INTRODUCTION

Research and development thrives in organizations where regular knowledge and information sharing and dissemination activities are encouraged and practiced among her staff in a cordial manner. NVRI seminar is one such avenue. Being a research based institution, seminar is a core activity in NVRI. It provides a meeting point for research staff to showcase their work and encourages the cross fertilization of research and diagnostic ideas. Interestingly, this was a subject of discourse in one of the seminars, where the need for knowledge sharing between health, environment and veterinary professionals for effective control of zoonoses was advocated. Therefore, seminars are especially pertinent to the actualization of the mandate of the Institute. More so, advances in science and technology have made scientific research one of the most dynamic terrains that require a constant update to be able to keep abreast with current trends. It is in line with these facts that the Institute encourage regular seminar presentations on topical issues related to her Vision and Mandate.

In this series, assessment of staff welfare, knowledge and attitude as it relates to biosecurity and biosafety in work place was analyzed and knowledge gaps highlighted with appropriate recommendations. The Institute was adjudged suitable for accreditation and registration with the National and International Standard Organization after a careful evaluation of facilities and procedures in the various Research and Production Divisions. Strategies for development and selection of vaccine candidates, effects of stabilizers and break in cold chain on vaccine potency, threat of the next pandemic and many more topics were covered in this series.

Ideas or knowledge shared is like a seed sown which has the proclivity of multiplication whereas ideas or knowledge shelved is bound to molder. Make a choice to share your findings and contribute to knowledge or shelve your results and lose your scientific relevance.

Joshua Kamani
Chairman Publications and Seminar Committee

OVERVIEW OF 2019 NVRI SEMINAR

The Institute recognizes the place of seminar in promoting the exchange of research ideas and showcasing her research, production and extension activities. Accordingly, management and staff of the Institute accorded priority to the fortnightly seminar presentations. During the year under review twelve seminars were presented; nine being technical and three on generalist subjects. This review covers the former. Interns of the NEF-NVRI Toxicology program also gave account of their three months' research experience and findings with the research community during one of the seminars. However, the details and abstract were not covered in this review. A guest researcher from the Centre for Tropical Livestock Genetics and Health, University of Edinburgh, UK, featured in one of the seminars and explicated on the prospects of NGS and 'omics' approaches to accelerate understanding of tropical livestock immunogenetics. The addition of these novel approaches to the Institutes' research portfolio is worth consideration.

Attendance to seminar in 2019 ranged from 33 to 181 with mean attendance of 106. Performance of the Divisions in terms of seminar attendance and presentation is depicted in figure 1. As it stands, considering the number of research staff in the Institute, there is room for improvement in attendance to seminars especially in terms of punctuality so as to maximize the time allocated. Furthermore, the presence of the Institute Management Committee (IMC) members, Assistant Directors and Heads of Divisions at the seminars will not only boost the quality of presentations but is one sure way of tackling some of the hiccups experienced.

Taken together the seminars have been enriching and rewarding. Looking forward to more presentations and active participation from all concerned in the coming years.

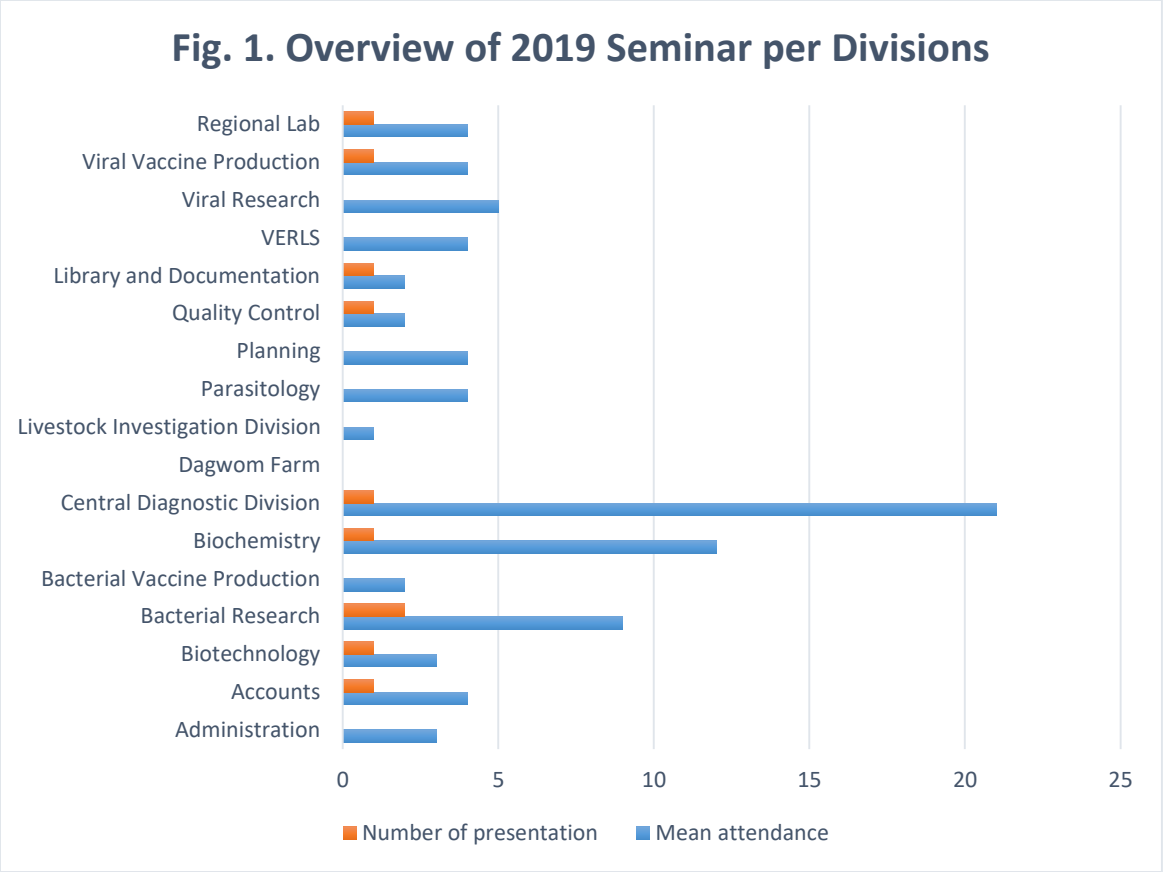
Fig. 1. Overview of 2019 Seminar per Divisions
Regional Lab
Viral Vaccine Production
Viral Research
VERLS
Library and Documentation
Quality Control
Planning
Parasitology
Livestock Investigation Division
Dagwom Farm
Central Diagnostic Division
Biochemistry
Bacterial Vaccine Production
Bacterial Research
Biotechnology
Accounts
Administration
0
5
10
15
20
25
Number of presentation
Mean attendance

Assessment of bio-risk knowledge and practices of laboratory staff of National Veterinary Research Institute, Vom, Nigeria.

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INTRODUCTION

The National Veterinary Research Institute (NVRI), Vom was established in 1924 to carry out research and diagnosis of animal diseases and production of animal vaccines. The Institute has 10 laboratory based Divisions to fulfill this mandate with about 250 research and laboratory staff. The staff works with a lot of infectious agents, some of which are zoonotic. Laboratory personnel handling samples containing highly infectious agents are at high risk of contracting laboratory – acquired infections (Weinstein and Singh, 2009). Some of these infectious agents could spread beyond the laboratory into the surrounding community (Gaudioso and Zanlo, 2007). Laboratory personnel are expected to act responsibly and not to expose themselves and the community to bio-risks, to follow safe working practices (biosafety) and practices that will help keep their work and materials safe and secure (biosecurity). There is generally low knowledge of bio-risk management by staff that work with infectious materials and the general public that visit or stay close to such facilities. The status of the laboratory staff in terms of bio-risk knowledge and perception is not adequately known. Newly recruited staff are not usually trained on biosafety/biosecurity before deployment to laboratories. This study was therefore, designed to assess the knowledge perceptions and practices of various levels of laboratory staff at NVRI as it relates to biosafety and biosecurity. This project will hopefully benefit NVRI Management by providing information to help design and develop bio-risk management policy and training at the Institute.

Methodology

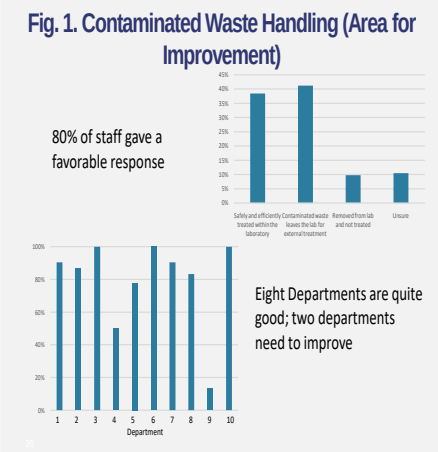
A survey was conducted with specific knowledge questions and questions related to certain areas of the CWA 15793. Forty-seven questions were included in the survey; four questions were on demographic data, 25 on a perceptual scale and 18 on specific knowledge. The areas covered were grouped into: Attitudes and awareness, laboratory practices, biosafety policies, biosecurity policies and waste management.

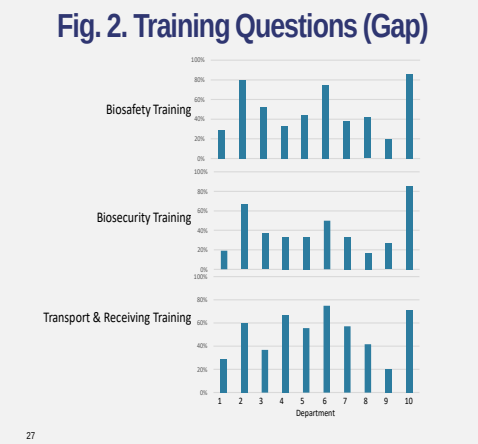
One hundred and fifty survey questionnaires were deployed to Laboratory Assistants, Laboratory Technicians, Laboratory Scientists and research staff of the Institute using the Divisional Biosafety Officers (BSOs). Excel spread sheet was used to enter and analyze the data. A Spotlight Analysis based on desired responses was used to present the results of the survey.

Results and Discussion

Out of 150 survey questionnaires distributed, 143 (95%) were returned. A good number of the respondents (92%) know what biosafety is while only 48% of the respondents know what biosecurity is. Results from the survey indicate that there is some level of awareness of biosafety policies, in particular with respect to segregation and storage of agents according to risk, availability of Standard Operating Procedures (SOPs) in the lab and waste handling (Fig. 1). However, there are some potential gaps in training, inventory of biological agents in the lab, Personal Protective equipment (PPE) availability and compliance, restriction of access to biological materials, keeping records of laboratory acquired infections (LAIs) and incidents reporting, transport/shipping and receiving of samples and occupational health. Most of these gaps can be addressed through training of the staff.

Some of the Divisions are doing quite good, while a few need improvements (Fig. 1 & 2). Those Divisions that are good may be as a result of upgrade of facilities in the labs and training received on biosafety/biosecurity by the staff of the Divisions (Fig. 2). Those Divisions that are doing well can be used as models for the Divisions that are lagging behind.





