (2006). This may be as a result of differences in cultivar and conditions under which the crop was cultivated. None of these authors have reported the presence of oxalates in pigeon pea seeds. The presence of oxalic acid in the seeds may be as a result of the type of soil upon which the crop was cultivated because it is one the factors that is said to affect chemical composition of the seeds as stated by Purdue, (2006). Boiling was more efficient in reducing the levels of antinutritional factors when compared with the other methods; it reduced the cyanide content by 24.00%, oxalate by 91.4%, phytic acid by 7.10%, tannins by 99.06% and trypsin inhibitor by 79.36%. Roasting and extrusion were however more efficient in eliminating the cyanide levels as they reduced it by 47.10 and 76.48% respectively.

The amino acid profile for the pigeon pea seeds (raw and processed) appeared not to be affected by processing.

Growth performance

The growth performance indices of the birds showed no significant differences ($P>0.05$) in the overall performance of the birds between dietary treatments. The birds fed the control diet appeared to have performed slightly but not significantly better than those fed the test diets in weight gain, feed conversion ratio and feed cost per kg gain.

The growth performances are generally below what has been reported with respect to growth performance of broilers. The final weights of the birds in this study were however, better than the range of 1220.00 – 2170.00g weights obtained by Etuk *et al*, (2003) and Etuk and Udedibie, (2006) using pigeon pea at 58 and 63 days respectively. These results were however, comparable with what was obtained by Ani and Okeke (2003) when they substituted pigeon pea for soyabean in broiler finisher diets. Feed intake values were comparatively higher than the rage of 98 – 112 g/day reported by NRC (1984) and Jagdish, (2005).

The FCR values in this study were higher than 1.8 to 2.0 reported as optimal for broilers by Oluyemi and Roberts (1979), Olomu, (1995) and Jagdish, (2005) in the tropics. The reason for this may be attributed to the comparatively low environmental temperature of Vom, which is said to cause the birds to increase feed intake due to increased energy requirements at lower temperatures as reported by Davidson, *et al*., (1961) and Smith (2001) respectively.

Carcass yield

The various meat yielding parts (Carcass, breast, wings, thighs and abdominal fat) and abdominal fat of the broiler chickens, expressed as percentages of the live weight were not affected by dietary treatments. Dressing percentages compared favourably with the range of 66-75% reported by Oluyemi and Roberts (1979), Aduku and Olukosi (2000), Anyaehie and Madubuike, (2007) and Tuleun and Igba (2007) for broiler chickens. The values for the breast, wings, thighs and drum sticks were also slightly better than previously reported for the same carcass components by the authors above. There was no particular pattern in the deposition of abdominal fat amongst the treatments. The comparatively better result may be due to genetic makeup of the birds and possibly quality of the feed. From these results, it can be concluded that pigeon pea can be included by 30% in broiler diets regardless of method

of processing without affecting growth performance, blood parameters and carcass characteristics.

References

Aduku, A. O. and Olukosi, J. O. (2000). Animal Products, Processing and Handling in the Tropics. Living Book Series. G. U. Publications. P. O. Box, 2280. Abuja.

Ani, A. O. and Okeke, G. C. (2003). Substitution of pigeon pea (*Cajanus cajan)* seed meal for soyabean in broiler finisher ration. In. Olatunji, E. A., Ayanwale, B. A., Shiawoya, E.L. and Aremu, **A. Sustainable Livestock Productivity and National Development. A Holistic Approach.** Proceedings of the 8th Annual Conference of Animal Science Association of Nigeria. September 15th – 18th Federal University of Technology, Minna.

Amaefule, K. U. and Obioha, F. C. (2001). "Performance and Nutrient Utilization of broiler starters fed diets containing raw, boiled or dehulled pigeon pea seeds. *Nigerian Journal of Animal Production.* **28**(1): 31 – 39.

