Editorial Board

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Forward

The review and planning meeting is a forum for discussing highlights of research activities that continued in 2012 and proposals for 2013 and beyond.A research review affords researchers the opportunity to interact with other stakeholders in the veterinary health care delivery system, agriculturalists and extensionists. This assists researchers improve their approach to research, making it more robust to generate relevant technologies for the livestock industry. As a national institute with a mandate covering the whole nation, the impact of the Institute continues to be felt in all the agro-ecological zones of the country.

The Institute's Mandate include

- To conduct research into all aspects of animal diseases, their treatment and control.
- To develop and produce animal vaccines, sera and biologicals to meet national demand
- To provide surveillance and diagnosis of animal diseases
- To introduce exotic stock for improved egg, meat and milk production
- To provide extension services to poultry and livestock farmers
- To training of intermediate manpower in veterinary and medical laboratory technology and animal health and production technology

The Federal Government's Agricultural Transformation Agenda is using a value chain development approach. Towards achieving this agenda in livestock, the Institute's role includes theproduction of animal vaccines and biologicals for the control of animal diseases and research for development in the treatment and control of animal diseases to support various value chainsin beef, poultry, dairy, sheep and goat, and the leather industry.

The proposed research projects in relation to the value chains are aimed at addressing identified gaps. These include:

i. Foot and mouth disease in ruminants
ii. Mastitis control and milk hygiene in dairy
iii. Contagious Bovine Pleuropneumonia(CBPP) in cattle
iv. Peste des Petits Ruminants (PPR) in small ruminants
v. Newcastle disease, *Salmonella* and Avian Influenza in commercial and rural poultry
vi. Dermatophilosis in cattle to improve the quality of hide for the leather industry

Research has commenced in all of these areas. After a long break it is envisaged that the annual research review meetings will become a regular scheduled activity of the Institute from 2013.

Let me use this opportunity to thank all our guests and participants for their contributions in making our research more robust and demand-driven.

Dr. M. S. Ahmed
Executive Director

Introduction

One of the mandates of the National Veterinary Research Institute Vom among others is "To conduct Research into all aspects of Animal Diseases, their Treatment and Control" In pursuance of this mandate, the Mission is *"To be the foremost veterinary research institute in Africa,producing international quality vaccinesand offering services for the identification, control,and eradication of economically important livestock diseases; through best practices, research excellence,and applying modern technology; with highly trained, experienced and motivated personnel".* The research projects of the Institute have therefore been designed to deliver on the Institute's mandate and mission statement, thus the main thrust and current areas of research interest are

Vaccine development
Research into emerging and re-emerging diseases
Disease surveillance and development of diagnostic and control tools
Livestock extension

For research to continue to be relevant to the needs of the people there is the need for regular self and peer review or evaluation to make sure that research is demand driven and generate innovations that can impact on the lives of people positively. The National Agricultural Research System in Nigeria has therefore institutionalised the in-house review process with the participation of other stakeholders to ensure that there is value addition in the process. The Institute in-house research review meeting therefore constitutes a forum of self-regulation by staff of the Institute to ensure maintenance of standards of quality, improve performance, and provide credibility to all research projects.

With the huge demand for vaccines by farmers, the Institute aims at aligning itself with the modern art of vaccine technology to produce international quality vaccines with the production of larger dose quantity and better quality products. With the 2006 Highly Pathogenic Avian Influenza (HPAI) H5N1 outbreak and the sudden upsurge of rabies outbreaks, research has also focused on emerging and re-emerging diseases.

The key to success in the control of any epidemic is early detection. If disease can be detected very early in the phase of epidemic development, the possibility exists that it can be arrested and eliminated before it actually inflicts damage. But early detection presupposes that there is a surveillance system in place that will bring infection to light when it is first seen.

A direct result of the Institute's diagnostic activities is the OIE recognition of NVRI as a ***Regional Laboratory for West and Central Africa for Avian Influenza and Trans-boundary Animal Diseases*** .The Institute is providing both diagnostic and training services to this sub region and improving its diagnostic capabilities by developing improved diagnostic and control tools.

The Institute has invited stakeholders and is also co-hosting the 2013 North-Central Zonal REFILS Workshop to conduct the 2013 in-house Annual Review Meeting review. It is therefore hoped that this exercise will yield the desired results.

David Shamaki (DVM, PhD, FCVSN)
Director Research

Project Title

Evaluation of Immunogenicity of Haemagglutinin Genes of H5N1 Sub lineages of Avian Influenza Virus from Nigeria for Vaccine Development

Introduction

Avian influenza (AI) is a major threat to animal and public health especially with the capacity of the AI virus to mutate and re-assort into different genetic lineages over the years. Distinct sub lineages of H5N1 (I, II, I/II, III, clade 2.2.1) have been detected in Nigeria during the 2006 - 2008 outbreaks (Ducatez *et al.,* 2007; Fusaro *et al.,* 2010). Although AI outbreaks appear to have been controlled in Nigeria, the risk factors (re-introduction by migratory wild birds, through international/national commercial activities in poultry & products) still makes the country vulnerable to re-introduction. Hence there is need for a comprehensive control programme (education, surveillance, biosecurity, diagnosis, culling, and vaccination) which will be effective in preventing or eradicating AI in Nigeria. WHO, OIE and FAO have raised an alert on the outbreaks of a new reassortantH5N1 particularly in Asia and the outbreak of a highly pathogenic H7N9 in China. Considering the devastating impact of the last AI outbreaks (over 368,000–1.2 million domestic birds were killed or culled) on the poultry industry in Nigeria (Monne*et al.,* 2008; Joannis *et al.,* 2008), all efforts should be made to have a comprehensive control programme anchored on the country's risk factors. This project was designed to investigate a component of a comprehensive control programme (decreasing susceptibility) which will hopefully lay a foundation for recombinant DNA vaccine technology for animal diseases.

Due to economic and practical reasons, the destruction of infected poultry alone is no longer considered the method of choice for the control of the disease especially with a very large population of poultry. Also the international organisations such as the World Organisation of Animal Health(OIE) and Food and Agricultural Organisation(FAO) have recommended that vaccination of poultry be considered for the control of AIV and countries like China and Italy have accepted vaccination as a component of AIV control programmed (Park *et al.,* 2006). Conventional and vectored AI vaccines are currently in circulation in many countries(OIE, 2009;Ge*et al.,* 2007; Rao *et al.,* 2009) and the vectored vaccines have been found to have faster production rate, safe and large scale production methods and the potential for enhanced efficacy against a broader range of influenza strains. Knowledge of the construction of vectored vaccines and their immunogenicity in poultry models will serve as an essential step in the development of vectored vaccines for inclusion in the national comprehensive control programme for AI. Although it is a new technology which is still in its early stage in Nigeria, it has great potentials to transform animal vaccine production in Nigeria. It is imperative to develop a national AI vaccine technology as there is no universal vaccine that will protect against all AI viruses because there are 16 HA subtypes and vaccines must be tailored against specific HA and/or NA subtypes and in some cases against specific lineages within an HA subtype.

It is imperative to develop a national AI vaccine technology. Because of the peculiarity of Nigeria & the epidemiology of AIV during the last outbreaks, it is only the new technologies that are suitable. The new technologies give vaccines with the required criteria of purity, safety, efficacy and potency (Rao et al, 2009). Response time and production capacity in pandemic situations. To achieve the objectives of the project,

the work will be carried out in collaboration with two research centers of excellence (OIE/FAO Reference Laboratory for AI and Newcastle Diseases [IZSVe] Padova, Italy and the Department of Veterinary Medicine, University of Maryland, USA). The IZSVe (Padova) is an OIE and FAO reference laboratory for AI, and is a centre of excellence for research on AIV. The Department of Veterinary Medicine is also a centre of excellence on vectored viral vaccine research particularly the bivalent AI-NDV vectored. The more technical research work and the training aspects of the project will be done in the above collaborating institutions, while the remaining aspects will be carried out at NVRI, Vom. By capitalizing on the resources and know-how from these diverse areas of expertise, the proposed research has very high potential for producing high quality results that will hopefully be of value to the poultry industry in Nigeria and both human and animal health. The overall goal of this project is to evaluate the HA & NA genes of H5N1 sub lineages in Nigeria for a bivalent AI-ND vaccine development; develop human and infrastructural capacity for recombinant and DNA vaccine for AI in Nigeria.

The period under review has isolation/characterisation of H5N1 sub lineages, full length HA & NA genes synthesis/purification and sequencing/analysis of HA & NA genes, HA & NA genes cloning/ expression of H5N1 sub lineages in Nigeria as the main objectives. Equipment, materials, reagents and biologicals required for the first two main objectives have been procured; isolates of H5N1 have been obtained and characterised; the protocols for RT-PCR synthesis of HA & NA genes have been validated, some full length HA & NA genes synthesised and NA gene cloned into 10G and BL21 cells.

Goal of the Project: To evaluate the immunogenicity of HA & NA genes of Nigerian isolates of H5N1 for a bivalent AI and ND vaccine development; and to develop human & infrastructural capacity for recombinant & DNA vaccine for AI in Nigeria.

Key Objectives:

1. To apply RT-PCR based approach to detect, amplify and isolate HA & NA genes from H5N1 subtypes identified in Nigeria.
2. To sequence & analyze the HA & NA genes of H5N1 subtypes in Nigeria.
3. To clone & express the HA & NA genes in various vectors to identify the best vectors for the genes.
4. To assess the immunogenicity of the recombinant HA, NA and the clones in chickens as models.
5. To develop one step H5 AIV antigen test protocol for on-the-field and laboratory rapid diagnosis of H5N1 AIV in Nigeria.
6. To construct & evaluate a recombinant NDV-HA clone as a bivalent AI & ND vaccine candidate for Nigeria

Work Done

Virus Propagation& characterization

Twenty-five representative outbreak isolates in the NVRI, Bio bank were selected, at least one from each sub lineage or reassortant classification of H5N1 isolates in Nigeria. The viruses were isolated in embryonating (9-11 days) chicken eggs and first characterized by agar gel immunodiffusion (AGID and heamagglutination-inhibition (HI) test and confirmed by RT-PCR.

Full length HA & NA genes synthesis

1. Full length haemagglutinin (HA) and neuraminidase genes were synthesized using Two-Steps RT-PCR according to the protocols of Fusaro*et al.,* (2010).
2. Two-Steps RT-PCR Protocols for the synthesis of full length HA and NA genes were optimized and validated. Full length HA (≈1778bp) gene and NA (≈1413bp) gene were synthesized from 5 isolates.
3. The results showed that protocols for HA and NA genes synthesis by Two-Steps RT-PCR have been established and will be used for synthesizing these genes from selected H5N1 isolates in Nigeria which will subsequently be used as naked DNA vaccination materials and inserts for cloning and expression experiments. These protocols will be used to generate the genes for sequencing and sequence analyses.