Conclusion

The study identified areas where the bio-risk management system is strong. There are some gaps in knowledge, practices and policies but there are individual Divisions that are strong. There are several areas that can be converted to strengths through awareness training. There is need to train the laboratory staff on Bio-risk Management and developing an Institutional Biosafety Manual.

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Isolation and molecular characterization of *Mycoplasma mycoides* subspecie *mycoides* in three agro ecological zones of Nasarawa state, Nigeria.

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INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is among the transboundary animal diseases classified under OIE list A. The disease is caused by *Mycoplasma mycoides* subspecie *mycoides*, and is characterized by respiratory disorders; dyspnea, polypnoea, pyrexia, anorexia, nasal discharges, extension of the neck and coughing when animal is forced to move (Thiaucourt *et al* 2003, OIE, 2014).

CBPP is endemic in sub Saharan African countries and Nigeria causing serious devastation on the economy (Chima *et al*., 1999; Aliyu, 2000; Nwankpa *et al*., 2004; Nicholas, 2004; Danbirni *et al*., 2010, Tambuwal *et al.*, 2011; Egwu, 2012; Alhaji, 2016; Ankeli, 2017; Francis *et al.*, 2017).

The aim of this study is to isolate, identify and characterize *mycoplasma mycoides* subspecie *mycoides* in 3 Local Government Areas of Nasarawa state, Nigeria. This will eventually lead to proper design of control measures of the disease in Nasarawa state.

Methodology

A total of 300 samples consisting of 150 nasal swabs and 150 pleural fluids were collected in duplicate from cattle manifesting signs of respiratory disorder suspected for CBPP in cattle markets and abattoirs in Nasarawa state, Nigeria. The samples were taken with sterile swab sticks and transferred to 2 ml of PPLO transport media. The collected samples in bijou bottles were

kept on ice and transported to the Mycoplasma Laboratory of N.V.R.I Vom for analysis or preserved at -20°C before analysis.

Isolation and identification**:** The samples were taken aseptically by sterile swab stick and transferred to PPLO broth. Cultural technique was according to the standard protocol of (OIE, 2012).

Biochemical assay**:** Biochemical assay of the 12 local isolates was carried out for the identification of *mycoplasma* species according to standard protocol of (Nicholas, 2008).

Polymerase Chain Reaction**:** The *Mycoplasma* isolates were subjected to DNA extraction for confirmation through PCR. The polymerase chain reaction was performed for the detection of *mycoplasma* species by using two set of primers; the *Mycoplasma* cluster and the specie specific primer as described by Miles *et al.* (2006).

Homology and phylogenetic analysis**:** The gel product of specific amplicon size were sequenced in both directions. Sequences were assembled and edited to a tool length of 1100 bp using Bio edit sequence alignment editor (Hall 1999). Furthermore, Phylogenetic tree was constructed using neighbor-joining algorithm of MEGA 7 (Tamura, 2013) for evolutionary study and to build a correlation with other strains of different regions.

Statistical Analysis**:** Data obtained were subjected to Chi square analysis using Open Epi (Version 3.02a) software to check statistical difference between different sources of samples.

Results and Discussion

Isolation of Mycoplasma**:** Out of 300 samples, 12(4%) were positive on culture for *Mycoplasma* species showing the classical comet with turbidity in PPLO broth media. A typical nipple like and fried egg colonies appeared on day four and seven post incubation on PPLO agar. Highest culture was obtained from nasal swab compared to pleural fluid. On statistical analysis of the data by Chi-square test (χ^2) there was no significant association ($P > 0.05$) between the two different samples obtained from cattle suspected of CBPP.

The positive culture of *Mycoplasma* species was sub cultured on PPLO agar till the characteristic typical fried egg appearance were observed. On biochemical analysis 7(58.3%) fermented glucose, reduced tetrazolium salt and hydrolyzed casein. They did not hydrolyze arginine, urea and negative for both phosphatase activity and film/spot formation. 3 (25%) hydrolyze arginine, positive for phosphatase activity, negative to tetrazolium salt, negative to casein, did not reduce glucose and negative for film /spot formation. 2(16.6%) reduced tetrazolium salt, positive for phosphatase activity and produced film/spot.

Molecular characterization**:** Based on PCR analysis, out of 300 samples, 203(67.7%) comprising of 98 nasal swab and 105 pleural fluids were positive for *Mycoplasma mycoides* subspecie *mycoides* DNA with amplicon size of 1.1kbp. *Mycoplasma mycoides* subspecie *mycoides* was identified for the first time in three LGAs of Nasarawa state, Nigeria. Nine of the PCR positive isolates were further amplified using specific primers and the amplicons were submitted for sequencing. Sequences obtained were 96% - 99% identical to 16S rRNA gene of *Mycoplasma mycoides* subspecie *mycoides* strains in the GenBank. Phylogenetic analysis indicated that the local isolates from this study form a two clades and clustered together with other strains in GenBank but differed from the T1/44 vaccine with 42% similarity (Fig 1).

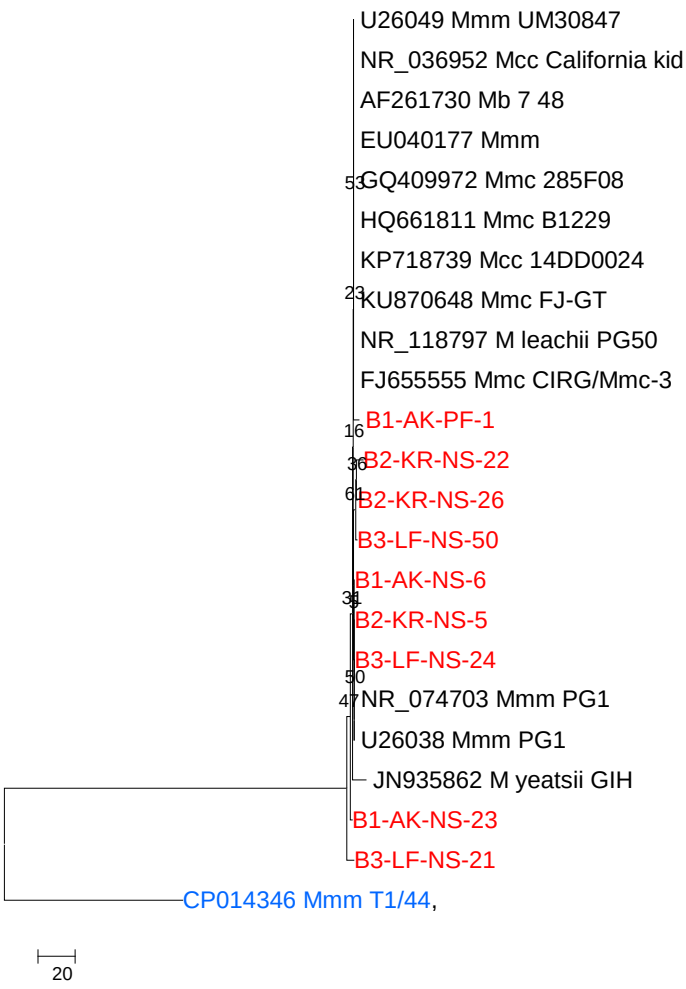

Fig 1. Phylogenetic relationships between 16S rRNA gene of *Mycoplasma mycoides* subspecies *mycoides* (Mmm) detected from nasal swabs and pleural fluid samples of cattle in Nasarawa State, Nigeria. Mmm sequenced in this study are B1-AK-PF1, B2-KR-NS5, B1-AK-NS6, B3-LF-NS21, B2-KR-NS22, B1-AK-NS23, B3-LF-NS24, B2-KR-NS26 and B3-LF-NS50. Phylogenetic analyses were completed with MEGA 6 software that used a neighbor-joining algorithm.

In conclusion *Mycoplasma mycoides* subspecie *mycoides* appears to be endemic in the study area. The nucleotide sequences of the isolates share high homology with strains of Mmm in the Gen Bank, but with evolutionary distance with the vaccine strain T1/44. Therefore, the successful isolation and characterization of local isolates of Mmm has provided an opportunity for the development of an indigenous multivalent vaccine containing the different strains for the control of CBPP in Nasarawa state, Nigeria.

Acknowledgement: I appreciate the advice and guidance of my supervisor Prof M.I Adah. The encouragements received from Director/ Chief Executive, NVRI, Vom Dr. D. Shamaki is acknowledged. I am grateful to all the staff of Mycoplasma laboratory and Biotechnology laboratory, National Veterinary Research Institute, Vom, Nigeria for technical support and to Inquaba biotech Ltd, for supplying the reagents.

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Stability studies and immunizing abilities of vaccines subjected to cold chain failures

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ABSTRACT

Stability of vaccines generally reduces more rapidly with exposure to hot storage temperatures hence they are kept in a cold chain in addition to the incorporation of stabilizers during their manufacture. The potencies of such vaccines also reduce consequently. Live Newcastle disease (Lasota strain) viruses were conventionally freeze dried with peptone and trehalose as stabilizers and subjected to changes/breaks in cold chain (37°C) for 2, 5 and 10 days. Their infectivity (EID_{50}) and haemagglutination (HA) titers were tested thereafter in embryonating eggs and Microtiter plates respectively to determine their stability over the storage duration. The trehalose stabilized ND Lasota vaccines had significantly better stabilizing ability than peptone stabilized vaccines. The immunogenicity of the stabilized vaccines was determined in minimum disease flock white cockerels (in non-controlled environment). The cockerels were vaccinated at 3-weeks old with the differently stored peptone (as positive control) and trehalose stabilized ND vaccines by oral route in drinking water. The antibodies titer was determined in the vaccinated birds using the haemagglutination inhibition test 21 days post vaccination. Trehalose stabilized ND vaccines conferred significantly larger total protective antibody levels than peptone stabilized ND vaccines did on vaccinated chickens even with subjection to changes in cold chain.

Keywords: Stability, Immunogenic, Newcastle Lasota vaccine, temperature, Stabilizer.

INTRODUCTION

Vaccines are formulations that could be liquid or dry and contain live attenuated or killed viruses or bacteria or immunogenic portions of the disease causing organism or attenuated toxins of the organism. They are administered to humans or animals for prophylactic purposes and to control epidemics and pandemics. Veterinary vaccines are administered to animals whether they are companion animals, wild animals or herds of livestock to prevent or reduce occurring problems as a result of exposure to field disease organisms (Lombard, *et al*., 2007; Anebo, *et al*., 2014).

Vaccines lose potency over time and this is rapid under temperatures outside of refrigeration and/or freezer (the cold chain). As such, they require proper storage conditions according to the lability of the immunogens they contain. The rate of loss of potency of vaccines is temperature dependent (Kissmann *et al.*, 2008; Chen and Kristensen, 2009; Knezevic, 2009). Consequently, various drying techniques and inclusion of stabilizing excipients are employed during production; and cold chains are used for storage and transportation to improve stability and shelf life of vaccines and ensure their potency ultimately (Iklizier and Wright, 2002; Rexroad *et al.*, 2002; Chen and Kristensen, 2009; Jaganathan *et al.*, 2010).