Amaefule, K.U. and Nwagbara, N.N. (2004). The effect of processing on nutrient utilization of pigeon pea (Cajanus cajan) sead meal and pigeon pea seed meal based diets by pullets. *International Journal of Poultry Science.* **3**(8): 543 – 564. Asian Network for Scientific Information.

Anyaehie, A. A. and Madubuike, F. N. (2007). Carcass yield and organoleptic parameters of broiler finishers fed maize cob diets supplemented with Nutri-Zym. In: Agiang, E. A., Agwunobi, L. N. and Olawoyin, O. O. **Sustainability of Livestock Industry in an Oil Economy.** Proceedings of the 32nd Annual Conference of Nigerian Society for Animal Production, University of Calabar, March 18 – 21 Pp 176 – 178.

A.O.A.C. (2006). Official Methods of Analysis of the AOAC (W. Horwitz, Editor). Association of Official Analytical Chemists. 18th Edition. (3) Arlington Va. USA.

D`Mello, J. F. P. (1995). Anti-nutritional Substances in Legume Seeds. In: D`Mello, J. F. P. and Devendra, C. (Eds). Tropical Legumes in Animal Nutrition. CAB International, Wallingford, U. K.

Etuk, E. B, Esonu, B. O. and Udedibie, A. B. I. (2003). Evaluation of toasted pigeon pea (*Cajanus cajan*) seed meal as replacement for soyabean meal and maize in broiler finisher diets. In: Olatunji, E. A., Ayanwale, B. A., Shiawoya, E. L. and Aremu, A. **Sustainable Livestock Productivity and National Development. A Holistic Approach.** Proceedings of the 8th Annual Conference of Animal Science Association of Nigeria, September 15th – 18th Federal University of Technology, Minna.

Jagdish, P. (2005). Poultry Production and Management. Second Revised Edition. Kilyani Publishers, New Delhi. 110 002.

N.R.C. (1984): National Research Council, Nutritional Requirements of Poultry. 8th Edition. National Academy of Sciences, Washington, D.C.

Olomu, J.M. (1995). Monogstric Animal Nutrition. Principles and Practice. Jachem Publication. Nigeria.

Oluyemi, J.A. and Roberts, F.A. (1979). Poultry Production in Warm Wet Climates. Macmillan Tropical Agriculture, Horticulture and Applied Ecology Series. Macmillan Press Limited, London and Basingstoke.

Onimawo, I.A. and Akpojovwo, A.E. (2006) Toasting (dry heat) and nutrient composition, functional properties and anti-nutritional factors pigeon pea (*Cajanus cajan*) seed meal. *Journal of Food Processing and Preservation.* **30** (12): 742-753. Blackwell Publishing.

Onwuka, G.I. (2006). "Soaking, boiling and anti-nutritional factors in pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*).

Journalof Food Processing and Preservation. **30** (5): 616-630. Blackwell Publishing.

Purseglove, J. W. (1984). Tropical Croops. "Dicotyledons". Volumes 1 & 2. Longman Group, LTD. Longman House Burntmill, Harlow Essex, CM 20 2JE. England

Salunkhe, D.K., Kadam, S.S. and Chavan, J.K. (1985). Post Harvest Biotechnology of Food Legumes. CRC Press Incorporated Boca Raton, U.S.A.

SPSS (2006). Statistical Package for Social Sciences. Pc Version 15. SPSS Inc. 444 N. MichiganAvenue Chicago, United States of America.

Tuleun, C.D. and Igba, F. (2007) Growth and carcass characteristics of broiler chickens fed water soaked and cooked velvet (*Mucuna utils*) meal. In Againg, C.A., Agwunobi, L.N. and Olawoyin, O.O. (eds). **Sustainability of the Livestock Industry in an Oil Economy**. Proceedings of 32nd Annual Conference of Nigeria Society for Animal Production. pp 240-243. Calabar, March 18-21.

Udedibie, A.B.I; and Carlini, C.R. (2002). Relative effects of dry and moist heat treatment on haemagglutinating and antitrypsic activities of selected legume grains. *Nigeria Journal of Poultry Science.* **1**:81-87.