Sequencing and Phylogenetic Analysis of AIV Genome

A member of the Project Team was trained on sequencing and phylogenetic analysis of AI genome at the OIE Reference Laboratory for Avian Influenza and Newcastle Disease, Legnaro, Padova, Italy. Hands on training: PCR amplification of gene segments using sets of internal primers; purification of products; sequencing reaction (in 313 x L Genetic Analyzer Applied Biosystems HITACHI); sequence editing (SeScape Software Appl. Biosystems®) & phylogenetic analysis (MEGA 4) was acquired.

To obtain HA and NA sequences needed for cloning & expression4HA & NA genes were amplified from 4 H5N1 isolates (VRD/07/131; VRD/07/275; VRD/07/187; VRD/07/263 using sets of primers(4 each).First Strand cDNA Synthesis and High Fidelity PCR Enzyme Mix kits were used (ThermoScientific®).Taq-polymerase catalysed cycle sequencing using fluorescent-labelled dye terminator reaction protocol was employed and analysis was on the 3130XL Genetic Analyser (ABI).Sequence editing was with FinchTV software; sequence analysis was with CLC Bio Version 5(at InqababiotechnicalIndustries (Pty) Ltd Pretoria SA).Phylogenetic analysis was with MEGA 4 software.

Cloning & Expression

Full length HA & NA genes were amplified using Two-Steps RT-PCR according to the protocols of Fusaro et al, (2010). Expresso T7 Cloning & Expression System(Lucigen®) was used to clone and express HA & NA genes. Cloning: pETite vector(2.2kb) + HA or NA gene; clone used to transform HI- Control 10G chemically competent cells *(E.coli*). Clones were isolated using GeneJET Plasmid Miniprep Kit Expression was in BL21 (DE3) High-Control cells (*E.coli*). Purification of recombinant HA & NA was using QIAexpress® Ni-NTA Fast Start Kit & Detection was using SDS-PAGE.

Application of Results

1. In ovo propagation of AIV and RT-PCR/sequencing based laboratory diagnosis of AI.
2. Two-Steps RT-PCR Protocols (SOPS) for the synthesis of full length HA and NA genes of H5N1 optimized and validated.
3. Developed Human capacity in sequencing & phylogenetic analysis of avian influenza virus genome.
4. Sequenced 4 HA and NA genes from 4 selected H5N1 subtypes (Nigerian isolates) for cloning, expression, banking & bivalent AI-NDV clone construction.
5. Recombinant *E. coli* Expression System Technology for NA expression based on Expresso™ T7 Cloning & Expression System was optimized and validated.

6. C-His pETite-NA clones in BL21 expression cells (*E. coli*) as DNA vaccine material (Figures 4, 5, 6).

Future Work

1. Cloning & expression of the HA & NA genes in various vectors to identify the best vectors for the genes.
2. Assessment of the immunogenicity of the recombinant HA, NA and the clones in chickens as models.
3. Measurement of avian cytokines responses following recombinant HA, NA and the clone immunization of chickens as model birds.
4. Development of one step H5 AIV antigen test protocol for on the field and laboratory rapid diagnosis of H5N1 AIV in Nigeria.
5. Construction & evaluation of a recombinant NDV-HA clone as a bivalent AI & ND vaccine candidate for Nigeria.

Mandate Area Development of vaccines, sera & biologicals	**Name of Programme Related to Mandate Area** Applied Biotechnology & Basic Biochemistry Programme
Title of Research Project Evaluation of Immunogenicity of Haemagglutinin Genes of H5N1Sublineages of Avian Influenza Virus from Nigeria for Vaccine Development.	**Expected Completion Date** 2014
Key Deliverables	1. H5N1 subtypes isolates. 2. HA clones as DNA vaccine materials. 3. Recombinant HA proteins. 4. Potential H5 subtype HA & DNA vaccine materials. 5. Rapid strip test protocol for H5 subtype of AIV in Nigeria developed 6. Bivalent (rNDV-HA) vaccine material.
Key Constraints & Challenges	1. Untimely release of funds and procurement of specialized reagents outside the country result in lack of achievement of set objectives within the stipulated time line. 2. Incessant strike actions (2011 - 2013). 3. Difficulty in shipment of Category A infectious substances for collaborative research in the foreign collaborating institutions.
Key Lessons	1. Objectives of the project are being achieved and on track. 2. Implementation of the remaining set of activities project will lead to successful evaluation of the immunogenicity of HA & NA genes of H5N1 isolates of Nigeria. 3. Development of a bivalent NDV-AI vaccine for decreased susceptibility of AI in Nigeria is feasible.
Key Recommendations	Timely release of funds

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Project Team Members and Institutional Affiliation

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Project Title
Development of rapid and effective diagnostic and control tools for African swine fever (ASF) in Nigeria

Introduction
African swine fever (ASF) is a viral disease of domestic and wild pigs responsible for a variety of clinical signs primarily in the domesticated pig population. The causative agent of the disease is ASF virus of the genus Asfivirus in the family Asfaviridae (Dixon *et al.,* 2005).

The typically acute form of the disease is characterized by severe lymphoreticular endothelia cells affection resulting in widespread haemorrhage, morbidity and mortality approaching 100%. The sub-acute form of the disease presents loss of condition; which may result in death of pigs due to complications like pneumonia. Chronic survivors may also present with stunted growth, emaciation and haemorrhagic necrosis of the skin overlaying the bony protuberances, abscessation and deep ulceration (Plowright *et al.,* 1994).

Conventionally, diagnosis of ASF is based on rapid and accurate laboratory diagnosis of ASFV positive and carrier animals and on the enforcement of strict sanitary measures since vaccines are not available (Sanchez-Vizcaino, 1999). The virus poses serious socio-economic problems especially in the African continent as confirmed in several reports on the outbreaks in many countries (Odemuyiwa *et al.,* 2000; Lubisi *et al.,* 2005; Gallardo *et al.,* 2011).

For a successful control and eradication of ASF, information will be required on the virus circulating in Nigeria and a rapid test kit that can be used on site for early detection. Studying ASF genotype will generate information on how to control and eradicate in the disease in Nigeria.

Specific objectives
1. To determine the prevalence of African swine fever (ASF) virus infection in Nigeria.
2. To carry out isolation, molecular characterization and sequencing of ASF virus strains from Nigeria.
3. To develop biological and technique for diagnosis of ASF
4. To recommend a national control strategy for ASF for possible implementation.

Work done so far
1. To determine the prevalence of African swine fever virus (ASFV) infection in Nigeria, sera samples were collected from different location in Nigeria. From the total sera samples collected through outstation Officer in charge of NVRI outstations, and send to the World Reference Laboratory for African swine fever CISA-INIA, Spain, a 9 % positivity was reported (Fasina *et al.,* 2011).

2. To carry out molecular characterization of ASF virus strains from Nigeria, a progress report was received from the World Reference Laboratory from the tissue samples submitted. From the tissues submitted, 48% were positive for ASF by PCR. Virus isolation is ongoing from all the positive tissues (Fasina *et al.,* 2011).

3. Amplification and sequencing of p72 gene and CVR of 26 positive samples respectively has been done.

4. Herbal plant has been collected and been extracted for analyse for ethno-veterinary properties on cell line.

Future work

1. To isolate the virus using pig macrophage cells
2. Sequence analysis of p72 and CVR of ASF isolates from Nigeria
3. Determine the efficacy of ethno-veterinary preparation in the management of ASF in Nigeria in vitro.
4. Develop a rapid and effective diagnostic kit for ASF.

Investigators

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<table>
<tr><td colspan="2">Mandate Area
To conduct Research into all aspect of animal diseases, their treatment and control</td><td>Programme(s) related to the mandate
Livestock disease diagnosis and control
Improved animal productivity</td></tr>
<tr><td colspan="2">Title of Research Project
Development of rapid and effective diagnostic and control tools for African Swine Fever (Epidemiology, Diagnostic and Control of African Swine Fever in Nigeria)</td><td>Expected completion date
2014</td></tr>
<tr><td>Key deliverables</td><td colspan="2">1. Determination of ASF prevalence in pigs in Nigeria
2. Isolation and molecular characterization of ASF viruses and sequencing of positive isolates
3. Development of a rapid and effective control tool</td></tr>
<tr><td>Key constraints and challenges</td><td colspan="2">1. Lack and reagents and material to work with
2. delay in obtaining the necessary material to work with making the delivery time line to be not feasible
3. Lack of proper documentation of previous work.
We recommend that materials and reagents critical for the advancement of the project should be made available when requested to reduce the unnecessary time spend in waiting for it and make the result outcome relevant.</td></tr>
<tr><td>Key lessons</td><td colspan="2">1. ASF is still prevalent in Nigeria
2. Multi-disciplinary approach to research could yield greater results</td></tr>
<tr><td colspan="2">Key recommendations</td><td>Funds should be made available to catch-up with the work plan</td></tr>
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Project Title
Isolation and characterization of *Salmonellae* for the development of a disease surveillance and control programme in commercial poultry in Nigeria:

Introduction
Salmonellosis is recognised as a major economic disease in animals and a major public health hazard in man. To improve poultry production and protect the public health, a*Salmonella* surveillance and control programme is necessary. The implementation of a monitoring programme for detecting the development of drug resistance is also needed. Accurate serotype identification is essential for effective epidemiological investigations. Serotyping, antimicrobial susceptibility determination and molecular genotyping will highlight the most common serotypes and clones of the organism and assist in developing control strategies including the development of a multivalent suited for the most prevalent serotypes in Nigeria. The aim of the study is to improve commercial poultry production and public health through the development of a surveillance and control programme for Salmonellosis.

Specific Objectives
1. To determine risk factors associated with *Salmonella*infections on commercial poultry farms.
2. To determine the predominant serotypes, their distribution and antimicrobial susceptibility patterns
3. To determine molecular and epidemiological associations amongst isolates from the different agro ecological zones of the country
4. To educate farmers and frontline extension staff on the role of *Salmonella*in poultry production and its public health significance in commercial poultry production.
5. To adopt suitable surveillance and control programmes for salmonellosis in commercial poultry in Nigeria in collaboration with farmer groups.

Sampling frame
The sampling frame covers primarily farms with at least 500 laying hens. Birds sampled at the end of their laying period within a maximum of 9 weeks before depopulation. Only one flock per farm was sampled. In case of presence of multiple age flocks sampling focused on the oldest laying hens. In the case of multi-age groups of birds within a single house, sampling concentrated on the oldest groups. Selection of farms took into account the risk that certain initially selected farms may not be sampled (e.g. because of early slaughtering) and therefore a slightly bigger numberwas considered.