Drying techniques used for the purpose of removing bulk water from the vaccines (for dried vaccines) and reducing stresses caused by excessively high or low temperatures (Chen and Kristensen, 2009) include conventional freeze drying (or lyophilization), spray drying, vacuum foam drying, membrane drying, spry-freeze drying, flake drying, supercritical fluid drying, etc. (Rowe, 1960; Meryman, 1960; Rexroad *et al.*, 2002; Chen and Kristensen, 2009).

Stabilizers are excipients added to vaccines in order to help protect them from adverse conditions like the freeze-drying process; elevated temperatures in the course of production, transportation, handling and storage (Kissmann *et al.*, 2008; Knezevic, 2009; Jagannathan *et al.*, 2010). Examples of excipients used as stabilizers in vaccines include amino acids; proteins such as human serum albumin or gelatin; and sugars such as sucrose, trehalose, maltose, fructose, glucose, sorbitol and lactose. They have been used singly or in combination with other excipients to stabilize vaccines (Barbour *et al.*, 2002; Sarkar *et al.*, 2003; Kadam *et al.*, 2005; Corbanie *et al.*, 2007; Farzamfar *et al.*, 2007; Jaganathan *et al.*, 2010; Best, 2010).

The cold chain is a system of transporting and storing vaccines within a recommended temperature range (-20^{0}C to 8^{0}C) and includes all materials, equipment and procedures used to maintain vaccines within the required temperature range from the time of manufacture until vaccines are administered (AGDHA, 2013; Ontario, 2013). There are no guarantees that live vaccines that leave the custody of the manufacturer are transported and stored under optimal conditions which ensure the potency is still high by the time of administration and that vaccine failure will not occur. Peptone stabilized vaccines still require a cold chain for optimal potency. Wambura (2008) used flake dried NDI2 stabilized with trehalose and stored at 25-34ºC to vaccinate chickens and found the vaccines stable for up to 16 weeks and able to confer protective immunity. Corbanie *et al.* (2008) used spray dried ND Lasota vaccines mixed with a combination of trehalose, polyvinylpyrrolidone and bovine serum albumin as stabilizer as coarse and fine powders and found the fine powders administered by inhalation to be more immunogenic than other forms of the vaccine. Okwor et al. (2009) studied the effects of good and poor vaccine storage on their stability and immunogenicity and found that good storage resulted in high antibody titers while the reverse resulted in low stability and low antibody titers.

This study compared conventionally freeze dried vaccines stabilized with peptone with those stabilized with trehalose with respect to stability under different storage temperatures, with breaks in cold chain and the immunogenic abilities (in uncontrolled environment) after subjection to cold chain failures. The aim is to compare the stabilities and immunizing abilities of conventionally freeze dried live Newcastle Disease Lasota vaccines stabilized with peptone and with trehalose under cold chain compliant and non-compliant conditions.

Methodology

The concentrations of trehalose stabilizers were prepared by weighing appropriate quantities of trehalose dihydrate, D-Mannitol and Na_2HPO_4. These were weighed into sterile 200mL media bottles and 100mL of sterile distilled water was added and stirred to form a solution. They were made up to the final volume of 200mL with sterile distilled water to yield the different trehalose stabilizers.

The pH of all stabilizers was 7.4±0.1. They were autoclaved at 121°C for 15 minutes and tested for sterility.

Vaccine production

This was done as indicated in table 1 and dispensed into vials.

Table 1: Composition of stabilized vaccines

Vaccine	Wet Virus (mL)	Vol. of Peptone Stabilizer (mL)	Vol. of Trehalose Stabilizer (mL)	Gelatin (mL)	Antibiotic solution (mL)
B	50	-	44.4	5	0.6
E	50	44.4	-	5	0.6

Freeze Drying (Lyophilization)

The vials were loaded onto pre-cooled shelves of a GT-40 Leybold-Heraeus GMBH Freeze Drying Plant (Germany). Primary drying involved freezing the vaccines to -50°C over 4 hours. Secondary drying involved application of vacuum (negative pressure) to evacuate bulk water from the vaccines during which shelf temperature increased to 30°C while the condenser temperature decreased to -40°C over 20-21 hours. Upon drying of the vaccines, they were automatically and completely stoppered and capped with aluminum caps.

Storage Temperatures

The freeze dried vaccines were grouped according to the type and concentration of stabilizer they contained, stored at 5±2°C for two days and samples taken for infectivity and potency tests then the rest were stored at 37°C for 10 days to simulate the break in cold chain and to determine whether or not trehalose and/or peptone is/are able to keep infectivity titers stable despite a breach in cold chain. Samples of the vaccines kept at 37°C were taken at 2, 5 and 10 days. The vaccines kept at 5±2°C then 37°C for two days each and those stored at 37°C for 5 and 10 days (Table 2.2) were tested for infectivity as well as antibody response in chickens.

Stability Evaluation and Potency Testing

Median (50%) Egg Infective Dose (EID_{50})

The infectivity titers of sampled vaccines were determined as median (50%) embryo infective dose fifty (EID_{50}) using the method of Thayer and Beard (OIE Manual, 2012; Young *et al.*, 2002) and infectivity titers were calculated using the Spearman-Kärber method.

Haemagglutination (HA) Test

The number of virus haemagglutinins in reconstituted vaccines was determined at each sampling of the vaccines in V-bottomed micro titer plates using the method of Thayer and Beard (1998).

Vaccination of Chickens

Day old minimum disease flock (MDF) White Shaver cockerels were obtained in two batches from a commercial poultry farms, Nigeria. Brooding was done under deep litter system and feed and water were given ad-libitum. The cockerels were fed with Hybrid® broiler starter throughout the experiment. They were not given any vaccines except vitamins and antibiotics till they were 3 weeks old when they were each given a single dose of 0.1ml of reconstituted vaccines made up to 20mL (equivalent to 200 doses with 0.1mL equal to a dose) orally. A number of them were bled before vaccination and their pre-vaccination antibody titers were determined. The birds were grouped and vaccinated and bleeding for collection of sera to test for antibody titers was done at 4, 8, 14, 16, and 21 days post vaccination.

Immunizing ability (Antibody Response): Haemagglutination Inhibition (HI) Test

The chickens were bled from the wing vein for sera which were tested using 4 haemagglutinating units(4HAU) of a ND Lasota antigen. Pre-vaccination bleeding was done in order to determine antibody titers of the birds before vaccination (OIE Manual, 2012; Young *et al.*, 2002). The plates were read as the highest dilution that showed complete inhibition of haemagglutination indicated by streaming. Samples with HI titers $\geq 2^4$ were considered protective (ACIAR Monograph, 2002; OIE Manual, 2012).

Statistical Analysis

GraphPad Prism® version 4.03, 2005 and Microsoft Office Excel 2007 were used for statistical analyses. Antibody response results were expressed as standard error of mean ($\pm$ SEM) antibody

titer. Comparison between groups of data was conducted using the student's unpaired t-test. P value <0.05 was considered significant.

Results and Discussion

Stability studies

Table 3: Stability of differently formulated vaccines subjected to failure/break in cold chain during storage.

Vaccine	Storage condition	Stability	
		EID_{50}/dose	HA
E_1	CC	7.9	8
B_1	CC	8.1	8
E_2	BR2	6.9*	8
B_2	BR2	9.1*	8
E_3	BR5	6.7*	7
B_3	BR5	8.7*	7
E_4	BR10	6.3*	8
B_4	BR10	8.3*	8

CC=cold chain (5-8°C) compliant vaccines; BR=break in cold chain (2, 5, 10 days at 37°C); E_0(8.7) and B_0 (8.9)=Peptone and trehalose stabilized vaccines after freeze drying; E and B= Peptone and trehalose stabilized vaccines; n=2; 1,2,3,4=vaccines at 5-8°C for 2 days, 37°C for 2,5 and 10 days respectively. Minimum allowed infectivity titer= 6LogEID_{50}/dose; *=significant p value

EID_{50} of cold chain compliant peptone and trehalose containing vaccines reduced to the same extent (Table 3). Thereafter, with cold chain failures, peptone containing vaccines lost infectivity faster than trehalose containing vaccines.

Among the trehalose containing vaccines, those outside cold chain lost infectivity titers more slowly than the cold chain compliant ones. Thus were more stable at hot temperatures. This was also observed by several authors (Farzamfar, 2007; Corbanie *et al.*, 2007; Corbanie *et al.*, 2008; Wambura, 2009; Alcock *et al.*, 2010). These studies were done with vaccines dried by different techniques. The better stability of trehalose is explained by its glass relaxation dynamics.

HA titers were not significantly different and remained basically the same. This agrees with previous reports (Nssien *et al.*, 2002; Abbas et al., 2003; Abbas *et al.,* 2006).

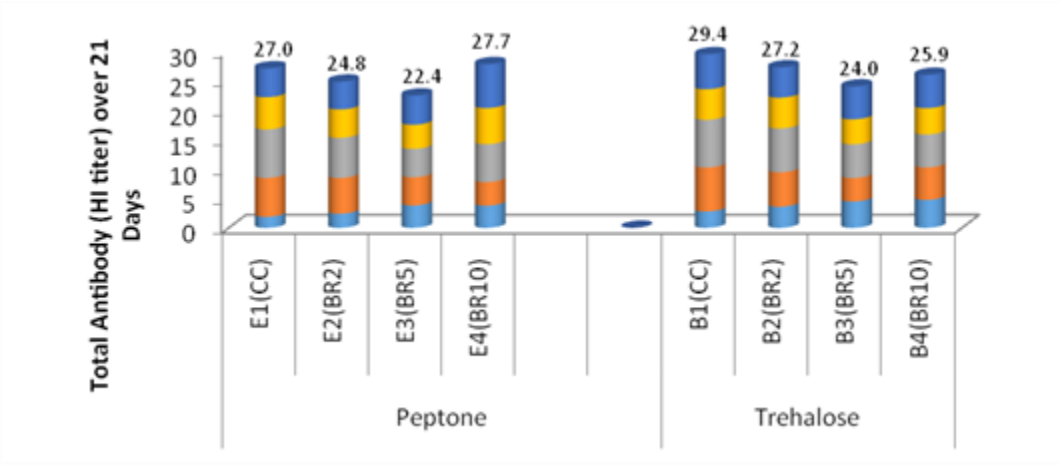

Figure 1: Total level of antibodies (HI Log_2) conferred by the different vaccine formulations before and following break in cold chain over 21 days.

Trehalose containing cold chain compliant vaccines elicited higher immune responses due to larger infectivity titers than peptone containing vaccines (Fig. 1). This behavior is because the magnitude of primary response to an antigen is directly proportional to the concentration of the antigen introduced. Vaccines subjected to failure in cold chain generally had lower HI titers with increasing duration at 37±1ºC but titers protected >80% of vaccinated birds. There was no significant difference in the number of birds protected in all the groups.