Davidson, J., McDonald, I., Mathieson, J. and Willians, R. S. (1961). Protein intake in birds. *Journal of Food Science and Agriculture* **12**: 425 – 439.

Etuk, E. B, Esonu, B. O. and Udedibie, A. B. I. (2003). Evaluation of toasted pigeon pea (*Cajanus cajan*) seed meal as replacement for soyabean meal and maize in broiler finisher diets. In: Olatunji, E. A., Ayanwale, B. A., Shiawoya, E. L. and Aremu, A. **Sustainable Livestock Productivity and National Development. A Holistic Approach.** Proceedings of the 8th Annual Conference of Animal Science Association of Nigeria, September 15th – 18th Federal University of Technology, Minna.

Etuk, E.B., Esonu, B.O and Njoku, C. (2003). Effect of methionine supplementation on the performance of broiler finishers fed pigeon pea (*C. cajan*) seed meal based diets. In Olatunji, E.A; Ayanwale, B.A; Shiawoya, E.L and Aremu, A. **Sustainable Livestock Productivity and National Development. A Holistic Approach.** Proceedings of the 8th Annual Conference of Animal Science Association of Nigeria, September 15th-18th Federal University of Technology, Minna.

Smith, A.J. (2001). Poultry. The Tropical Agriculturist. Centre for Tropical Veterinary Medicine, University of Edinburgh, U.K. C.T.A. Publication. Macmillan Education LTD, London and Oxford. Second (revised) Edition.

Table 1. Effect of Processing on Levels of Antinutritional Factors in Pigeon Pea.

Antinutritional Factor (ANF)	Processing Method			
	RPP	ROPP	BDPP	EPP
Hydrogen Cyanide (mg/g)	0.17	0.09	0.13	0.04
Oxalate (mg/g)	19.60	5.04	1.68	14.00
Phytic acid (mg/100g)	24.03	24.76	22.57	--
Tannins (mg/100g)	2.13	0.67	0.02	--
Trypsin Inhibitor (HUI/g)	5.62	3.87	1.16	3.12
Haemagglutinin (mg/g)	15.10	10.64	5.80	9.72

RPP=Raw Pigeon Pea
BDPP=Boiled and Dried Pigeon Pea
ROPP = Roasted Pigeon Pea
EPP=Extruded Pigeon Pea.
-- = Not analysed

Table 2. Combined Growth Performance of the Broiler Chickens in Experiment 1

Parameter	Level of Differently Processed Pigeon Pea Seed Meal (%)					
	0 (Control)	30RPP	30 ROPP	30 BDPP	30 EPP	SEM
Initial Weight (g/bird)	90.25	88.75	88.47	89.25	92.75	2.14NS
Final Weight (g/bird)	2299.11	2075.98	2104.79	2039.93	2028.11	102.31NS
Daily Feed Intake (g/bird)	119.05	121.36	119.79	124.23	116.10	6.41NS
Daily weight Gain (g/bird)	38.19	35.94	35.01	36.07	34.57	1.73NS
Feed Conversion Ratio (FCR)	3.12	3.37	3.44	3.46	3.38	0.24NS
Feed Cost (N/kg)	41.81	43.57	43.60	43.57	43.54	
[1]Feed Cost (N/kg gain)	135.26	155.04	158.15	159.41	155.73	11.08NS
Mortality (Number)	5	4	7	2	4	

S.E.M. = Standard Error of the Mean. NS = Not significant (P>0.05)
[1]Calculated based on market price of the ingredients (N/kg) at the time of the experiment (Maize = 25.00; Wheat = 22.00; soyabean cake = 55.00; pigeon pea + 36.00; fishmeal = 200.00; premix = 380.00; salt = 24.00; lysine = 700.00; methionine = 750.00)
RPP = Raw Pigeon Pea BDPP = Boiled and Dried Pigeon Pea
ROPP = Roasted Pigeon Pea EPP = Extruded Pigeon Pea.