THE SAFETY AND SERO-CONVERSION OF A MIXED *PESTE DES PETITS RUMINANTS* AND SHEEP AND GOAT POX VACCINE IN SHEEP AND GOATS IN KWALE COUNTY, KENYA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR MASTERS DEGREE OF UNIVERSITY OF NAIROBI [APPLIED MICROBIOLOGY (BACTERIOLOGY OPTION)].

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MAY 2023

DECLARATION

Declaration by the student

This thesis is my original work and has not been presented for the award of a degree in any other University or institution of higher learning

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TITLE OF THE WORK: SAFETY AND SERO-CONVERSION OF A MIXED *PESTE DES PETITS RUMINANTS* AND SHEEP AND GOAT POX VACCINE IN SHEEP AND GOATS IN KWALE COUNTY, KENYA

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DEDICATION

This Thesis is dedicated to my loving wife Rael Ndisi Wambua, my daughter Rinnah Luvuno, my son Robinson Ndegwa, my wonderful mother Grace Kanze Karisa and the Rains of Harvest Church ministries' Holy family Kinango, for their price less support that made this work a great success.

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LIST OF ABBREVIATIONS AND ACRONYMS

CVL – Central veterinary laboratories

LSD – Lumpy skin disease

PPR – *Peste des petits ruminants*

SGP – Sheep and goat pox

HS – Hemorrhagic septicemia

BQ – Black quarter

CBPP – Contagious bovine pleuropneumonia

CCPP – Contagious caprine pleuropneumonia

GP – Goat Pox

SP – Sheep Pox

ASAL – Arid and semi-arid lands

PPRV – *Peste des petits ruminants* virus

OIE – World organization for animal health

ELISA – Enzyme linked immunosorbent assays

VNT – Virus neutralization test

CPV – Capripox virus

RT – Reverse Transcriptase

PCR – Polymerase chain reactions

DNA – Deoxyribonucleic acid

GALVmed – Global alliance for livestock veterinary medicine

MCI – Moroccan animal health company

LYOPOX - Freeze dried pellet live attenuated sheep pox virus

PPRTM - Live attenuated pestes des petitis ruminants virus

KEVEVAPI – Kenya veterinary vaccine production institute

FAWC – Farm animal welfare

WQP – Welfare quality project

RM - Residual moisture

KSGP - Kenya sheep and goat pox

PNPP - para-Nitrophenylphosphate

CFT – Complement fixation test

Ab – Antibody

Ag – Antigen

NGO – Non-governmental organization(s)

ANOVA – Analysis of variance

ODs – Optic densities

NC – Negative control

PC – positive control

USA – United states of America

SHP – Sheep

GT – Goat

S – Sample

N – Negative

S/N% - Competition inhibition

S/P% – Percentage inhibition

D – Diluent

SPP – Sheep and goat pox and peste de petits ruminants mixed vaccine

FMD – Foot and mouth vaccine

RVF – Rift valley fever

AD – Anno domini

DTP – Tetanus and Pertusis vaccine

ILRI – International livestock research institute

WOSU – Washington state University

UN-FAO – Food and Agricultural Organization of the United Nations

GEP – Global eradication plan

WFF – World food forum

ABSTRACT

Kwale County is endowed with livestock wealth owing to her vast rangelands in the tropical climate and with areas that are not suitable for crop production, hence farmers solely relying on livestock livelihoods. The County has a population of about 377,047 sheep and goats from the previous livestock census conducted in 2019: (2019 census report) that are distributed across ranches and various households. Therefore, a disease affecting sheep and goats is a huge threat to most Kwale inhabitants whose income is dependent on the small stock. *Peste des Petits Ruminants* is an upcoming disease in Kwale County as per previous laboratory investigations conducted in Mwavumbo Ward Kinango sub-county in Kwale (Client Ref. No. 0112/2020) by the Central veterinary investigation laboratories (CVL) in Kabete, Kenya. Those findings confirmed the disease as emerging among affected sheep and goats across various households that were losing high numbers of the small ruminants. The current study was carried out in Kinango Sub-County, Kwale County in Kenya, to determine the immunogenicity of *Peste des Petits Ruminants* (PPR) and Sheep and Goat Pox (SGP) vaccine mixture in both sheep and goats, under normal field conditions. This was geared towards reducing pain and vaccine administration expenses, for cost effective disease control. A total of 47 sheep and 45 goats were first screened for PPR and SGP antibodies, to rule out previously vaccinated animals. The animals were also screened for any suspected underlying conditions associated with the disease and whether they had previous history of infections involving the two diseases. All of them were found to be negative for both PPR and SGP antibodies; also all the parameters monitored, based on body temperatures, were found to be within the normal range. However, only 41 sheep and 42 goats which were sero-negative (naïve) were recruited for the research because some of the animals were younger (less than three months) hence were eliminated from the study. The target animals were dewormed and