Conclusion

Infectivity titers of all vaccines decreased with increased exposure to cold chain failure. This was more rapid with peptone containing vaccines. Trehalose vaccines were significantly more stable. The vaccines conferred protective immunity on vaccinated chickens (70-100%) with trehalose being slightly more immunogenic.

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Knowledge sharing among public health practitioners on zoonotic diseases in Plateau State, Nigeria

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INTRODUCTION

Zoonotic diseases are a major cause of morbidity and mortality which has resulted in the death of over 14 million people across the globe annually (WHO, 2003; Tekki *et al*., 2013). Beside morbidity and mortality, zoonotic diseases have other negative consequences such as restrictions in international trade and travels. Similarly, in countries with limited resources where zoonotic diseases are high, the health systems are usually overstretched (Jaffry et al., 2009; Katare and Kumar, 2010).

To reduce the adverse effects of zoonotic diseases, several studies have been conducted with appropriate recommendations (Kujul *et al.*, 2010; Ehizibolo *et al*., 2011; Aiyedun and Olugasa 2012; Tekki *et al.* 2013). In spite of these studies, the incidence of zoonotic diseases still persists. To advance the control of zoonotic diseases, scholars have stressed the need for collaborative efforts across veterinary, medical and environmental health professionals under the One Health paradigm (Joshi, 2008; Ehizibolo *et al.,* 2011; Karshima, 2012). One-way collaborative effort is made possible is through multidisciplinary knowledge sharing among various health professionals, specifically veterinary, medical and environmental health professionals. This study explores multidisciplinary knowledge sharing among public health professionals in managing zoonotic diseases in Plateau State.

To achieve the objective of this study five research questions were raised to include: What is the perception of public health professionals about knowledge sharing in managing zoonotic diseases? What is the absorptive capacity of health professionals in managing zoonotic diseases?

(To determine the absorptive capacity of public health professionals in managing zoonotic diseases; the following sub question was asked: How do Public health professionals acquire external knowledge in managing zoonotic disease?). What factors motivate public health professionals to share knowledge in managing zoonotic diseases? What factors limit knowledge sharing of public health professionals in managing zoonotic diseases? How do the constructs of social exchange theory explain the perception of public health professionals about knowledge sharing in managing zoonotic disease? Social Exchange Theory provided the theoretical framework for the study.

Methodology

The study adopted a qualitative research method. Choosing a qualitative approach is appropriate when emphasis is on describing, understanding, and explaining complex phenomena (Yin, 2004). The study was conducted in a natural setting as it attempts to gain an in-depth understanding of the perception of public health professionals about multidisciplinary knowledge sharing in managing zoonotic diseases. This study adopted semi- structured interview and focus group discussion (FGD) for data collection.

Data analysis

Data in the form of phrases and sentences were collected through semi- structured interview from the participants of this study. The interviews were recorded using a tape recorder. The audio recordings were then transcribed. All transcripts of the interviews were analyses using qualitative content analysis.

Findings revealed positive perception about knowledge sharing among public health professionals in managing zoonotic diseases as follows; effective management, knowledge gaps exist, conform to the notion of “One Health Initiative”, save humans lives. Also, the study found that; public health professionals acquire external knowledge in managing zoonotic disease through multidisciplinary networks and professional associations; attending seminars, workshops, symposia and conferences; consulting books, journals, and internet databases and during work routine and processes. Findings also revealed factors that motivate public health professionals to share knowledge in managing zoonotic diseases in Plateau state to include:

Forum to share knowledge; adequate funding; professional ethics; good policy guidelines; and organizational support. Similarly, factors that limit knowledge sharing among public health professionals in managing zoonotic diseases in Plateau state are: professional dichotomy; negative traits; mono-disciplinary training; lack of policy; lack of funds; and leadership issues. The study found that there is a convergence between the two constructs of social exchange theory "reciprocity" and "trust" and the perception of public health professionals about knowledge sharing in managing zoonotic diseases.

Discussion

The perception of public health professional about knowledge sharing is positive. This is rooted in the desire of public health professionals to reduce the negative impact of these diseases in humans in terms of morbidity and mortality. There is no doubt that the positive perception of public health professionals about multidisciplinary knowledge sharing will facilitate the absorption of external knowledge. Acquiring knowledge from other public health professionals will fill the knowledge gaps that exist and also increase the efficiency in managing of zoonotic diseases. The knowledge gaps that exist in managing zoonotic diseases occurs between what health professionals know about zoonotic diseases within the context of their professional training and what they need to know about zoonotic diseases from other public health professionals. Therefore, acquiring knowledge from other public health professionals to fill this gap is about incorporating knowledge which a professional lack but is identified to be critically important for the advancement of the professional and the profession (Kristensen *et al*., 2016).

The approach to multidisciplinary knowledge sharing will be positive when factors that motivate public health professionals to share knowledge are adequately in place (Dessie, 2017). Factors limiting multidisciplinary knowledge sharing constitutes major setback which has led to increase in human and economic losses (Lee and Hong, 2014).

Recommendation

Stakeholders should take advantage of the positive perception of public health professionals by strengthening the capacities in the human and animal health sectors and also create the

mechanism necessary to share knowledge among public health professionals in order to respond to emerging health threats of zoonotic diseases efficiently.

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Implementing the ISO 9001: 2015 Quality Management System (QMS) Standard in NVRI VOM

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INTRODUCTION

The ISO 9001: 2015 QMS standard is just one out of the many standards published by the International Organization of Standards (ISO) headquartered in Geneva, Switzerland. The ISO is an international non-governmental standard-setting body that is composed of representatives from various national standards organizations founded on 23 February 1947 with 164 countries as members. The organization tends to promotes worldwide proprietary, industrial and commercial standards. It has published over 22599 International Standards and issued over 1 million certificates worldwide on ISO 9000 with the ISO 9001: 2015 being the most popular world standard as at 2019.

ISO 9001: 2015 QMS is the most recent of the ISO 9000 series of QMS and is designed to provide all types of organizations (businesses, schools, government and its parastatals and the private sectors etc.) with the tools necessary to improve performance and continually meet customer satisfaction.

The ISO 9001: 2015 Standard- Quality Management System- Requirements

This is a 40-page document (standard) that was last updated in 2015 and provides stepwise instructions and guidelines to organizations on how to implement and sustain a QMS that will continually ensure customer satisfaction and improvements in the quality of goods and services. This ultimately leads to ISO 9001 certification issued to such attaining body by the standards organization body in the concerned country.

The ISO 9001: 2015 certification is a written guarantee, showcasing that an organization is qualified, committed and capable in the continual production of quality goods and services to meet (and even exceed) customers' requirements at all times. The attributes of the standard are that it is voluntary, market (customer) driven, consensus and worldwide. Consequently, the instructions in it can be summed up as; say what you do, do what you say, write it down (records) and check it and act appropriately.

All the requirements of ISO 9001:2015 are generic and are intended to be applicable to any organization, regardless of its type or size, or the products and services it provides. This standard is based on a number of quality management principles including a strong customer focus, the motivation and implication of top management, the process approach and continual improvement.

Principles of Quality Management

QM is hinged on the following core principles for all prospecting organizations.

1. **Customer Focus:** Understand the needs of existing and future customers and align organizational objectives in tandem with customer needs and expectations. Meet customer requirements. In addition, measure customer satisfaction while managing customer relationships and striving to exceed customer expectations.
2. **Leadership:** Establish a vision and direction for the organization and set challenging goals by modelling organizational values while establishing trust. Equip and empower employees and recognize employee contributions.
3. **Engagement of People:** Ensure that people's abilities are used and valued. Make people accountable for their actions as well as inactions. Enable and encourage participation in continual improvement and also evaluate individual performance. Enable learning and knowledge sharing as well as open discussion of problems and constraints.
4. **Process Approach:** Manage activities as processes while measuring the capability of activities. Ensure you identify linkages between activities. Prioritize improvement opportunities. And always deploy resources effectively.

5. **Improvement:** Improve organizational performance and capabilities and align improvement activities. Empower people to make improvements while ensuring you measure such and other improvements consistently. Celebrate improvements as this has been shown to lead to further improvements.

6. **Evidence-based decision making:** Ensure the accessibility of accurate and reliable data. Only use appropriate methods to analyze data and make decisions based on analysis. Also balance data analysis with practical experience and record all findings accordingly.

7. **Relationship Management:** Identify and select suppliers to manage costs, optimize resources, and create value. Establish relationships considering both the short and long term effects. Share expertise, resources, information, and plans with partners while collaborating on improvement and development activities. Recognize supplier successes.

Benefits of ISO 9001: 2015 Certification: these include but not limited to:

- Increased revenue
- Increased efficiency
- Boost employee morale
- International recognition
- Factual approach to decision making
- Good supplier relationship
- Accurate, timely and reliable documentation
- Consistency
- Customer satisfaction
- Improvement in processes

Conclusion and Recommendations

Despite not being compulsory, many organizations choose not to transact businesses with firms that are not ISO 9001: 2015 certified. This consideration justifies so a win-win situation for both parties in meeting maximal customer satisfaction and subsequently profit. Considering the benefits afore-listed, it is pertinent that the National Veterinary Research Institute, Vom should

embark upon and obtain this all important certification. A feat that can be achieved based on recent audit reports submitted to management and the level of interest and commitment shown by the management and staff of the Institute.

Acknowledgements

The authors wish to recognize the immense contributions of the management committee of the Institute in sponsoring both the technical and non-technical trainings of the designated staff from all Divisions in the Institute and the provision of materials needed by the Quality Control Division towards certification the ISO 9001: 2015 by the Standards Organization of Nigeria (SON). The authors also wish to thank all heads of division and their respective staff especially the Quality Managers and Document Controllers for the zeal and enthusiasm so far displayed towards achieving this very important task document.

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Strategies for generating high growth reassortant (HGR) Influenza Virus for the production of candidate vaccine virus (CVV)

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INTRODUCTION

Influenza viruses are negative sense single stranded RNA virus and their genome composed of eight segments that encodes eleven proteins. It is the segmented structure of the influenza genome that allows for the exchange of the individual gene segments between influenza viruses; a process known as REASSORTMENT (Cox and Subbarao, 2000). The classical method of HGR generation takes advantage of the process of this reassortment to produce high yielding influenza viruses which is used for vaccine production. Reassortment has been used to investigate functions of viral proteins and to generate candidate vaccine viruses for the production of influenza vaccines for decades (Othmar, 2012).

Seasonal flu vaccines are inactivated trivalent vaccines which contain antigen from H1N1, H3N2 (i.e. the A strains) and an influenza B strain. The changing character of influenza viruses makes manufacture of influenza vaccine exceptionally challenging (Minor, 2010). Due to antigenic drift and shift, the influenza viruses present in the vaccine may no longer be protective against the current circulating strain (Fulvini *et al*., 2011). Therefore, there is always the need to change the composition of flu vaccine annually for both the northern and southern hemisphere. It is recommended that a quadrivalent vaccine containing another influenza B strain should be used during the 2019-2020 northern and southern hemisphere flu season (GISAID, 2019).