Work done
Five hundred and thirty four farms(534)out of a projected 683 farms have been surveyed. Laboratory analysis of 2680 samples completed out of which 182 were serotyped as *Salmonella* comprising 53 different serotypes. Major serotypes include *S.* Kentucky (17%), *S.* Enteritidis (9.9%), *S.* Elizabethville (6%), *S.* Poona (6%), *S.* Agama (5.5%), *S.* Larochelle (4.4%).

Capacities developed
Training was conducted for project team on questionnaire administration, data handling and analysis. Training was also conducted on sample collection and processing in order to standardise all procedures.

Future Work
Questionnaire survey, sample collection and laboratory analysis will be completed. Data analysis, seminar/workshop and stakeholders' meeting are due to take place

Implications for agricultural transformation in Nigeria
1. Project will provide a scientific basis for setting targets for *Salmonella* in food animals in Nigeria
2. Data generated will identify factors related to*Salmonella* contamination in commercial poultry farms
3. Project could lead to the development of a comprehensive farm management and biosecurity programme to reduce *Salmonella* contamination
4. Laboratory testing of poultry and poultry products and their certification for international trade
5. Development of a multivalent *Salmonella* vaccine to control the most common serotypes present in commercial poultry farms

Mandate Area Conduct research into all aspects of animal disease, their treatment and control	**Name of Programme** Bacterial Research
Title of Research Project Isolation and characterization of *Salmonellae* for the development of a disease surveillance and control programme in commercial poultry in Nigeria	**Expected completion date** 2014
Key deliverables	1. Epidemiology of avian salmonellosis determined 2. Avian *Salmonella* serotypes in Nigeria determined 3. Antibiotic sensitivity of isolates documented 4.Genotypes of isolates of *Salmonella* identified 5. Frontline extension staff and poultry farmers trained 6. Stakeholder meeting held
Key constraints and challenges	1. Late delivery of procured materials to carry out planned activities 2. Funding not at level required 3. Security challenges have hampered field work in some areas
Key lessons	1. An urgent need to develop multivalent vaccine for Salmonella control 2. Poultry farmers easily accessible with use of local staff 3. Biosecurity measures appear relaxed after AI enlightenment 4. Awareness of economic and zoonotic importance of *Salmonella* established.
Key recommendations	There is a need to institute a*Salmonella* surveillance and control programme in Nigeria. Need to develop a novel multivalent *Salmonella* vaccine

Investigators

S/N	Investigator	Affiliation	Role On Project
1	M. Muhammad	N.V.R.I, Vom	Overall Coordinator, Questionnaire survey & sample collection, Laboratory analysis, Stakeholder workshop, Data analysis,

			Publication and report writing
2	L.U. Muhammad	N.V.R.I, Vom	Questionnaire survey, Workshop for Extension staff & farmers, Stakeholder workshop, Data analysis, and report writing
3	S. S. Ngulukun	N.V.R.I, Vom	Questionnaire survey & sample collection, Laboratory analysis, Stakeholder workshop, Data analysis, and report writing
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15	C. Ugbong	N.V.R.I, Vom	Laboratory analysis
16	G. Gullek	N.V.R.I, Vom	Laboratory analysis
17	A.G. Ambali	UNIlorin	Data analysis, and report writing
18	J.K.P Kwaga	A.B.U, Zaria	Molecular typing of isolates, Data analysis, Publication and report writing
19	E.I. Ikani	A.B.U, Zaria	Workshop for Extension staff & farmers, Stakeholder workshop
20	A. Ricci	Italian Ref Lab for *Salmonella*, Padova, Italy	Serotyping of *Salmonella* isolates

Project Title
Development of Killed Adjuvanted Rabies Cell Culture Vaccine

Introduction
Rabies is an acute fatal zoonotic viral infection of the central nervous system (WHO, 1992; Cardoso and Pilz, 2004.) it is one of the oldest zoonotic disease known to mankind (Nottidge *et al,* 1992; Van Zyl et al., 2010; Hayman et al., 2011.). Rabies a zoonotic disease, is transmitted from the wild to domestic animals and constitute a great threat and major problem to the public health in Nigeria (Chukwuedo and Olabode, 2000; Ogo *et al.*, 2011).

Rabies is caused by viruses of the genus *Lyssavirus* in the family Rhabdoviridae. Although all mammals are thought to be susceptible to rabies infection, the primary hosts are carnivores and bats. (Freuling *et al.,*2011). Most human exposure to rabies occurs via an animal bite in which the skin is broken and the virus is transmitted from the infected animal's saliva to the blood and tissues of the victim. The rabies virus infects the human nervous system causing acute encephalomyelitis, an inflammation of the brain and spinal cord (Kammer and Ertl, 2002; CDC, 2002; Lembo *et al.*, 2008). Death, usually by respiratory failure occurs within seven to 10 days after appearance of the first symptoms. The average incubation period before symptoms of the disease appear is three to seven weeks, with a range of 10 days to seven years (Nottidge, 1994; WHO, 2004; Ogo *et al.,* 2011).

Globally Rabies is common in some parts of the world, particularly in the developing countries of Africa, Asia, and Latin America. Rabies has been eradicated in the United Kingdom. It is considered to be a re-emerging viral disease because it is poorly controlled in many developing countries despite widely available human and animal vaccines (Fabiyi and Louc, 1991; Hayman *etal.,* 2011). WHO estimates that every year about 10–12 million people worldwide receive post-exposure prophylaxis and that about 35,000 people; primarily children die of rabies every year. However the incidence of rabies in the developing world is believed to be severely underreported. Most rabies exposures are from bites by unvaccinated dogs (Plokin and Clark, 1997; WHO, 1993; 2004a, b).

The symptoms includes change in temperament, irritability, restlessness, photophobia, excitability, convulsion, paralysis depends on the amount of virus delivered, host susceptibility and distance of bite to the brain (Wiktor and Clark, 1975) infection of rabies is prevented by vaccination with potent rabies vaccine or by administration of rabies immune globulins (CDC, 1991) Although there is no cure for rabies once symptoms of the disease have appeared, a highly effective vaccines that provides protection from the virus both pre-exposure prophylaxis and post-exposure prophylaxis have been developed.

The vaccine consists of killed rabies virus that, when injected, induces the immune system to produce antibodies that bind to and destroy the virus. The antibody response develops within seven to 10 days of vaccination and provides protection for up to two years. A second type of rabies vaccine, rabies immune globulin (RIG), provides immediate, short-term protection after exposure to the virus.

Some of the existing vaccines include

1. Human Diploid Cell Vaccines (HDCVs) use inactivated rabies viruses. Comes in two formulations: one for IM injection and one ID injection.
2. Purified chick embryo cell (PCEC). The vaccine is made from rabies virus grown in cultures of chicken embryo and then inactivated.
3. Rabies Vaccine Adsorbed (RVA) is made from virus grown in cell cultures of foetal rhesus monkey lung cells and then inactivated.
4. Human Rabies Immune Globulin (HRIG) is a vaccine made from human serum that contains high levels of antibodies against rabies (Roumiantzeff, 1998; Kammer and Ertl, 2002; Talbi *et al.*, 2010; CDC, 2004a,b).

In Nigeria, Low Egg Passage (LEP) and High Egg Passage (HEP) flurry strain vaccines are produced against dog and cat rabies respectively, but previous research finding have shown that dogs in Nigeria succumb to clinical rabies in spite of previous vaccinations (Stites *et aI.,* 1978; Pay *et al.,* 1985).

These recent studies are designed to produce inactivated cell culture adjuvanted rabies vaccines using the egg adapted LEP flurry strain of rabies virus. Tissue culture techniques have long been used in studies related to rabies virus, and there are now a number of continuous cell line (BHK-21, MNA VERO and PCER) used in research on pathogenesis, vaccine production and diagnosis of rabies (Wiktor and Clark 1975; King, 1996).

Cell culture rabies vaccines are cleaner, devoid of egg lipids and protein. It is more potent with longer shelf life and will give greater quantity of vaccines. The Aim of this project is:

1. To Develop killed adjuvanted rabies cell culture vaccine in Nigeria.

Specific Objectives

1. To adapt the previous egg based LEP rabies virus to cell culture (BHK-21 CL 13, Vero).
2. To formulate killed adjuvanted rabies cell culture vaccine.
3. To make recommendations and produce protocol for the vaccine production.

Work Done So Far

MIT

The LEP vaccine seed virus was reactivated in 1-7 day old baby mice and in young adult mice (3–6 wk old). The ex-mouse brain harvest form the initial working seed
For the development of killed adjuvanted rabies cell culture vaccine

Vero cell

The rabies virus (LEP strain) was grown into vero cells up to 4^{th} passage.
The 4^{th} passage was harvest and freeze dried to serve as cell culture rabies vaccine seed and stored at $-80^{0}C$ deep freezer.
The Vero adapted freeze dried has been titrated in both baby mice (1-7 days old) and adult mice (3-6 wk old).

BHK-21

The rabies virus was also grown in BHK-21 cells up to 2^{nd} passage.
The cell culture harvest was titrated in both baby and adult mice.

The 2nd passage harvest from BHK-21 cell is ready for further passage and titrations in BHK-21 cells.