confirmed to be worm free before start of the experiment. The *Peste des Petits Ruminants* and Sheep and Goats Pox vaccines used were prepared using Nigerian 75/1 strain and Kenyan Sheep and Goat Pox strains KSGP 0240 respectively, from Kenya Veterinary Vaccine Production Institute (KEVEVAPI); both of which were live attenuated vaccines. The two vaccines were reconstituted separately, adhering to manufacturer's guidelines and vaccination on the herd done 14 days post deworming and 3 days after confirming the herd to be worm free respectively. Vaccine combination was undertaken during vaccination by drawing respective doses of each vaccine type using separate needles into one syringe, forming the desired mixed vaccine under test. The vaccine mixture was then injected as a single bolus to segregated groups of sheep and goats and animals' body temperatures monitored daily for a period of 14 days, after which blood samples for serum were collected from the jugular vein. The serum samples were analyzed using competitive ELISA and Double Antigen ELISA tests for *PPR* and SGP antibodies respectively. This trial was carried out alongside positive controls where *PPR* and SGP vaccines were administered separately and respective immune responses monitored (to establish their respective immunogenicity), and negative control, where diluent (normal saline) was used. In general vaccination of both sheep and goats, using the mixed vaccine, conferred immunity to both *PPR* and SGP at 100%; the immunity being detected fourteen days post vaccination. The study showed that, there were no reactions or interactions when *PPR* and SGP vaccines were mixed and that the immune system could detect individual vaccine components from the mixed vaccine to generate immunity against each without interference. On the same breath, there were no significant differences in immunity generation between groups given the PPR-SGP bivalent mixed vaccine and those groups given monovalent PPR/SGP vaccine, confirming that; no interactions existed when the two vaccines were mixed and injected as a single shot. It was noted, from the manufacturer,

that the PPR and SGP vaccines were prepared in powder form, to be reconstituted just prior to vaccination by dissolving in specified diluent, and that the two vaccines shared the same diluent along with other vaccine types such as Lumpy skin Disease (LSD) vaccine. During mixing of the two experimental vaccines, it was observed that the resultant mixture was homogenous and it could be injected easily into the animal bodies, and there were no reactions noted at the injection sites. On assessment of the animals' body temperatures, the vaccinated ones did not show deviation from the normal, even though some of them were in their third trimester of pregnancy; some of them gave birth to healthy kids and lambs without complications. This study therefore, confirmed that PPR and SGP vaccines could be formulated into one vaccine mixture and administered as a bivalent vaccine without affecting their efficacy. This reduces vaccination cost, promotes animal welfare, through reduction of number of injections per vaccine, and enhances economic gains. The findings of this study will serve as eye openers to manufacturers and all concerned parties on possibilities of reducing vaccine costs for effective disease control and safeguarding small ruminants' livelihoods. It will, thus, help in the PPR and SGP eradication efforts and improve food security in Emerging Countries.

CHAPTER ONE: INTRODUCTION

Disease control through vaccination is a critical tool employed in sheep and goats' health management for the various disease infections that they are predisposed to. In many cases, it is a common scenario for various vaccines to be administered concurrently in animals to offer immunity against the prevailing endemic diseases and also against any anticipated disease epidemics, based on advisories from governments and other stakeholders (Alex Mabirizi *e.t al*., 2022). So far there is no product in Kenyan market that contains a combination of *Peste des Petits Ruminants* (PPR) and Sheep and Goat Pox (SGP) vaccines. Despite government and donor funded vaccination programs against PPR and SGP in small ruminants, the diseases have persisted and continued to cause losses in the Arid and semi-arid areas of Kenya due to poor farmers' cooperation (Kihu *et al*., 2012).

1.1 Background and Problem statement

From my personal experience in the field, working in Kenya and in South Sudan where livestock is a mainstay for most of the poor communities living in rural areas, veterinarians have encountered challenges while implementing Food security and Livelihoods programs through provision of veterinary services in rehabilitation and protection of livestock resources. While livestock keepers accept vaccination of their livestock against various diseases, they do not like seeing their animals getting multiple injections on various body sites concurrently. This was a major drawback towards animal vaccinations as most farmers disapproved it like in scenarios where; cattle were immunized against; Hemorrhagic Septicemia (HS), Anthrax, Black Quarter (BQ) and Contagious Bovine Pleuropneumonia (CBPP); goats were vaccinated against; Contagious Caprine Pleuropneumonia (CCPP), Goat Pox (GP) and *Peste des Petits Ruminants* (PPR) and Sheep were vaccinated against; Sheep Pox (SP) and *Peste des Petits Ruminanats*. The *Peste des petits ruminants* disease is reported

in over seventy Countries and World-wide the approximated population of sheep and goats is 2.5 billion benefitting over 300million families. Given the high morbidity rate (100%) and mortality rates (80%) associated with PPR disease outbreak, the estimated economic losses are at US$ 2.1 Billion (Felix Njeumi *et. al*., 2015). On the other hand losses that are attributed to sheep and goat pox outbreaks globally results from mortalities, devaluation of meat and skin as well as loss in domestic and international markets. These losses are is approximately US$ 48 Million (Yune N, Abdela N 2017). In Kenya, the losses attributed to PPR disease outbreak are approximated at US$ 19.1 Million annually, caused by morbidity and mortalities (88%) and production and market (12%) losses (Kihu *et. al*., 2015).