HGRs are faster growing, higher yielding influenza viruses. They are viruses produced by mixture of genes from the wild-type (wt) and the high yielding laboratory 'donor' strain having the surface

heamagglutinin (HA) and neuraminidase (NA) genes from the wt parent as well as the core or internal (backbone) genes from the donor or lab-adapted parent; PR8 or IVR-145 (Baez *et al*., 1980). The genes of the reassortants can range in composition, from the highly undesirable 0:8 (wt) to 8:0 and anything in between. Usually a gene constellation of 6:2 (donor: wt) is preferred for making good HGRs for CVV production but this isn't always possible via classical reassortment, nor is it always the most suitable composition for reassortant growth (Fulvini *et al*., 2011). Thus this presentation described the steps of generating HGRs by classical reassortment method.

METHODOLOGY

There are two methods of producing HGR virus:

1. Classical reassortment method

This is a method of HGR production where genes from viruses of different parental backgrounds are exchanged naturally in a living system such as embryonated egg as illustrated below.

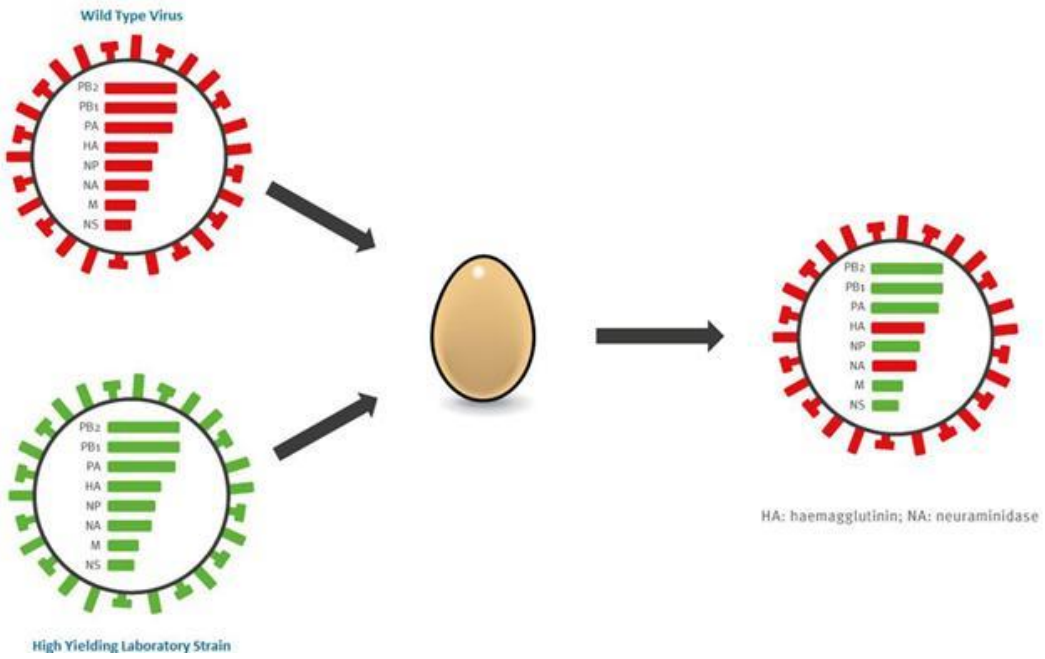


Fig 1: Classical reassortment of wild-type and laboratory adapted influenza virus in egg

2. Reverse genetics method

This is important for viruses that do not adapt and grow well in embryonated eggs as well as for highly pathogenic influenza viruses such as H5N1 and H7N9 (Nicolson *et al*., 2005; Dong *et al*., 2009; Minor *et al*., 2009). This method allows for cloning and mixing the genes as desired using cell culture based system for virus rescue as illustrated (Neumann and Kawaoka, 2002; Nicolson *et al*., 2005, Robertson and Engelhardt, 2010).

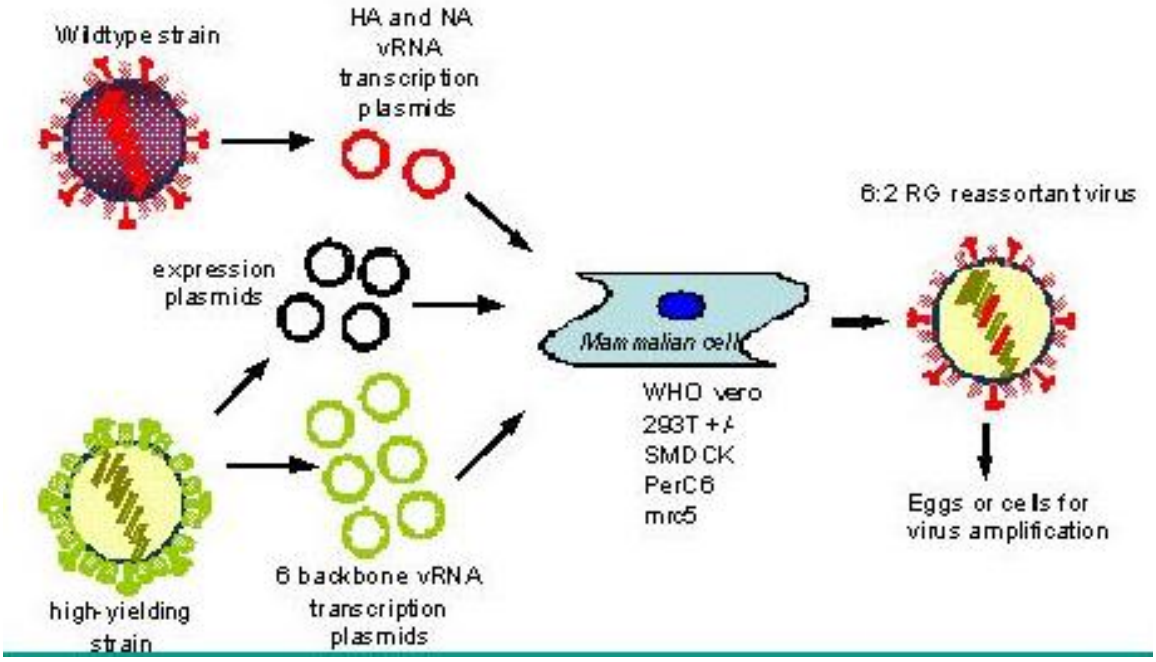


Fig 2: Generation of HGRs by reverse genetics approach using 12 plasmid systems

In classical re-assortment method, there are 2 ways of HGRs characterization. One is by conventional method using multiplex RT-PCR coupled with enzyme restriction analysis (restriction fragment length polymorphism- RFLP), and the other is by a newly developed Real-Time qPCR method (developed and validated at National Institute for Biological Standards and Controls, UK).

A) Characterization by conventional multiplex RT-PCR and RFLP:

Three HGRs were produced by the conventional method of Fulvini *et al*. (2011) and were re-analyzed and characterized by a more and highly sensitive, quicker and cheaper method of real-time qPCR.

The 3 HGRs are from H3N2 viruses of the following strains:

A/Sri Lanka/61/2015 (which do not grow very well in eggs)

A/Hong Kong/50/2016

A/South Australia/135/2016

Steps: The steps below illustrate the classical reassortment method (Fulvini *et al*., 2011). The different co-infection of embryonated eggs with both wild-type and lab-adapted (donor) parent viruses at different dilutions as shown on table 1.

Table 1. Co-infection of eggs with wild-type and donor parent viruses

Embryonated eggs (11-day old)		Wild type (e.g. A/Sri Lanka/61/2015)		Donor (e.g. IVR-145)	
		10^{-1}	**10^{-2}**	**10^{-4}**	**10^{-5}**
1	2	-	100µl	-	100µl
3	4	-	100µl	100µl	
5	6	100µl	-	-	100µl
7	8	100µl	-	100µl	

i. Eggs were candled then followed by co-infection of viruses (wt and lab parents) as shown in table 1.

ii. Incubation of infected eggs at 37^{O}C for 72 hours

iii. Chilling of eggs for 20 minutes at -20^{O}C and harvesting the allantoic fluid (ALF) for heamagglutinin (HA) test (using 0.7% guinea pig RBC containing 0.1% BSA). Then selection of group(s) of eggs with high HA titre (at least 2 groups) which are then carried forward.

iv. Treatment of ALF with antisera, i.e. 1st antisera passage (using anti-HA and anti-NA antisera against Solomon Island). There are 2 types of antisera for each gene which are used at 50µl each with 50µl ALF and 250µl of PBSA, then incubated for 1 hour at room temperature.

v. A 10-fold serial dilution of the virus-anisera mixture using PBSA is prepared and then re-infected another sets of embryonated eggs in duplicate each with viral dilution 10^{-3}, 10^{-4} and 10^{-5} (making total of 12 eggs for 2 groups).

vi. Incubation of eggs at 37°C for 72 hours, ALF was harvested thereafter and tested for HA titre.

vii. Selection of group(s) with high titre and carried forward for 2nd antisera passage treatment as in step iv and continued to step vi.

viii. Random selection of representative of medium-to-high titres (as many as possible) from the groups for viral RNA isolation from the respective ALF and synthesis of cDNA according to the standard methods, for PCR analysis of HGR for HA and NA genes using diagnostic primer (di-plex) sets.

ix. 1st Limiting dilution stage: Selected good candidate with clean HA & NA from wt and good HA titre and made 10-fold dilution of the virus mixture. Dilutions 10^{-4} to 10^{-9} were inoculated separately in SPF eggs in triplicate (total of 18 eggs) and incubated at 35°C for 72 hrs. Eggs were chilled and HA test was performed on ALF as described above.

x. 2nd Limiting dilution stage: At least 2 groups with high or good titre (and preferably at highest dilution) were selected and a 10-fold dilution of each was made. Dilutions 10^{-6} to 10^{-10} were inoculated in SPF eggs in triplicate (total 15 eggs). Eggs were chilled and HA test was performed on ALF as earlier described.

xi. Random selection of representative of medium-to-high titres from the groups for viral RNA isolation from the respective ALF and synthesis of cDNA according to the

standard methods, for PCR analysis of HGR for all internal genes (i.e. PB2, PB1, PA, NP, MP & NS) using their respective non-diagnostic primers for characterization.

xii. Enzyme restriction analysis (RFLP) was performed on all the internal genes of selected HGRs using appropriate restriction enzymes according to standard protocol.

xiii. HGRs with desired gene constellation for CVV were selected and carried forward for further analysis of 2-way HI test (antigenicity testing) and full length sequencing of HA & NA and using AlignIR software for sequence analysis. Correct HGRs were then sent to Crick Lab for further confirmation of sequence analysis.

xiv. HGR candidate that passed all the tests were inoculated into ferret for antiserum production and collection.

xv. Lyophilization of HGR candidate which was assigned NIB reference number and kept at -76^{o}C until when needed by vaccine manufacturers.