Other activities

1. Development of 21 Standard Operating Procedures (SOPs) for Rabies research work
2. CVS Titration in mice
3. LEP rabies virus titration in 5 days old baby mice.
4. LEP cell culture harvest titration in cell culture
5. Titration of CVS in cell culture
6. Standardization and titration FITC conjugate using BHK-21 cell culture

Results

1. The CVS titration in mice was $10^{4.75}$/ 0.03ml titre
2. The CVS is awaiting titration in BHK-21 cell culture
3. The Cell culture Adapted rabies seed in mice gave $10^{2.75}$/ 0.03ml in 5 days old baby mice
4. All cell culture based tests were not conclusive
5. Reactivated cell culture rabies seed has under gone 2nd passage in BHK-21 for further titration using cell culture

Investigators

A. A. Chukwuedo	(B.SC, MSC)	**Principal Investigator**
M.S. Ahmed	(DVM, MVSC, PhD)	**Co-ordinator**
C. Nwosuh	(DVM, MVSC, PhD)	
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J. Kaigama	(MLT)	

Mandate Area	Development of biological, sera and vaccines for the control of livestock diseases
Name of Programme related to mandate area	Virus Disease Research programme
Title of Research project	Development of killed adjuvanted rabies cell culture vaccine. **Expected completion date** 2013
Key deliverables	1. Cell culture rabies seed produced. 2. Rabies immune anti serum. 3. Cell culture rabies vaccine. 4. Vaccine production protocol developed 5. Personnel capacity for vaccine production built
KeyConstraints and challenges	1. Delay in releasing funds 2. Inadequate supply of BHK-21 cell. 3. Mice cannibalism after inoculation 4. Lack of regular water supply to the laboratory. 5. Erratic power supply to the rabies laboratory. **Recommendation to mitigate constraints/challenges** 1. Prompt release of funds**.** 2. Create a separate cell culture unit 3. Source for a new breed of mice 4. Adequate supply of water to the laboratory 5. Check and repair the electrical faults in the building
Key lessons	New technology will be acquired on killed adjuvanted viral vaccine development
Key Recommendations	1. There is a need for training in rabies vaccine development in a specialized laboratory. 2. All dogs, cats and other livestock should be vaccinated annually against rabies 3. There is the need for strong campaigns to create awareness on the danger of contracting rabies 4. Only qualified vendors should be allowed to handle market and administer rabies vaccines to animals

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Project Title
Isolation and Molecular Characterization of Peste Des Petits Ruminants (PPR) in Sheep, Goats and Camels and the Adoption of a Thermostable PPR Vaccine in Nigeria

Principal Investigator: Dr. Woma Timothy Yusufu

Introduction
Peste des petits ruminants (PPR), is an acute contagious disease caused by a Morbillivirus in the family Paramyxoviridae. It affects mainly sheep and goats and occasionally small ruminants living in the wild. PPR occurs in most of Africa, especially in countries lying between the Equator and the Sahara, in the Arabian Peninsula, throughout most of the Near East and Middle East, and in south-west Asia. These areas encompasses much of the developing world that relies heavily on subsistence farming to supply food or goods for trade, and small ruminants provide an excellent supply of both. With its associated high morbidity and mortality, PPRV constitutes one of the major obstacles to subsistence farming; mortality from infection reaching 50–80% in a naïve population.

In the past, control of PPR was ensured through vaccination with the rinderpest tissue culture vaccine because of the existence of a strong antigenic relationship between PPR and rinderpest viruses. The use of this heterologous vaccine has been abandoned in favour of the live attenuated PPR virus vaccine, which is now widely commercially available. However, there is the need for improved PPR vaccines for better control of PPR in Nigeria using the currently available technologies like the Xerovacc.

Specific Objectives
The main aim of the project is to Increase Small Ruminants Production in Nigeria through the following specific objectives:

1. To serosurvey for PPR in selected states of Nigeria
2. To isolatePPR Viruses using cell culture
3. Molecular Characterization of PPR Viruses Isolated using RT-PCR technique
4. Sequencing of the PPR full length genome for genetic analysis
5. Adoption of a Thermostable PPR Vaccine for control and
6. To build capacity toward quality research and production of potent PPR vaccine

Work Done So Far
The major works done so far are highlighted below:

1. A Pre-inception meeting was successfully held were the entire work plan was reviewed in line with the approved budget and specific tasks were allocated to each team member.
2. Some basic equipment, reagents and consumables have been purchased, installed, optimised and are functional.
3. Sampling staff have been recruited and trained.
4. The Virus transport medium and all other reagents required for sample collection has been prepared.
5. Wet and dry season sera samples (5,685 from sheep and goats; and 1,137 from camels) from all the six agro-ecological zones of Nigeria (Adamawa, Taraba,

Yobe, Imo, Anambra, Oyo, Osun, Ogun, Ondo, Sokoto, Kano, Kaduna, Kwara, Plateau, Akwa Ibom and Cross Rivers States) have been collected.

6. All the sera samples have been analysed for PPR antibody detection.
7. The serological analysis by competitive ELISA test shows a prevalence of about 26% (1,506 positive sera out of 5,685) in sheep and goats and 3% (39 positive sera out of 1137) in camels.
8. 140 tissue samples have been collected from all the agro-ecological zones of Nigeria out of which 41 were positive for PPR by RT-PCR.
9. Thirty seven of the RT-PCR positive tissues have been sequenced and the sequences deposited in GenBank awaiting accession numbers.
10. Phylogenetic analysis showed that more than one lineage of PPR may be circulating in Nigeria currently.
11. Thirty two PPR viral isolates has been obtained using the CV-20 cell line developed by our collaborators in Austria.
12. The process of thermostable PPR vaccine production has begun and is ongoing.
13. A mid-term review meeting was successfully held where the strategies for attaining the remaining main objective of Xerovacc thermostable vaccine production was outlined.

In conclusion, we have established from the over 5,600 sera samples analysed from the different agro-ecological zones of the country that the current PPR seroprevalence rate in sheep and goats is about 26% while that of camels is about 3%. Total eradication of PPR from Nigeria is possible through massive vaccination campaigns using the thermostable vaccine to be produced indigenously and adequate border control of animal movements.

Future Work Plan

1. To conclude thermostable vaccine production (currently on going).
2. To finish quality assessment tests on produced vaccine.
3. Epidemiological data analysis and extensive village hall meetings with farmers from selected areas from each agro-ecological zone to create awareness about PPR control via vaccination
4. Scientific conferences, report writing, winding-up and journal publications
5. Collection of tissue samples from camels with clinical signs of respiratory distress for molecular diagnosis and possible virus isolation.
6. Continuous virus isolation using the recently developed Monkey CV1 cell line expressing the sheep-goat SLAM and to sequence greater portions of the viral genomes so as to further elucidate the nature of the currently circulating viruses in Nigeria. These information, when obtained, will be useful in the control and eradication strategies for PPR in Nigeria and elsewhere.

Mandate Area Research into Livestock Diseases, their prevention and control	**Division Related to mandate area** Viral Research Division
Title of research project: Isolation and Molecular Characterization of Peste Des Petits Ruminants (PPR) in Sheep, Goats and Camels and the Adoption of a Thermostable PPR Vaccine in Nigeria	**Expected Completion date:** December 2014
Key deliverables	1. Determine the seroprevalence of PPR in sheep, goats and camel from selected states across all agro-ecological zones of the country 2. Isolate and bank 50 PPR viruses from current outbreaks in sheep, goats and camels 3. Determine the lineage(s) to which current PPR viruses from Nigeria belong 4. Enlarge the sequences of PPR viruses from Nigeria in GenBank 5. Thermostable PPR vaccine is available for use and possibly export
Key Constraints/Challenges	1. Lack of a Biotechnology company producing reagents in Nigeria means a long wait for purchased or ordered materials to arrive Nigeria thus delaying certain aspects of project implementation unavoidably 2. Insecurity in parts of the country decrease the options of states available for sample collection 3. Lack of industrial harmony (strikes) negatively affects work speed
Recommendation to mitigate constraints	1. Government should do more to encourage biotechnology companies to open their offices in the country through provision of electricity and better transportation network, tax rebate etc 2. All necessary steps should be implemented to forestall avoidable strike action by any segment of staff 3. Government should secure the country as its primary objective
Key Lessons	1. The prevalence of PPR in Nigeria is about 26% in sheep and goats and 3% in camels. 2. Total eradication of PPR from Nigeria is possible through massive vaccination campaigns using the thermostable vaccine to be produced indigenously 3. The Nigerian PPR isolates bank is the largest owned by any PPR endemic Nation
Key Recommendations	1. Adequate border control of animal movements 2. Massive vaccination campaigns Nationwide **3.** Effective Veterinary Posts on major roads

List of Investigators

	Name		Office	Role on Project
1	A. Diallo	DVM, PhD	FAO/IAEA Labs, Austria	Sequencing, Phylogenetics & training
2	D. Shamaki	DVM, PhD	Director, Research, NVRI	Co-ordination &training
3	H. M. Kazeem	DVM, PhD	Professor ABU Zaria	Co-ordination & training
4	T.Y. Woma	DVM, MSc, Cert (Mol Biol)	Prin. Vet. Res. Officer, NVRI	PI/Virologist
5	C. Nwosuh	DVM, PhD	Asst. Director NVRI	Vaccine Production trainer

6	D.G. Bwala	DVM, MSc	Prin. Vet. Res. Officer, NVRI	Vaccine Production
7	A.N. Egbuji	DVM, MSc	Prin. Vet. Res. Officer, NVRI	Vaccine Quality Control
8	H.G Ularamu	DVM, MSc	Prin. Vet. Res. Officer, NVRI	Molecular Virologist
9	P. L. Dachung	DVM, MSc	Prin. Vet. Res. Officer, NVRI	Molecular Virologist
10	O.D. Olalekan	B.Tech	Tech. Officer, NVRI	Sampling and Field trials
11	S.E. Mantip	DVM	Sen. Vet. Res. Officer, NVRI	Laboratory Analysis
12	B.J Audu	DVM	Sen. Vet. Res. Officer, NVRI	Molecular Biologist
13	B.B. Dogonyaro	BSc, MSc	Research Officer, NVRI	Molecular Virologist
14	S.C. Chollom	BMLS	Lab. Scientist, NVRI	Laboratory Analysis
15	D.C. Nyam	HND	Tech. Officer, NVRI	Sampling and Field trials
16	G.H. Bature	MLT	Lab. Technician, NVRI	Technician
17	D.N. Tom	MLT	Lab. Technician, NVRI	Technician
18	L. Saleh	MLT	Lab. Technician, NVRI	Media Preparation
19	J. Ogbona	BSc	Secretary, NVRI	Secretarial Co-ordination

Project Title
Multivalent Vaccines Research and Development

Sub-Project Title **Development of Multivalent Black Quarter (BQ), Hantavac and Haemorragic Septicaemia (HS) Vaccine for Large Animals**

Sub-Project Title **Development Of Multivalent Fowl Typhoid (FT) & New Castle Disease Virus-Komarov (NDVK), Infectious Bursa Disease and New Castle Disease Virus – Lasota (NDVL) Vaccines For Poultry**

Introduction
Blackquarter, Black disease and Shipping fever remain diseases of national economic importance in Nigeria considering the havoc done in the livestock industry as a result of persistent outbreaks (Princewill, 1985; Itodo, *et al., 2009*; Odugbo, *et al.,* 2007). Newcastle disease and Infectious Bursal Disease are both viral infections of poultry with a wide geographical distribution causing great economic losses in the poultry industry, while Fowl Typhoid (FT) and Fowl Cholera (FC) are septicaemic disease that most often affects mature domestic poultry with a worldwide distribution caused by *Salmonella enterica* subsp. *enterica serotype gallinarum* and *Pasteurella multocida* serotype A, respectively.

Vaccines produced in The Institute to control these diseases in Nigeria are produced singly, monovalent and specific for each disease. The production and usage of monovalent vaccines are full of challenges. The human and material resources involved are outrageous, thus making it impossible to sustain production of vaccines to meet national demands. Also, the end users find it extremely difficult to transport the products to the field while at the same time maintaining cold-chain. Moreover, the inconveniences from repeated injection of animals with the various monovalent vaccines results in the inability to adequately cover all the animals with such vaccines. It is also labour intensive. This project therefore intends to develop and evaluate multivalent vaccines that will have combinations of the antigenic and immunogenic properties of all the three, as is proposed in the large animal vaccine, and two in the poultry vaccine for the control of these diseases.