B) Characterization by probe/dye-based real-time qPCR

This second method was necessitated due to few drawbacks with conventional method such as longer time of analysis and ambiguity in interpretation of the results of some HGRs. It is quicker, robust, more precise and above all cheaper method.

Optimization and validation of probe/dye-based assay

i. Primers and probes were designed by Locked Nucleic Acid (LNA) or Allelic Discrimination assay method: This was used for only one gene (PB2)

ii. Primers and dye/probe designed by SYBR green/Taqman assay: Here we tested and validated the primers and probe sets for all the 8 genes designed by our collaborator; the New York Medical Centre (NYMC).

RESULTS AND DISCUSSION

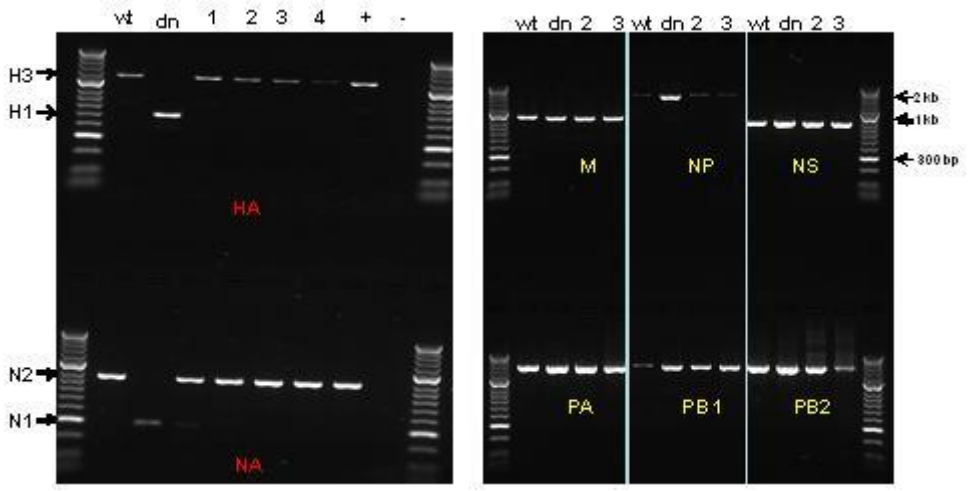
Haemagglutination (HA) assay

Table 2: Result of HI test showing high titre in all the eggs but egg 2 & 8 were carried forward.

Eggs	1	2	3	4	5	6	7	8
HA titres	1024	1024	1024	1024	1024	1024	1024	2048

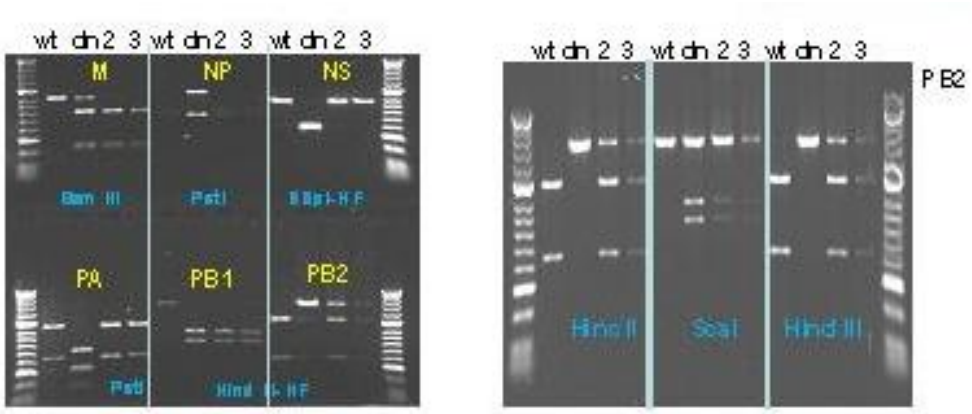
A). Conventional PCR/RFLP method

i) A/Sri Lanka/61/2015



A B

Fig 3. Characterization by conventional method (RT-PCR/RFLP) for A/Sri Lanka/61/2015 Virus reassortant. Diagnostic (di-plex) RT-PCR analysis of HA & NA gene segment (A) as well as analysis of internal genes (B) of HGRs from H3N2 reassortant. Keys; wt = wild-type, dn = donor, 1-4 = HGRs, + (positive control), - (negative control)



A B

Fig 4: Characterization by conventional method (RT-PCR/RFLP) for A/Sri Lanka/61/2015 Virus reassortant. Enzyme restriction analysis (RFLP) of internal genes of HGRs for M, NP, NS, PA, PB1 & PB2 using BamHI, PstI, Hind-HF (A) and for PB2 gene using Hinc II, Sca I and Hind III (B). Keys; wt = wild-type, dn = donor, 2 & 3 = HGRs.

Table 3: Gene constellation of HGRs 2 & 3 by conventional method for A/Sri Lanka/61/2015 Virus reassortant.

HGR	PB2	PB1	PA	NA	NP	HA	MP	NS	Total
2	wt	dn	wt	wt	wt	wt	dn	wt	**2:6**
3	wt	dn	wt	wt	wt	wt	dn	wt	**2:6**

Note that both HGRs showed total gene constellation of 2:6 for donor and wild-type respectively. This may be considered for CVV since it contained HA & NA wild-types and at least 2 internal genes.

ii) A/Hong Kong/50/2016

Table 4: Gene constellation of HGRs 1, 2, 3 & 4 by Conventional method for A/Hong Kong/50/2016 reassortant.

HGR	PB2	PB1	PA	NA	NP	HA	MP	NS	Total
1	wt	wt	wt	wt	wt	wt	wt	wt	0:8
2	dn	dn	dn	wt	dn	wt	dn	dn	**6:2**
3	wt	wt	wt	wt	wt	wt	wt	wt	0:8
4	dn	dn	dn	wt	dn	wt	dn	dn	**6:2**

Note that only HGRs 2 & 4 contained good gene constellations for donor and wild-type (6:2) and are considered as better candidate for CVV. HGRs 1 & 2 have poor gene constellation because they did not contain any internal genes.

B). Real-Time qPCR method:

Result of optimization and validation of probe based assays (LNA) gave good sensitivity and specificity of the primer and probe to wild-type (wt) and donor (dn) viruses (Fig 5). The qPCR method gives a clear gene constellation of PB2 gene of the HGRs 2 & 3 (Fig 6A) compared to ambiguous result by conventional method (Fig 6B) probably due to incomplete enzyme digestion.

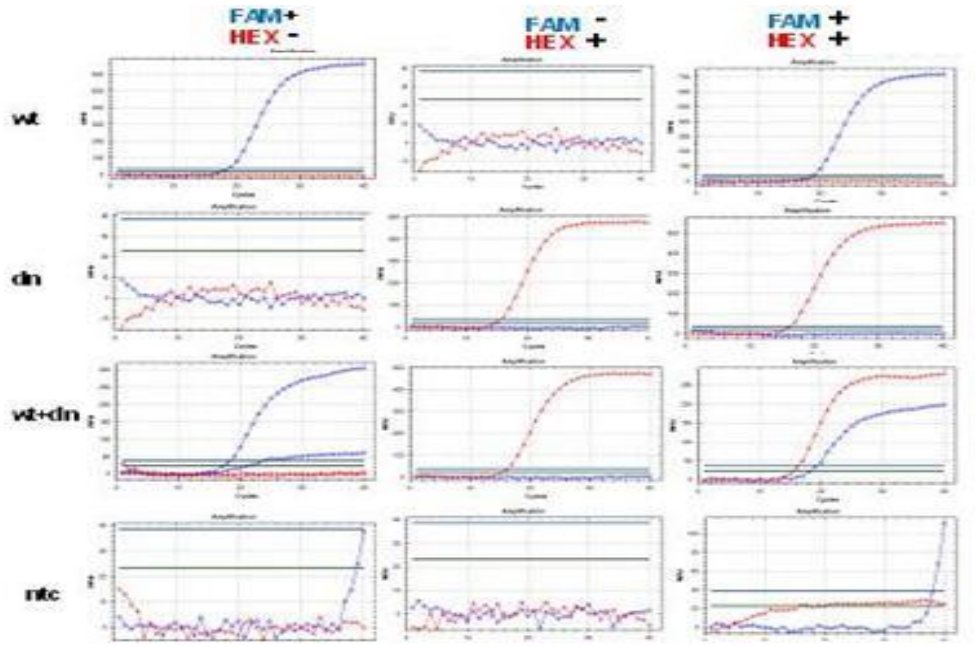


Fig 5: Assay validation by qPCR method with HGRs of A/Sri Lanka (wt) and IVR-145 (donor) labelled with FAM & HEX probe respectively. Keys; wt = wild-type, dn = donor, ntc = non-template control.

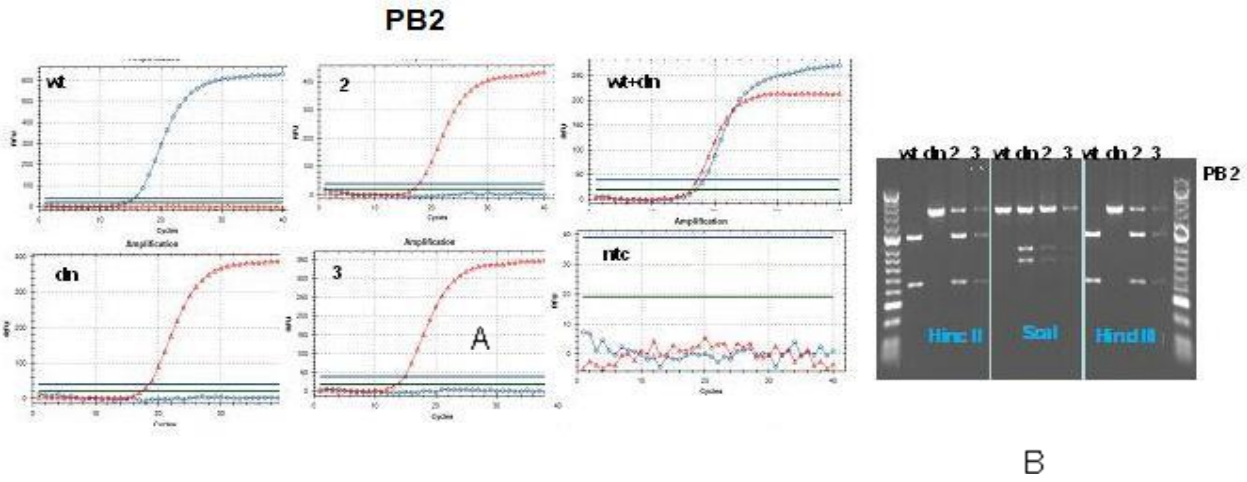


Fig 6: Comparison of analysis of HGRs from A/Sri Lanka wt and IVR-145 donor by qPCR and conventional method. Keys; wt = wild-type, dn = donor, 2 & 3 = HGRs, ntc = non-template control.

Table 5: Result of optimization and validation of NYMC primer-probe (Taqman) assays for A/Hong Kong/50/2016 reassortant.