Specific Objectives
1. To develop a Multivalent Black Quarter (BQ), HANTAVAC and Haemorrhagic Septicaemia (HS) Vaccine

2. To Develop Multivalent Fowl Typhoid (FT),Fowl Cholera and New Castle Disease Virus-Komarov (NDVK), Infectious Bursa disease and New Castle Disease Virus – Lasota (NDVL) Vaccines.

Work Done So Far
Pure cultures of the vaccine seeds were confirmed by morphology and biochemical tests. The pure cultures were stored in 3% glycerine, cooked meat medium and Nutrient agar slants awaiting the commencement of vaccine manufacture. Virulence tests of the purified seed cultures for *P. multocida* types B (3, 4) (standard), and *P. multocida* E: 2 (Obudu) was concluded.

Fowl cholera, Fowl typhoid and NDVL vaccine seed characterization, Inactivation of culture and Culture validation have been concluded.
The FTV strain used was the 9R, which is attenuated and not virulent enough for use in a killed vaccine preparation.

Vaccine Formulation
Previously, the proposed formulation for the poultry multivalent vaccine was FT/FC/NDVK as a killed vaccine; a pilot study to the main work was conducted.The FCV, NDVL strains and the 9R strain of the FTV were mixed in different ratio with the 2 adjuvants, Aluminium Hydroxide and Montanide ISA 70. The preparations were done according to protocols from LANAVET and SEPPIC. These products are undergoing observations at varying temperatures, 25^0C& 4^0 C to ascertain stability of the 2 emulsions and shelf life.The study has progressed to different formulations for the poultry vaccines, and the large animal vaccine. These formulations were arrived at after a month's Technical Transfer visit by Collaborators from LABIOFAM, Cuba.

1. Combinations of Black Quarter, Hantavac, Haemorrhagic Septicaemia and adjuvant (Sodium Alginate) at different ratios to give 4 different formulations.
2. Combinations of IBDV & NDVL, FTV& NDVK with different stabilizers to give 6 different formulations.

The vaccine manufacture was carried out according to protocols adopted from both LABIOFAM and NVRI. The vaccines formulated are currently undergoing Quality Control tests.

Future work
Standardization of formulated vaccines
The isolation of field strains of Salmonella *enterica* subsp. *enterica* serotype *gallinarum*. The isolate will then be tested for virulence in chickens before use in the multivalent vaccine production.

Laboratory and experimental house trials
1. Construction of isolator pens for the experimental birds
2. Procurement of adjuvants, Elisa kits and CFT kits.
3. Procurement of experimental chickens, guinea pigs, mice, sheep and cattle.
4. Preparation of antigens for screening of chickens.
5. Validation of manufactured multivalent vaccine through Quality Control tests, Potency, Safety tests.

List of principal investigators
Dr E. O. Irokanulo PhD (App. Microbiology) Overall Coordinator

Sub Project - 1

Name	**Qualification**
1. Dr. Audu E. Itodo	DVM, MSc, PhD (Vet. Prev. Med.) Coordinator
2. Mal. Gimba Haruna	B.Sc., MSc, (Microbiology) Team Leader
3. Dr. M.O.Odugbo	B.Sc., MSc, PhD (Microbiology)
4. Dr. T.H.T.Spencer	FMLSCN, M.Sc., PhD (Applied Microbiology)
5. Dr. Manasa Sugun	DVM, MSc
6. Mrs Lilian Okeke	BMLS (Medical Microbiology)

7. Mr Latim Nimbut	BMLS (Medical Microbiology)
8. Mr Ishaya D. Bismoyi	Med. Lab. Technician

Sub-Group 2

Name	**Qualification**
1. Dr. Chika Nwosuh	DVM, MSc, PhD (Vet. Prev. Med.)Coordinator
2. Dr Yewande Akalusi	DVM, MSc (Microbiology) Team Leader
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4. Dr. Leo Shedua	DVM
5. Dr. Asala	DVM
6. Dr. I. S. Tekki	DVM (Medical Microbiology)
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12. Mrs G Gwong	MLT
13. Mr I A Shittu	MSc

Mandate area	**Vaccine Production, N.V.R.I. Vom**	
Title of research	**Multivalent vaccines research and development** ***Sub-project title*** *Development of multivalent black quarter (BQ), hantavac and haemorrhagic septicaemia (HS) vaccine for large animals* ***Sub-project title*** *Development of multivalent fowl typhoid (FT) fowl cholera and new castle disease virus-komarov(NDVK),Gumboro (IBDV)& new castle disease virus – lasota (NDVl) vaccine for poultry*	**Expected completion date** 2014
Key deliverables	1. Screened and evaluated vaccine strains for production of multivalent NDVK,FTV + FCV; NDVL+ IBDV; HSV,BQ + hantavac vaccines 2. Capacity building in basic immunology and vaccinology 3. Adjuvanted vaccine technology acquired 4. Prototype multivalent vaccines developed and produced 5. Reports ,publications and implementation	
Key constraints and challenges	1. Inadequate training in multivalent vaccines production 2. Non availability of laboratory animals (guinea pigs and pigeons), and SPF chickens in the institute. 3. Non availability of appropriate experimental house. 4. Inadequate quantity of seppic adjuvant	
Key lessons	A need for more collaboration between the research and production labs for further works on existing vaccines and novel vaccines	
Key recommendations	Evaluation of master seeds of vaccines produced in the institute	

Project Title

Survey for Prevalent Strains of Mycoplasma Mycoides subspecies Mycoides Small Colony in Nigeria

Introduction

Contagious bovine pleuropneumonia (CBPP) is a disease of cattle caused by Mycoplasma mycoides subsp. mycoides SC (bovine biotype) (MmmSC; SC = small colonies). It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges. Diagnosis depends on the isolation of the aetiological agent. Identification of the agent: Samples to be taken from live animals are nasal swabs and/or broncho alveolar washings or pleural fluid obtained by puncture. Samples to be taken at necropsy are lung lesions, lymph nodes, pleural fluid and synovial fluid from those animals with arthritis. For cultivation of the pathogen, the tissues are ground in medium with antibiotics and inoculated into media that contain inhibitors to prevent the growth of contaminating bacteria. The growth of MmmSC takes several days.

The main problems for control or eradication are the frequent occurrence of sub-acute or asymptomatic infections and the persistence of chronic carriers after the clinical phase. The disease is prevalent in Africa where it is responsible for high economic losses and is a limiting factor in cattle. NVRI is responsible for the development and production of animal vaccines in Nigeria. Currently available MmmSC vaccines are attenuated live strains which induce immunity, but have the drawbacks of the need for annual revaccination and a relatively high incidence of post vaccinal reactions. However, the institute does not have the necessary research and development capacity to further improve the efficacy and safety of the T1/44 strain of MmmSC vaccine that is currently in use in Nigeria, and in other developing countries in Africa.

Specific Objectives

i. To isolate and characterize strains of MmmSC in Nigeria
ii. To determine the prevalent strain of MmmSC in Nigeria.
iii. To develop disease map for Contagious Bovine Pleuropneumonia (CBPP) in Nigeria.

This proposal will combine basic research on the molecular genetics and genomics of the organism, and modern approaches to epidemiology using geographic information systems (GIS) to focus vaccination and control procedures.

Work done so far

Essential items required for the field work have been provided by the collaborating institute.

Research scientist had in-house epidemiological training in surveillance and GIS techniques (session I).

Research team held a pre-surveillance meeting with FLD field officers, VIOs (NVRI Out- stations), State veterinary officers, Agricultural Extension Workers (community animal health workers).

Passive sampling of 5 Agro ecological zones, namely: South-West, South-East, South-South, North-Central &North-East have been completed where 2 states per zone were visited and samples collected from major cattle markets and abattoirs.

Total number of samples collected and still being analysed are 1,403 (319 Ear swabs & 344 Nasal swabs, 674 sera, 27 pleural fluid, 25 lungs, 6 lymph nodes and 8 thoracic swab. The Coordinates of sampled sites were recorded.

Fifty (50) presumptive MmmSC organisms have been identified.

Enhanced research performance--survey trips and extension links established

Future Work

1. Viability test on presumptive isolates
2. Freeze drying of identified isolates
3. Molecular characterization of these isolates at collaborating institutions Active surveillance to the Northwest Agro ecological zone. Field survey, sample collection and GIS mapping of reported outbreaks and Culture and isolation of MmmSC (from tissue samples).
4. Procurement of equipment and consumables.
5. Training
6. Post-surveillance meeting with FLD field officers, VIOs (NVRI Out- stations), State veterinary officers, Agricultural Extension Workers (community animal health workers) and other stakeholders
7. Publications and report writing

List of Team Members:	**Role of Personnel**
Dr. Y. Akalusi (PI)	Team Leader and Principal Investigator
Dr L Muhammad	Assessment of Field Extension activities
Dr C Kennedy (UNN)	Epidemiology
Dr Nwaigwe (UNN)	Field Survey\Microbiology
Dr. Roth (Iowa)	Immunology
Dr Minion (Iowa)	Molecular and Cellular Biology
Dr A Jambalang	Field Survey and Laboratory Processing of Materials
Dr J Dalis	Field Survey and Laboratory Processing of Materials
Dr P Ekong	Field Survey and Laboratory Processing of Materials
Dr L Ikpa	Field Survey and Laboratory Processing of Materials
Dr P Ankeli	Field Survey and Laboratory Processing of Materials
Dr O Asala	Field Survey and Laboratory Processing of Materials
Mr. S Kigbu	Laboratory Processing of Materials
Mrs. M Gyang	Laboratory Processing of Materials
Mr. A Adeleke	Field Survey
Mr. E Ogundeji	Laboratory Processing of Materials
Mr. S Makinde	Laboratory Processing of Materials
Mrs. R Tanko	Laboratory Processing of Materials
*Field Veterinarians (FLD, State-VOs, VROs)	Field Survey/Monitoring outbreaks and impact of CBPP

Mandate Area	**Mycoplasma Research Unit**, Bacterial Research Division, N.V.R.I. Vom.