	HA	NA	PB2	PB1	PA	NP	MP	NS
H3N2	yes	yes	yes	yes	yes	yes	no	no
IVR-145	no	no	yes	no	yes	yes	no	no

Note only 9 out of 16 primer-probe sets designed by NYMC are good for our assay after validation.

Table 6: Result of optimization and validation of primer-dye (SYBR Green) assays for A/Hong Kong/50/2016 reassortant.

	HA	NA	PB2	PB1	PA	NP	MP	NS
H3N2	-	-	-	-	-	-	yes	yes
IVR-145	yes	yes	-	yes	-	-	yes	yes

The 7 other remaining primer-dye sets designed by our group passed the validation test

Table 7: Validation of combination of primer-probe/dye assay on 7 Clades of H3N2 viruses (3c.2a, 3c.3a & 3c.3b).

Clades	PB2	PB1	PA	NA	NP	HA	MP	NS
A/Sri Lanka/2015 (3c.2a)	+	+	+	+	+	+	+	+
A/Hong Kong/16 & 14 (,,)	+	+	+	+	+	+	+	+
A/New Caledonia/14 (,,)	+	+	+	+	+	+	+	+
A/Victoria/2014 (,,)	+	+	+	+	+	+	+	+
A/Norway/2014 (,,)	+	+	+	+	+	+	+	+
A/Stockholm/14 (3c.3a)	+	+	+	+	+	+	+	+
A/Newcastle/14 (3c.3b)	+	+	+	+	+	+	+	+

Note that all the 7 gave positive detection to H3N2 for each gene and negative detection to IVR-145 primer-probe-dye set, signifying specificity of H3N2 primer-probe/dye design in detecting H3N2 clades of viruses.

iii. Real-Time qPCR Analysis of HGRs co-infection of A/South Australia and IVR-145 virus

Table 8: HA and NA analysis at 2nd and 1st antisera treatment (HGR 1 was discarded while 2 was carried over)

	HGR	H3	H1	N2	N1	
2nd Antisera passage	1	-	+	-	+	Failed
	2	**+**	**-**	**+**	**-**	Passed
1st Antisera passage	1	-	+	-	+	Failed
	2	**+**	**+**	**+**	**+**	Mixed pop

Table 9: 2nd Limiting Dilution showing analysis of internal genes and HA & NA with acceptable gene constellations for HGR 2.

HGR	PB2	PB1	PA	NA	NP	HA	MP	NS	
2	dn	dn	dn	wt	dn	wt	dn	dn	**6:2**

C. Benefits of different qPCR primer-probe/dye designs

Table 10: The advantages and cost-benefit ratio of HGRs analysis by qPCR method against conventional method.

Criteria	LNA primer-probe	NYMC primer-probe (Taqman)	Primer-dye (SYBR)
Cost per gene without supermix	£155 + £40 design charge by the company	£104.2 with some	£3.6
Ease of reaction per gene per HGRs	Very simple with only 1 pair of primer and 2 distinct probes in a reaction tube	Complex with 2 pairs of primers and 2 distinct probes in 2 separate reaction tubes	Less complex with 2 pairs of primers and a similar dye in 2 separate reaction tubes
Virus detection in one tube reaction	Assay can easily detects both parents (wt & dn) in one tube reaction	Assay fails to detect both parents in one tube reaction (can only pick 1 parent)	Assay fails to detect both parents in one tube reaction (cannot distinguish btw parents)
Result (outcome)	Good	Good	Good
Supermix cost	£51 per 1ml vial (Biorad)	£51 per 1ml vial (Biorad)	£66 per 1ml vial Biorad)
Total cost	£246	£155	£70

CONCLUSION

Overall, the qPCR assay has advantage over conventional PCR for HGR characterization in that it is very sensitive and can pick lowest concentration of mixed population or contamination of HA (H1 & H3) from the start which may be missed by conventional method and can only be detected at a later sequencing stage (as witnessed during analysis of A/Hong Kong/61/2016 HGRs). Real-Time PCR method is cheaper and robust in clearly elucidating gene constellation of HGRs.

In conclusion, the production of influenza vaccines requires careful coordination of a highly complex process involving a wide range of technical expertise in both public health laboratories and vaccine companies in order to provide on time safe and effective vaccines.

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Zoonoses and the Next Pandemic

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BACKGROUND

In 1918, a pandemic was caused by influenza A virus subtype H1N1 that infected about 500 million people and killed over 50 million of them. Till date, that pandemic is still referred to as the mother of all pandemics (Taubenberger, 2006). Three other pandemics were reported in 1957/58, 1968/69 and 1977 (Beveridge, 1991) and the first pandemic of the twenty-first century was also caused by Influenza A/H1N1, which began in Mexico in April 2009 (Meseko *et al*., 2013). Historically, most pandemics are caused by zoonotic pathogens that are easily transmitted. Influenza viruses possess these traits and since 1996, influenza H5Nx arising from waterfowls and poultry has been transmitted to human (Zoonoses). The avian influenza virus subtype H5N1 was the harbinger of a pandemic until influenza H1N1 earlier referred to as swine flu emerged in 2009. The virus was a product of gene reassortments amongst avian, swine and human influenza viruses that circulated in pigs before it emerged as a pandemic in human.

Methods

In previous studies, influenza H5N1 clade 2.2 was first described in Nigeria from 2006 and the country witnessed resurgence of H5N1 clade 2.3.2.1c in 2015 (Joannis *et al.,* 2006; Meseko *et al*., 2007; Monne *et al*., 2015; Shittu *et al*., 2016). Till date H5N8 clade 2.3.4.4 is still being detected in the country particularly from Live bird markets (OIE, 2019). Risk factors for the introduction, spread and evolution of zoonotic avian influenza in Nigeria include: movement of migratory birds, trade in Live birds and co-mingling of multiple animal species. In another study, we described inter-species transmission of H5N1 from avian to swine hosts. In the investigation, we tested 129 tracheal swabs by conventional and Real-time PCR assays. Multiplex and duplex realtime PCR and clade specific RT-PCR assay for influenza HA and NA genes. Sequencing of the HA fragment by

Sanger method and BLAST in GenBank was done. Sera were also screened for flu A nucleoprotein and H5 specific using IDvet ELISA kit. Selected positive sera were further tested by Heamaglutination Inhibition (HI).

Results

The results obtained showed that forty-three of 129 (33%) tracheal swab specimens from pigs were positive for influenza A virus (M and NP genes). Of the thirty positive samples that were tested in multiplex RT-qPCR assays specific for HA and NA subtypes of European porcine influenza viruses, all were negative in the HA test (i.e. H1pdm-, H1av-, H1hu- and H3), but 26 were positive for N1. Out of the 23 samples tested by the H5N1 duplex assay, 22 were positive in the H5 reaction and two in the N1 part. In addition, those 22 samples could be determined as belonging to the H5N1 clade 2.3.2.1c by clade-specific RT-qPCR. Query BLAST of 5 sequences that were generated via conventional RT-PCR and Sanger sequencing of an HAII fragment (about 650 nucleotides) returned closely related viruses. These include A/Chicken/Nigeria/15VIR399-1/2015, other poultry sequences from Nigeria and from other African countries in 2015 as well as from Asian countries (all H5N1) with 99% sequence homology. As determined with the clade-specific RT-qPCR and by sequence analysis, the swine H5N1 virus genomes detected in this study clustered together and along avian H5N1 clade 2.3.2.1c. NA gene sequencing attempts repeatedly failed even with different primer sets including conventional panNA RT-PCR.

Serological tests on five hundred sera showed 222 (44.4%) were positive by IDvet NP-ELISA. Among the 300 investigated serum samples from 2016, 183 (61%) reacted positive. Of those, 42 (14%) showed positivity in the IDvet H5 ELISA. All 200 serum samples from 2013 were negative in the H5-ELISA though 39 (19.5%) were NP positive. HI test against antigen for HPAI H5N1 clade 2.3.2.1.c confirmed H5 antibodies with HI titres ranging from 160 to 1280 in selected 6 of the 42 H5-ELISA positive sera from year 2016. Sera reacted also against LPAI H5N3 virus confirming that the specific component of the sera must be antibodies against H5. No cross reactivity was obtained with H5 HPAI viruses of other clades, i.e. 2.3.4.4b (H5N8) or 2.2 (H5N1). Fourteen NP-positive /H5 negative samples that were tested against swine-specific influenza virus antigens

showed positive reaction predominantly for A/H1N1pdm09 with HI titres ranging from 320 to1280 (Table1).

Table 1: Sero-reactivity of swine influenza strains H1av, N1av, H1huN2, H1N1pdm and H3N2 on selected NP-positive sera.

Antigens		Field sera ID (titre)													
Subtype	**Virus strain**	**353**	**186**	**30**	**24**	**100**	**202**	**15**	**56**	**286**	**72**	**48**	**129**	**204**	**293**
H1avN1av	A/sw/Germany/R1738/2011	80	40	160	20	80	40	20	20	160	40	160	160	40	40
H1huN2	A/sw/Germany/R2107/2010	40	20	640	40	80	10	20	80	40	40	80	20	20	20
H1N1pdm	A/sw/Germany/R26/2011	1280	1280	640	320	320	320	320	320	1280	640	1280	640	640	640
H3N2	A/sw/Germany/R96/2011	10	10	80	10	10	20	10	10	10	20	10	10	10	10

Discussion and recommendation

This investigation revealed interspecies transmission of H5N1 and endemic H1N1 in traditional mixing vessel (pig) in Nigeria (Meseko *et al*., 2018); a recipe for reassortment and emergence of novel and zoonotic influenza virus with pandemic potential. Interestingly, Nigeria is also considered as a global hotspot for emerging diseases with a huge population of human and animals and less than optimum biosecurity. The risk of disease emergence from reservoir hosts and zoonotic transmission to human and human to human transmission across many countries is what makes a pandemic. Persistent circulation and mutations of influenza virus (antigenic shift) and co-mingling of multi species leading to reassortments (antigenic drift) are indicators of emerging virus with pandemic potential. The next flu pandemic according to WHO is a matter of ***'when', not 'if***. (http://www.emro.who.int/pandemic-epidemic-diseases/news/the-next-flu-pandemic-a-matter-of-when-not-if.html). Importantly, Influenza has a bigger impact than many other infectious diseases like Lassa, Monkeypox or Ebola because apart from its pathogenicity

and transmissibility, Influenza virus is also an airborne pathogen. Pandemic influenza preparedness and response may avert or reduce the magnitude of future catastrophe.

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Leptospirosis: Reemerging Zoonosis

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INTRODUCTION

Leptospirosis (LEP-toe-sp-ROW-sis) is the most widespread zoonotic disease in the world found in almost all species of mammals (Bezerra *et al*., 2011). Zoonotic disease of public health importance listed by WHO as "neglected" with great impact on animal and human health as well as socio-economic impact in Developing countries (Cheema *et al*., 2007). The disease is caused by Spirochaetes of the genus *Leptospira*. They are thin, highly mobile, slow-growing obligate aerobic bacteria with an optimal growth temperature of 28-30^{0}C, measuring 6-20µm long and 0.1 – 0.15µm in diameter, and can be distinguished from other spirochetes on the basis of their unique hook or question mark- shape ends (Li *et al*., 2000).

The genus *Leptospira* (Greeklepto, meaning fine or thin and Latin spiral, coil) is classified serologically into two species, the pathogenic species *Leptospira interrogans* and the saprophytic species *Leptospira biflexa* however the genus has recently been reorganized and pathogenic leptospires are now identified in 17 named species and four genomospecies of *Leptospira* (Morey *et al.,* 2006), with more than 250 serovars of *L. interrogans* arranged in 23 serogroups (Vijayachari *et al.,* 2004). While *Leptospira biflexa* are more than 60 serovars (Kmety, 1993).

The other names for leptospirosis are weil's disease, rice field fever, cane cutter's disease, swineherd's disease, dairy farm fever, mud fever, which reflect transmission conditions (James *et al.,* 2006).

Risk groups of Leptospirosis infection

Leptospirosis recognized as an important occupational hazard of agriculture manual laborers, sewage workers, animal handlers, forestry workers and other outdoor workers who work in wet conditions, butchers, veterinarians and veterinary public health workers *(*Collares-Pereira *et al.,*

2000). Indirect occupational exposure occurs with many sewer workers, miners, soldiers, septic tank cleaners, fish farmers, and game keepers (Levett, 2001).

Many cases of leptospirosis in humans come about from activities of daily life, such as walking barefoot in damp conditions or by drinking contaminated water or contact with animals and infected animal product (Levett, 2001). Stray dogs have been classified as a significant reservoir for human infection (Weekes *et al*., 1997). Another obstacle developing countries face is the spread of contaminated water during flooding. Many studies have documented instances where numbers of leptospirosis infected individuals increased soon after flooding (Levett, 2001).

Chronic carriers of leptospirosis are usually wild or domestic animals, such as rats, dogs, cattle, pig, Sheep and goat. (Adler *et al*., 2010). The hosts (infected animals) can be classified into definitive or reservoir (maintenance) hosts, to which the persistence of the enzootic cycle is attributed, or into accidental hosts, with no impact on the dissemination of this zoonosis (Levett, 2001). In rural and urban ecosystems, synanthropic rodents play the role of main reservoirs of this disease since they house *Leptospira* in the kidneys, eliminating them alive through the urine to the environment, which contaminates the water, soil and foods. Among domestic rodents (*Rattus norvegicus, Rattus rattus* and *Mus musculus*), *R. norvegicus* is noteworthy because it is a classic carrier of Icterohaemorrhagiae, the serovar most pathogenic to humans (Adler *et al*., 2010). Rats are the first recognized carriers of Leptospires and often incriminated as the source of infection to humans. Other rodents such as bandicoots, mouse, mongoose, shrews and the aquatic rodent coypu of France are the source of infection in different ecological niches. Several domestic animal species can harbor leptospires and act as source of infection to humans and cattle (Bezerra *et al*., 2011). Leptospiral seropositivity has been observed in many wild animal species including opossums and sea lions (Bezerra *et al*., 2011).

Pathogenicity

Any serovar of L. interrogans can cause mild or severe illness depending on the virulence and concentration of the infecting serovar and the immune response of the infected person. They colonize the kidneys of infected animals and, in females; they also colonize the reproductive tract at birth or abortion, thus contaminating the animals' environment (Faine *et al*., 1999). The

Pathogenic Leptospira colonize the kidneys of infected animals reside in the proximal renal tubules of the kidneys of carriers and are subsequently excreted in urine. Urine tainted with leptospires can then contaminate soils, surface water, streams, and rivers (Adler *et al*., 2010). Leptospirosis usually results from contact with the urine of infected animal (Faine *et al*., 1999).

Transmission

Exposure may occur through either direct contact with an infected animal or through indirect contact via soil, food or water contaminated with urine, blood and tissue of an infected animal. Portal of entry include cuts and abrasions mucous membrane of the eyes, nose and mouth, by bathing or accidental immersion in the fresh water, lakes or rivers or canals contaminated with the urine of the infected livestock. Transmission in livestock is via grazing on fields contaminated by the urine of infected rats and other mammals and drinking water from an infected puddle, muddy river-banks, ditches, gullies with regular passage of either wild or farm mammals (Ahmad et *al.,* 2005; Vijayachari *et al.,* 2008).

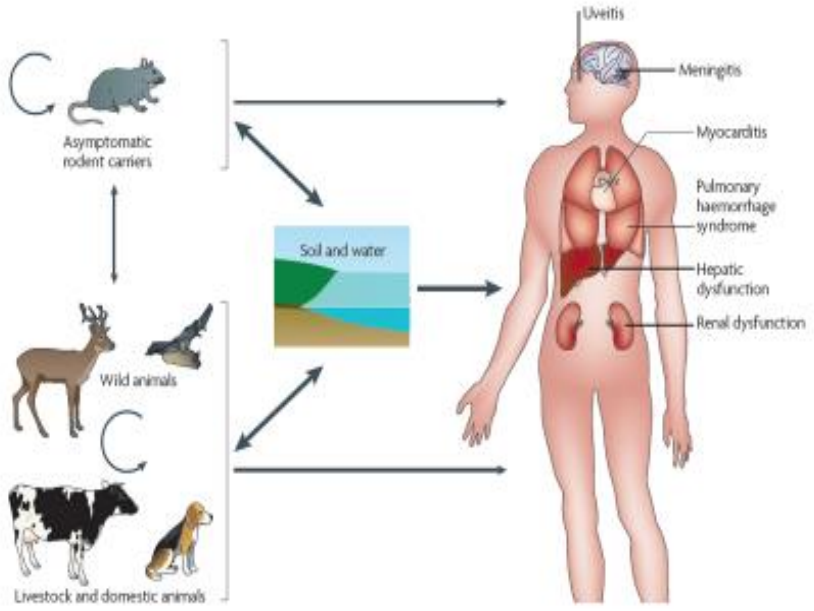

Plate 3: Transmission Cycle of Leptospirosis.

The transmission cycle involves interaction between one or more animal hosts harboring leptospires, with environment favorable for its survival. It is prevalent in both developed and developing countries; however, the disease is primarily associated with poor communities that lack proper sanitation facilities and flood-prone regions (Fraga *et al.,* 2011). The symptoms of leptospirosis in human appear after 4-14 days incubation period. The incubation period in animals is from 2 to 20 days.

Clinical Signs

Leptospira infection in humans causes a wide range of symptoms and some infected persons may or may not have symptoms. Leptospirosis is a biphasic disease that begins with flu-like symptoms such as high fever, chills, myalgia, intense headache, anorexia, conjunctivitis, muscle pain, abdominal pain and constipation (Plank & Dean, 2000). Complications in humans include meningitis, azotemia, extreme fatigue, hearing loss, and respiratory distress, liver failure, cardiovascular problems and renal interstitial tubular necrosis results in renal failure.

Symptoms in animals include fever, Jaundice and haemoglobinuria, which may result in death. Complications in Livestock include abortions, stillbirths, infertility, reduced milk production, 'red water' disease and death. (Langoni *et al*., 2008).

Diagnostics: Laboratory diagnosis is difficult to obtain; it is only performed in the event of strongly suspected leptospirosis (on a blood sample):
Early diagnosis: Occurs between days 0 and 7 using PCR
Presumptive diagnosis: After 7- 10 days: microscopic agglutination test (MAT); IgM ELISA test provides and culture (bacteria grow slowly, specific culture medium).
Other investigations: Complete blood count: possible polymorphonuclear leukocytosis, thrombocytopenia or anaemia.
Urinalysis: proteinuria, leukocyturia, possible microscopic haematuria.

Treatment: The drug of choice is Penicillin, Tetracycline, Streptomycin and Erythromycin

Prevention

Rodent control

Vaccination of domestic animals

Avoid bathing in endemic areas.

The use of protection clothing

In Nigeria Leptospirosis has been reported in livestock and humans from several parts of the country (Dialo, 1979; Eze *et al.,* 1990; Agunloye, 2002; Ngbede *et al.,* 2012; Abiayi *et al*., 2012).

The current concerns

- In Nigeria, a national surveillance programs for leptospirosis does not exist despite the substantial global burden of disease because of limited information available on both human and animal Lepospirosis.
- The number of reviews and updates on both human and animal leptospirosis is insufficient compare to the developed countries. This disparity may be as result of under reporting and lack of integration between the diseases reporting system in animal and human.
- To promote research and control of leptospirosis in Nigeria, review information on human and animal leptospirosis in these countries are urgently needed.
- Leptospirosis can cause severe economic loss in dairy farms leading to outbreaks of mastitis with a significant decrease in milk production, decreased calving percentage due to abortions and high death rate.
- There is a further cost if a farmer, a family member or a farm worker is infected with the disease. The considerable time spent off work and the medical expenses incurred during recovery from leptospirosis add to the economic losses in animal production.
- Decision makers at local, national and international levels rely on data on morbidity and mortality to make decisions about budgetary allocation.
- When data are lacking diseases cannot be prioritized

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Using NGS and 'omics' approaches to accelerate understanding of tropical livestock immunogenetics

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The MHC plays a key role in determining the peptides that are presented to T-cells and therefore plays a critical function in dictating the epitope-specificity of T-cell responses. Thus understanding MHC is pivotal in the rational development of novel subunit vaccines that aim to elicit protective T-cell mediated immunity. A key feature of the MHC is a high level of genetic diversity (polymorphism), which is predominantly focused on the exons that encode the protein domains that form the peptide binding groove; a consequence of this diversity is variation between different MHC molecules in the 'motifs' of peptides that they are able to bind and present.

At present our knowledge of the repertoire of MHC alleles present in cattle is very limited, with intensive analysis largely restricted to breeds that are of high economic value in Europe and North America, such as the Holstein-Friesian. A primary factor in this knowledge gap has been that previous techniques to sequence MHC genes have been expensive and laborious and so constrained large scale studies in other breeds of cattle. At the moment only 5 MHCI alleles from African cattle breeds have be submitted to the official IPD-MHC database.

We have recently developed a high-throughput next-generation-sequencing approach to analysing cattle MHCI and MHCII diversity and have begun applying this to studies of cattle breeds from across the globe including animals from Uganda, Zambia, Brazil, Pakistan, Ghana, Italy, Cameroon and Nigeria. We have sampled >5000 animals and have identified many new MHCI and MHCII alleles. In tandem with this we have, in collaboration with colleagues from the University of Oxford (UK) and University of Buenos Aires (Argentina), begun to use mass spectrometry analysis of MHC-eluted peptides to characterise the peptide repertoires presented

by different cattle MHC molecules (immune-peptidomics). Using this combined NGS and 'omics' approach we have started to rapidly expand our knowledge of bovine MHC diversity and how this determines the presentation of peptides to T-cells in this species; in future studies the functional consequences of this, how it influences T-cell mediated immunity will be studied and exploited to accelerate vaccine development.

References

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