Title of Research **Survey For Prevalent Strains of Mycoplasma mycoides subspecies mycoides small colony In Nigeria**	**Expected completion date** 2014

Key Deliverables	**Deliverables of Project** 1. Capacity building in basic epidemiological concepts (GIS mapping and surveillance for disease outbreaks). 2. Collation of data on cattle population and distribution in Nigeria. 3. Epidemiological survey map for national surveillance of CBPP. 4. Establishment of surveillance linkage for CBPP disease reporting, diagnosis and data dissemination. 5. MmmSC isolate bank produced. 6. Prevalent MmmSC strain(s) in Nigeria determined
Key Constraints &Challenges	1. Delay in disbursement of funds from ARCN. 2. Insecurity situation in the nation affects field trips 3. The work plan could not follow the proposed timelines due to the security situation in the state thus adjustments are being made. There was delay in the release of funds. 4. Delay in the purchase of items/equipment for the project, due to the procurement act. (Due process taking several months). **Recommendation to mitigate constraints/challenges** Direct purchase of categorized items should be permitted
Key Lessons	1. To establish the most prevalent strains of MmmSC as basis for the development of future attenuated vaccines 2. Effective and efficient methods for control of CBPP in Nigeria by surveillance and GIS mapping should be adopted.
Key Recommendations	1. There is a need to build capacity through workshops and laboratory trainings for members of the group 2. There is a need to determine the immunological and molecular effect of the different isolates of *MmmSC* in cattle in Nigeria. 3. Disbursement of funds should be demand-driven.

Project Title

Improving Brucellosis control in Nigeria through the production and standardization of *Brucella* antigens for disease Diagnosis

Introduction

Brucellosis is an infectious disease of animals and man caused by fastidious, intracellular Gram negative bacteria of the genus *Brucella*. It is a reproductive disease causing serious economic losses in the livestock industry due to abortion, infertility, high neonatal mortality, and reduction in milk production and culling of infected animals. Humans are also highly susceptible to brucellosis through direct contact with infected animals and consumption of infected raw milk resulting in clinical signs such as intermittent fever, headache, night sweat, muscle and joint pains. While the disease has been eradicated in most developed countries, it is still a major problem in developing countries such as Sub-Saharan Africa, Asia and Latin America. In Nigeria, the disease is endemic having been reported in all parts of the country since the 1970s to date

Vaccine for immunization of animals against brucellosis is produced at the National Veterinary Research Institute, Vom but its use is at the discretion of the farmers. Only about less than 5% of farmers in Nigeria are aware of the vaccine and are the only ones that have their animals vaccinated against brucellosis. There has been no effort by government to embark on national vaccination campaign for the control and eradication of the disease. Diagnosis of brucellosis is mainly based on isolation of the organism and serology based on detection of antibodies in sera of infected animals. Serological tests provide rapid diagnosis of brucellosis and are required for surveillance purposes. The most commonly used antigens for the diagnosis of Brucellosis in Nigeria are Rose Bengal Plate Test (RBPT) for serum and Milk Ring Test (MRT) antigen for Milk. They however, are normally imported and therefore very difficult and expensive to procure. This makes availability unsustainable.

Control programme for brucellosis could only be successful when there is availability of antigens for the rapid serological detection of antibodies in infected animals.

The only possible means of providing adequate antigens for use in Nigeria and other African countries is by producing our antigens. This will enable us have enough antigen for our use and for commercial purposes.

Specific objectives

1. The specific objectives of the projects are:
2. To produce Rose Bengal plate test (RBPT) antigen.
3. To produce milk ring test (MRT) antigen.
4. Test and evaluate the antigens.

Work done so far:

1. Propagation of seed culture
2. Propagation of seed lot
3. Harvesting and washing of cells
4. One thousand five hundred millilitre (1500ml) millilitres of *Brucella* harvested cells was produced.

Future plans

i. Standardization of cells already produced.
ii. Preliminary field evaluation of antigens.

Investigators

1. Dr. R.A Ocholi
2. Dr. W.J Bertu
3. Dr. Ehizibolo, D.
4. Dr.A.A Masduq
5. Dr. A.M Gusi
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8. Mr Moses Hassan
9. Mr Abdul Hamid Muazu.
10. Miss Lubabatu Ibrahim

Mandate Area	To produce sera and biological for diseases diagnosis	
Title of research project	Improving Brucellosis control in Nigeria through the production and standardization of *Brucella* antigens for disease Diagnosis	**Expected completion date** December 2014.
Key deliverables	1. Number of 20ml bottles of RBPT antigens produced. 2. Number of 20ml bottles of MRT antigen produced. 3. Antigens standardized and evaluated.	
Key constraints and challenges	1. Major equipment that will enhance the production of cells, harvesting and washing is lacking. These include refrigerated centrifuge, incubator shaker. 2. Equipment for standardization of antigen such as pH meter is yet to be procured. 3. Limited quantity of cell is produced per batch with use of roux flasks.	
Key lessons	Preliminary work showed that the project is viable.	
Key recommendation	The project is progressing despite the challenges. We recommend that funds be released on time to buy equipment that will mitigate these challenges, leading to completion of the project successfully.	

Project Title

Isolation and characterization of Foot-and-mouth disease (FMD) virus in Nigeria for improved vaccine development.

Foot and Mouth Disease (FMD) is an endemic trans-boundary animal disease (TAD) in Nigeria. It is one of the major animal diseases that impact very negatively on trades in livestock and livestock products in the country. To date, four of the seven serotypes had been found in circulation in Nigeria. These include serotypes O, A, SAT1 and SAT2.

FMD results in death of calves, reduces milk production and causes psychological trauma for the farmers. It comes at huge costs and has delimited the effort of the herdsmen and resource poor farmers, (including the women and children) to benefit from the genetic potentials of Nigerian cattle

In Nigeria, like in many other developing countries, where eradication of FMD seems too costly, FMD control is mainly achieved through vaccination and control of animal movement. Currently, limited vaccine development effort has been documented in Nigeria. For an effective control and eradication of FMD in Nigeria, and by extension, West Africa, vaccines that incorporate the antigenic strains from the sub-region must be developed. At the same time, the current trek routes being used for planning purposes for cattle was developed in late 1970 and cannot meet the current demand in view of rapid urbanizations, changed ecology and climatic factors in most parts of Africa. It will therefore be necessary to plan to undertake the development of new cattle trek routes.

Specific Objectives

1. To train field and laboratory staff on the effective sampling procedure and diagnosis of FMD in Nigeria.
2. To develop a comprehensive archives of diagnostic samples (tissues and sera) for the diagnoses and characterization of circulating FMD virus in Nigeria, with the ultimate aim of vaccine production.
3. To generate scientifically sound current information on FMD in Nigeria and an effective communication infrastructures.
4. To innovatively map the country for disease transmission risks, demarcate the FMD hotspots, create templates that may assist in the control and advise the necessary government agencies appropriately.
5. To improve field to laboratory sample dispatch under the best conditions and in the shortest time possible.
6. To enhance collaborations and trainings in the selection of appropriate vaccine candidate based on phylogenetic analyses and promote technology transfer on FMD vaccine production.

Work Done

1. Planned activities include the training of personnel on disease recognition, detection and sample collection achieved.
2. Epidemiological units created.
3. Questionnaire drafted and administered.
4. Clinical samples which include a total of 79 epithelial tissues, 190 sera samples, 130 probang samples and 4 Vesicular fluids were collected across the country.
5. Laboratory analyses carried out using RT-PCR, Antigen and antibody ELISA revealed the presence of serotypes A, O, SAT-1 and SAT-2 circulating in Nigeria.

6. ZZR and IBRS-2 cell-lines have been obtained multiplied and banked for FMD virus isolation.

Future Work

1. Continues administration of questionnaire, samples collection, further laboratory analyses, and Field data sample analysis by GIS and statistical analyses.
2. Laboratory work will consist of serology, isolation and characterization of FMDV in order to generate FMDV sequences and vaccine candidates.
3. Vaccine development and animal trial

Mandate Area To conduct research into all aspects of animal diseases, their treatment and control	**Name of Programme** Viral Research
Title of Research Project Isolation and characterization of Foot-and-mouth disease (FMD) virus in Nigeria for improved vaccine development.	**Expected completion date** December, 2013
Key Deliverables	1. Field veterinarian trained on disease recognition, detection sample collection and diagnosis achieved. 2. Questionnaires administered 3. Suspected samples collected, Tissue and Sera bank created 4. Current FMD serotypes circulating in Nigeria determined (A,O,SAT1 and SAT2)
Key constraints and challenges	The nomadic pastoralist are not always located in one place and they are reluctant in reporting suspected FMD outbreaks to relevant authority for prompt action and sampling. 2. Lack of awareness and public enlightenment on the need to report FMD outbreaks to relevant authorities. 3. Under funding of research project and training of man power. 4. Lack of Project vehicle for prompt movement to reported outbreaks
Key lessons	1. FMD is endemic in Nigeria and so far we have collected samples from the following States that had an outbreaks: Adamawa, Plateau, Nasarawa, Kogi, Benue, Kaduna, Taraba, Bauchi, Oyo and Enugu 2. Lack of reporting FMD outbreaks in Nigeria has contributed to not knowing the current existing serotypes in the country. 3. Farmers are complaining that government has not intervened in the situation. 4. The movement of the nomads is another source of spreading the disease. 5. There is need for manpower training. 6. There is also need to create awareness through mass media, radio jingles rural and community awareness of the disease.
Key Recommendations	1. Government should make a policy on the control of FMD either through vaccination or test and slaughter. 2. Control the movement of the nomads by having grazing reserves. 3. Educate stakeholders and farmers in livestock industry that FMD can be controlled through massive vaccination campaign.

Project Team

1. Dr.Shamaki D.
2. Dr.Wungak Y.
3. Dr.Damina M.S.
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24. Dr.BalaAkawu.
25. Dr.Bwala D.G.

Project Title

Effect of *Moringa oleifera* on performance, carcass characteristics, immune response, blood chemistry of broilers and cockerels

Introduction
The leaves of *Moringa oleifera* (MO) contain carbohydrates, amino acids, minerals and vitamins. The phytochemicals in MO have antioxidant, inflammatory, helminthic, cancer and biotic properties. The chemicals lower blood pressure and sugar levels and modulate immune response.

Specific objectives
The objectives of the study were to determine the chemicals and nutrients in MO leaves grown in Nasarawa state and the effects of different inclusion levels of MO leaves on the performance indices, carcass characteristics immune response, blood chemistry and some endoparasites of broilers and cockerels.

Work done so far
***Moringa oleifera* leaves**
A tonne (1000kg) of fresh MO leaves, twigs and pods were obtained from a farmer in Nasarawa State, Nigeria. The MO parts were air dried in shade, then in a store and the leaves, twigs and pods were separated. The leaves were ground into powder.

Chicks
Two hundred and fifty Hubbard broiler and 250 Black Nera cockerel day old chicks were purchased from a hatchery in Jos. The chicks were housed on deep litter at the Poultry Division of the National Veterinary Research Institute (NVRI), Vom with 2 sq/bird provided as floor space.

Feed and Feeding
On arrival the broilers and cockerels were conveniently divided into 4 groups of 57 chicks each. The groups were control (0%), 5%, 10% and 20% corresponding to the inclusion rate of dried MO leaves in chick mash or broiler starter prepared by Dagwom Farms. Feed and water were provided to the chicks *ad libitum.*

Vaccines and Vaccinations
Live infectious bursal disease (IBD) and Newcastle disease (ND) La Sota vaccine were obtained from the NVRI. The IBD vaccine was administered in water at 7 and 14 and La Sota in water at 21 days of age.

Phytochemicals, Proximate and Amino acid Analysis of *Moringa oleifera* Leaves
The phytochemicals screening of MO leaves was done as described by Trease and Evans in 1998, proximate composition by the method of AOAC in 1990 and amino acid analysis by the method of Spackman *et al.* (1958).

Parasitology
Faeces were monitored for presence of helminth eggs and coccidia oocysts by floatation McMaster technique.

Assessment of Carcass Characteristics
Characteristics were assessed by determining weight of dressed carcass, heart, liver, gizzard, spleen, thymus, pancreas and intestine.

Determination of Feed Conversion
Initial and final body weight, weekly body weight, body weight gain, and feed consumption were determined. Feed conversion ratio (FCR) was obtained as: FCR = Total amount of feed consumed/Total body weight gain.

Data Analysis
Data generated were entered into Excel spread sheet, cleaned and transferred to SPSS version 17. The data were analysed using descriptive statistics. Differences in means were compared using Student T-test. p value of $\leq$ 0.05 was considered as significantly different.

Results and Discussion
The leaves of MO leaves contained high concentrations of protein (28.14%) and energy (4293.90 calorie/g) and negligible crude fibre (7.24%), lipid (4.37%) and moisture (7.92%) content. *Moringa oleifera* contained alkaloids, cardiac glycosides, flavonoids, saponins and steroid/tarpenes. Eight essential amino acids Including lysine (4.21 g/16gN) and methionine (2.03 g/16gN) were demonstrated in MO leaves. *Moringa oleifera* leaves are highly nutritious and can be included in chicken feed. Examination of the faeces of control and MO supplemented chickens did not reveal helminth eggs or oocysts. It was not possible to assess the antiparasitic effect of MO because chickens not supplemented had no oocysts and eggs. Broilers and cockerels fed with ration containing 20 % MO leaves consumed more feed then controls and those fed MO at 10% and 5% inclusion. The body weight of broilers feed MO at 5% was significantly higher ($p < 0.05$) than controls and those fed with MO at 10% and 20% inclusion rates at 5, 6, 7 and 8 weeks of age. The body weight of cockerels fed MO at 20% was significantly higher ($p < 0.05$) than controls and other inclusion rates at 7 and 8 weeks of age.The feed conversion ratio of broilers fed MO at 5 % was lower than that of controls and those fed with MO at 10% and 20% inclusion rates. The feed conversion ratio of cockerels fed MO at 20 % was lower than that of controls and those fed with MO at 5% and 10% inclusion rates. Although MO at 20% inclusion improved feed consumption in chickens the feed conversion ratio depends on the type of bird with broilers performing better at lower inclusion rate. The thymus, gizzard and intestine of broilers not supplemented with MO were lighter than the thymus, gizzard and intestine of broilers supplemented at 5%, 10% and 20%. The heart, liver and spleen, of broilers not supplemented with MO were heavier than the heart, liver and spleen of broilers supplemented at 5%, 10% and 20%. The heart,liver, thymus, pancreas and intestine of broilers supplemented with MO at 5% were heavier than the hearts, liver, thymus, pancreas and intestine of broilers supplemented with MO at 10% and 20%. The heart, liver, thymus, spleen, pancreas, gizzard and intestine of cockerels not supplemented with MO were lighter than the heart, liver, thymus, spleen, pancreas, gizzard and intestine of cockerels supplemented at 5%, 10% and 20%. The heart,liver, thymus, spleen, pancreas and intestine of cockerels supplemented with MO at 20% were heavier than the hearts, liver, thymus, spleen, pancreas and intestine of cockerels supplemented with MO at 5% and 10%. Organ weights were positively influenced by inclusion of MO in feed with 5% having a greater effect in broilers and 20% in cockerels. Its effect on the spleen and thymus may potentiate immunity.

Conclusions

The leaves of MO leaves contained high concentration of protein and energy and low crude fibre, fat and moisture. *Moringa oleifera* contained essential amino acids and bioactive chemicals. *Moringa oleifera* improved feed consumption, body weight gain, feed efficiency, organ weights and dress weight in broilers and cockerels. The effect of MO on body weight was more apparent at 5 weeks of age and older in broilers and 7 and 8 weeks of age in cockerels.

Future work

Extensive study with 5% MO in broilers and 20% in cockerels is recommended. The effect of MO on immune response following vaccination against ND and Gumboro disease, performance of breeders and layers and the possible acute and chronic toxicity of MO leaves in chickens need be investigated.

Investigators

S/N	Investigator	Affiliation	Role on the project
1	P. A. Abdu	ABU, Zaria	Coordination, data analysis, publication and report writing
2	U. Musa	NVRI, Vom	Coordination and report writing
3	N.M. Sati	NVRI, Vom	Experimentation and data collection
4	P. E. Emennaa	NVRI, Vom	Experimentation and data collection
5	A. Ahmed	NVRI, Vom	Coordination and report writing
6	P.R. Kumbish	NVRI, Vom	Sample analyses
7	N.I. Ogo	NVRI, Vom	Sample analyses
8	Oga Onoja	NVRI, Vom	Sample analyses
9	G. Forcados	NVRI, Vom	Sample analyses
10	E. Payi	NVRI, Vom	Coordination and feed compounding
11	H. M. Miapyen	NVRI, Vom	Experimentation and data collection
12	A.G. Yisa	NVRI, Vom	Nutritionist
13	S.A. Ogedegbe	UNIBENIN	Data Analysis
14	G. Agida	NVRI, Vom	Sample analyses
15	H.B. Yusuf	NVRI, Vom	Sample analyses
16	A. M. Qasim	NVRI, Vom	Experimentation and data collection

Mandate Area Conduct research into all aspects of animal disease, their treatment and control	**Name of Programme** *Moringa oleifera* Research
Title of Research Project: Effect of *Moringa oleifera* on performance, carcass characteristics, immune response, blood chemistry of broilers and cockerels	**Expected completion date** December, 2013
Key deliverables	1. Effect of MO on performance indices 2. Evidence of immune potentiating effect of MO 3. Evidence of effect of MO on helminths and coccidia 4. Effect of MO on haematological parameters 5. Human resource development 6. Stakeholder meeting
Key constraints and challenges	1. Late delivery of procured materials to carry out planned activities 2. No regular budget for MO research 3. Slow and incomplete laboratory analysis and submission of findings
Key lessons	The level of commitment of staff involved with MO research needs to improve
Key recommendations	1. Extensive study with 5% MO in broilers and 20% in cockerels 2. The effect of MO on the immune response following vaccination against ND and Gumboro disease 3. The effect of MO performance of breeders and layers 4. The possible acute and chronic toxicity of MO leaves in chickens need be investigated 5. Include MO research into NVRI regular research

Project Title

Development of pelleted thermostable NDV-I_2 Clone vaccine for the control of Newcastle disease in rural poultry using local foodstuff as carrier.

Introduction

Productivity of rural poultry is constrained by annual outbreaks of the Newcastle disease (ND). ND is the most important infectious disease affecting village poultry with mortality rates of up to 90% in susceptible flocks (Janviriyasopak *et al*, 1989, Johnson and Cumming, 1992, Echeonwu, 1993).

Control of ND is by vaccination. Current vaccines which come in large dose units are targeted towards large commercial flocks and have little relevance in village flocks which are often small, scattered, multi-aged, and free-roaming with minimal control. Maintenance of cold chain for vaccine viability is also near impossible in the rural set-up. A viable solution to this problem is the formulation of a pelleted food-based vaccine using a thermostable virus strain in small doses using locally available feedstuff.The introduction of such a vaccine will enable rural dwellers especially the women folk take charge of their poultry disease problems as little expertise will be required in the administration of pelleted food vaccine. The demand for cold chain will be minimal. The stress associated with the handling of individual birds for vaccination or water deprivation before vaccines are given through drinking water are eliminated by the food-based vaccines.

Specific objectives

1. To select a suitable thermostable clone from $NDVI_2$ vaccine strain that will withstand higher temperature than the parent virus.
2. To screen locally available foodstuff and binders and obtain the least detrimental to viral viability for incorporation of $NDVI_2$ vaccine clone
3. To pellet the food vaccine and assess its efficacy.
4. To carry out on-station and field trials of the pelleted food-based vaccine for protection against Newcastle disease.
5. To make recommendation on technological transfer and model for industrial pelleted food-based vaccine production and use.

Work done so far (Progress report)

1. A more thermostable $NDVI_2$ virus clone has been developed.
2. A suitable food-based vaccine carrier with minimal anti-viral elements has been obtained.
3. An $NDVI_2$ pelleted food vaccine has been developed.
4. On-station trials of the pelleted vaccine in susceptible chickens has been successfully carried out.
5. Challenge studies of vaccinated birds has been successfully carried out.

Future work

1. Revalidation of the on-station trial.
2. Field trial of the pelleted feed vaccine in rural Field trials of the vaccine in five states.
3. Publication of research findings in leading scientific journals will be undertaken.

Investigators

1. Dr. J.O. Ibu
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12. Dr. Didacus Eze
13. Dr. E. C. Okwor
14. Prof. P. Abdu

Mandate area Vaccine Development	**Name of programmes related to mandate area** Viral Research Division
Title of Research Project; Development of pelleted thermostable NDV-I_2 Clone vaccine for the control of Newcastle disease in rural poultry using local foodstuff as carrier.	**Expected Completion Date** April 2014
Key Deliverables	1. A more thermostable $NDVI_2$ virus clone has been developed. 2. A suitable food-based vaccine carrier and with minimal anti-viral elements have been obtained. 3. An $NDVI_2$ pelleted food vaccine has been developed. 4. On-station trials of the pelleted vaccine in susceptible chickens have been successfully carried out.
Key Constraints and Challenges	1. Breakdown of our egg incubator. 2. Fear of non-expected co-operation from villagers. 3. Security challenges in Nigeria as regards carrying out field trial of this work in villages. **Recommendation to mitigate Constraints/Challenges** 1. Repair or purchase of a new incubator 2. Advocacy visits to rural poultry farmers prior to field trials 3. Improvement on the security situation across the country
Key Lessons	1. Control of Newcastle Disease outbreaks in rural poultry populations through vaccination with minimal expertise and risks is now possible. 2. With the use of the pelleted food vaccine, there will be a marked reduction in heavy losses during seasonal outbreaks of the disease. 3. There will be greater income for rural dwellers. 4. There will be an abundant and more affordable poultry products in the Nigerian market 5. With greater confidence in disease control, more village women and youths will take to backyard poultry keeping thereby reducing unemployment, rural-urban migration, and youth restiveness.
Key Recommendation	1. Regular disbursement of fund to facilitate early completion of the project 2. Improvement on the country's security situation for adequate coverage of selected rural areas during field trials

RESEARCH PROPOSALS

Project Title
Diagnostic survey for the establishment of livestock bench-mark disease information for the control of economically important diseases

Introduction
The livestock sub-sector is transforming in response to the economy and societal expectations. The population expects livestock to provide safe and plentiful food and other by products for the growing population. Increased livestock production will improve the livelihoods of the producers, traders, processors as well as impact on global public goods related to environmental sustainability, food security and animal borne diseases. Livestock production is largely still traditional and in the hands of rural people, where they draw their livelihoods and household food security.

Theexpected change in pace of livestock productivity will lead to an imbalance in the growth of the subsector with consequent problems of intensity, inefficient production systems and unforeseen animal health implications. Animals, humans and their pathogens have always coexisted side-by-side, but recent economic and environmental trends are creating new disease risks and intensifying old ones. The risks are emerging due to geographic clustering of intensive livestock production facilities near urban populations and the movement of animals, people and pathogens between traditional and intensive production systems. These systems have different disease control and prevent strategies and the exchange of pathogens between them can cause major disease outbreaks.

The dearth of a bench mark livestock disease information in the country complicates disease control and prevention as climate change is altering patterns of livestock incidence. This is because pathogens, insects and other vectors that carry them enter new ecological zones.

To mitigate the challenges of disease control and prevention, a bench mark information on livestock diseases needs to be established. Once this is achieved it will facilitate planning to increase the flow of technologies, capital, live animals and products of animal origin around the country. It will also reduce the risk of spreading diseases of animal origin to the human population. Attention will focus on principles of prevention, progressive disease containment, or elimination of a new emerging disease before they spread.

Objectives
1. To establish the bench-mark information of livestock diseases for each agro-ecological zone.
2. To determine control measures common to each agro-ecological zone.
3. To target vaccine production and timely delivery.
4. Data generated will form basis for instituting surveillance and control programmes for economically important diseases in each agro-ecological zone.

Justification
Animals, humans and their pathogens have coexisted for years. Lack of data on endemic diseases in each of the agro-ecological zone of the country seriously affects

vaccine research, development, production and distribution. This has affected planning for control and prevention of disease outbreaks. The problem becomes more serious with the current economic and environmental changes which might aid outbreaks of some endemic diseases and create risks of epidemics of new ones. This is in addition to climate change that alters patterns of incidence of livestock diseases. As pathogens, insects and other vectors that carry them enter new ecological zones.

Methodology

The study will cover all the 6 agro-ecological zones of the country involving 24 states selected based on livestock population. In each state 50 farmers, 5 private practicing veterinarians will be randomly selected and the state director of veterinary services. Farmers will be selected in each state on pro rata basis, in a predominantly ruminant livestock 35 livestock farmers will be sampled and 15 poultry farmers. In a state where poultry pre-dominate 35 poultry and 15 ruminant farmers will be surveyed respectively.

Activities

1. Pre-inception meeting with collaborators to discuss project implementation guidelines.
2. In-house meeting and training of project team on questionnaire administration and data collection under field conditions.
3. Pilot survey to test the questionnaire, results will be analysed and adjustment to be made where necessary before the general survey takes off.
4. National survey
5. Data analysis and final report writing.

Expected contribution to knowledge

1. Diseases peculiar to each agro-ecological zone will be readily available and vaccine requirement will be known.
2. NVRI that will use the information to target vaccine production to meet seasonal demand.
3. Data generated will form basis for research to generate new technologies for disease prevention, vaccine research and production.
4. Information will be available to guide and direct vaccine distribution relevant to each agro-ecological zone.
5. Data available will enable the institution of an active surveillance programme for some economically important diseases.
6. Capacity would have been built with the field experience acquired by staff. It will also provide mentoring for junior researchers and vaccine producers.
7. Policy makers will use the data generated to formulate policy for the control or eradication of certain diseases.

National relevance of study

The data generated will be used to establish disease data bank for future use in planning research. Effective vaccine distribution channels will be put in place to reach farmer as and when needed. Disease reporting will be carried efficiently to avert outbreaks. Active surveillance will be mounted to check on emerging and re-emerging diseases. Wholesome livestock products for human consumption will be guaranteed. The socio-economic status of the producers and others connected with the livestock industry such as processing and trade will improve.

Project Team

Dr Lawal U Muhammad	Principal investigator
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Miss Patience Ajegena	
Dr Moses Gyang	
Dr Peterside P Kumbish	

Project title
Toxicity Profiling Of Some Dry-Season Plants in Mining Area of Zamfara State

Introduction
Lead, a well-known environmental toxicant has been used since ancient times as a component of domestic, agricultural and industrial products. Today, this heavy metal is one of the ubiquitous environmental pollutants particularly widespread in industrial areas. The use of lead in developing countries such as Nigeria is still a major problem (Igwilo *et al.*, 2006; Nduka and Orisakwe, 2007). Man and animals are exposed to lead through feed from numerous sources as well as from the general environment. Lead bioaccumulates and circulates in the food chain and ingestion of grazing and browsing plants by ruminants has resulted in poisoning. Public health implication of consuming products from such animals is a concern. Lead is highly toxic and main heavy metal causing intoxication of birds (Martins *et al.*, 2010). Main target organs of lead toxicity are kidney, liver, spleen and testes inducing a wide range of physiological, biochemical and behavioural dysfunctions (Suradkar *et al.*, 2010). Acute and chronic exposure of chickens to lead has been well documented (Mazliah *et al.*, 1989; Pain *et al.*, 1995; Trampel *et al.*, 2003). Sources of lead exposure in chickens are lead bullet shots (Hirai *et al.*, 1991; Nakade *et al.*, 2005), contaminated feed, water and soil (Donaldson and Leaming, 1984), industrial pollution and agricultural technology (Brams and Anthony, 1983).

Mining activities have contributed immensely to the release of heavy metals such as lead into the environment (Chakravorty, 2001; Spencer *et al.*, 2008). Recently, environmental lead contamination as a result of artisanal mining has been reported in some Nigerian villages (UNE/OCHA, 2010; Ezeh and Chukwu, 2011). This community and others alike, have recorded high human mortality due to lead, especially in children less than 5 years of age (CDC, 2010; UNE/OCHA, 2010; Dooyema *et al.*, 2012).

Infectious diseases have remained among the top five causes of death worldwide (WHO, 2008) and recent reports have suggested that exposure to common chemicals from our daily environment may be an overlooked factor that contributes to this global burden of disease (Spannhake *et al.*, 2002,Winnans *et al.*, 2011; Colosio *et al.*, 2005; Singh, 2005). Preliminary investigation of animal involvement in the Zamfara lead poisoning revealed that animals are equally affected as humans and exposure to lead is of a chronic nature (Oladipo *et al.*, 2012).

Objectives
1. To identify and sample plants grazed/browsed by animals during both dry and wet season in mining areas of Zamfara state.
2. To determine the concentration of toxic principles present in plants collected.
3. To carry out toxicity profiling of plants collected.

Justification
The information generated from this study is expected to helping determining the level of success achieved in lead poisoning remedy measures so far taken in Zamfara State. A data bank of grazing plant profile in Zamfara State will be generated in addition to maintaining a chemical and toxic profile of plants growing in contaminated areas of

the State. The information will also be useful to non-affected areas and States by obtaining data on grazing and browse plants.

Methodology

1. Preparation of structured questionnaires: A well-structured open-ended questionnaire will be designed by the team based on the needed information and validated by the epidemiology and extension units of the Institute. This will be administered to farmers, nomads and small-holder farmers via a guided dialogue interview technique.
2. Plants collection and identification:
   Efforts will be made to collect specimen samples of plants mentioned in the questionnaire and these shall be identified via Voucher specimen numbers the department of Biological Sciences, Ahmadu Bello University, Zaria.
3. Plant preparation and extraction:
   Plant parts collected will be air-dried under room temperature and grounded to fine powder using a laboratory milling machine (Fritsch 65 13.1030, Germany) after crushing with pestle and mortar. These will be stored in air-tight containers pending use. Various types of extraction solvents will be used for extraction. Acetone, Methanol, Ethanol, Petroleum ether, Methyl chloroform and water will be used to extract the plant samples according to the method of Sofowora (1979). The filtrate obtained will be lyophylized in a freeze-dryer and stored in a 68 wide mouthed screw capped glass bottles at 4oC until needed.
4. Phytochemical screening:
   The extracts obtained from the plants will be subjected to qualitative phytochemical screening using the method of Trease and Evans (1989). Further analysis to determine the chemical structure and composition of the extracts will be done using a Shimadzu Gas chromatography-mass spectrometer (at NARICT, Zaria).
5. Anti-nutritional
   The following anti-nutritional factors will be determined qualitatively according to the spectrometric methods described by AOAC (1990); Phytic acids, tannins and oxalates.
6. Trace and heavy metal analysis:
   The levels of Lead, Cadmium, Chromium, Manganese, Zinc, Arsenic, Mercury, Magnesium, Copper, Iron, Selenium and Potassium will be determined grounded plants according to the protocols by AOAC (1996).
7. Acute and sub-chronic toxicity studies:
   The extracts obtained will be tested in laboratory rats using the standard protocols as described by OECD (2001). The mean lethal dose of each extract will be determined in these species of animals. In addition, the acute and sub-chronic effects of these extracts will be determined using the same protocols.
8. Statistical Analysis
   All data obtained were expressed as mean ± SEM. Student t-test and one-way analysis of variance (ANOVA) was used to determine differences between the test samples and sample locations, respectively. Values of $P < 0.05$ were considered significant.

Expected contribution to knowledge:

1. The data generated from this study will help validate the risk factors of contamination in Zamfara state.

2. Public health education of the human populations.
3. The data generated will help advice on policy.
4. The data generated will help quantify the magnitude of animal exposure in Zamfara state.
5. The data will help in concerted efforts to ameliorate the environmental pollution and alert for the environment and animal health.

National relevance of the study:

The information generated from this study will be useful in planning and remediation of the lead poisoning outbreak in Zamfara state. A data bank of grazing plant profile in Zamfara state will be generated in addition to maintaining a chemical and toxic profile of plants growing in contaminated areas of Zamfara state.

Project team

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