1.2 Justification

Vaccination as an approach to disease control in livestock should endeavor to cover all animals or at least 75-90% of the population (Yune N, Abdela N 2017). To realize herd immunity and effective disease control, all animals including those crossing borders should be vaccinated as well (ElArbi *et. al*., 2019). Since the farmers have sometimes cited suffering of their animals that were vaccinated against several diseases, emanating from numerous injection inflictions during immunizations, there is need for efficient mechanisms that improve livestock keepers' cooperation and trust in vaccine delivery regime. The time and cost spent by both the veterinarians and the farmers can be prohibitive especially in arid areas where travels of over 100 km are common. There are many challenges in communicating to farmers in order to get them to bring their animals in those arid areas for vaccinations. Therefore it is in every one's interest to pilot multi-valent vaccines to control numerous diseases with one injection rather than single dose vaccines. There are economic cost benefits for in using multiple dose vaccines compared to monovalent vaccines that have to be administered by several injection or injections given in different days apart. The concurrent

administration of PPR and SGP vaccine has been proven to reduce vaccination costs by up to 70% (Alex Mabirizi *e.t al*., 2022). This research was aimed at establishing a possibility of mixing two vaccines [Sheep and Goat Pox (SGP) and *Peste des Petits Ruminants* (PPR)] without affecting their potency, and reducing vaccination costs and animal suffering as previously shown in Ethiopia (Ayalet *et al*. 2012). However, this study was carried out in actual uninterrupted field environment, unlike previous studies that had been conducted in controlled field and laboratory settings.

1.3 Objectives

1.3.1 General Objective

To determine whether mixing *Peste des petits* ruminants and sheep and goat pox vaccines will affect their respective sero-conversion:

1.3.2 Specific Objectives

1. To assess the clinical body safety when a mixed vaccine comprising *Pestes des Petits Ruminants* and Sheep and Goat Pox vaccine strains was used:
2. To determine and compare respective sero-conversions when the two vaccines were administered together and separately:

1.4 Hypothesis

There is no significant difference, with respect to sero-conversion (humoral immune responses), whether the two vaccines are administered together, as mixed vaccine, or separately.

CHAPTER TWO: LITERATURE REVIEW

2.1 Role of Sheep and Goats vaccination in alleviating poverty

Most emerging countries are usually characterized by high poverty index that is greatly contributed by food insecurity among the poor populations who are the majority in those regions. Kenya as a member of the middle income countries is adversely affected by poverty owing to a large portion of her geographic area being under Arid and Semi-Arid lands (ASAL) climatic weather conditions. Most communities residing in ASAL areas depend on livestock livelihoods, since crop farming performs poorly. These community's cultural practices are livestock oriented, giving livestock great values.

Livestock populations in the Kenyan ASAL are greatly comprised of sheep (14,354,925) and goats (25,250,865) compared to cattle (12,155,974), as shown by the 2019 Census report. This is because sheep and goats are well adapted to environmental conditions of those regions (Bergevoet and Engelen, 2014) where they contribute to resilience building of the communities. Thus, major strides made in enhancing food security in the ASALs: are apparently through sheep and goat production (Monteiro *et al*., 2017). However, disease outbreaks are biggest drawbacks that have resulted in loss of livestock livelihoods, increasing food insecurity and vulnerabilities of the local communities (Balamurugan *et al*., 2014). Some of the fatal diseases of sheep and goats include; *Pestes des Petits Ruminants* (PPR), Sheep and Goat Pox (SGP) and Contagious Caprine Pleuropneumonia (CCPP). The two diseases, PPR and SGP, affect both sheep and goats (Coetzer et. al., 2004; Boshra *et. al*., 2015), while CCPP only affects goats (Parray *et. al*., 2019).

The PPR and SGP diseases are however, preventable by vaccinations that offer immunity against active endemic infections or anticipated future epidemics. Vaccination is therefore a vital component in livestock rearing; it should be undertaken with seriousness for the

protection of these livelihoods and rehabilitation to build resilience. Sheep and goat species are very prolific with high growth rates; a factor which makes their turnover rates very high. This necessitates frequent vaccinations against PPR and SGP among other diseases of economic and public health significance. It has also necessitated administration of various kinds of vaccine for the different diseases of interest at the same time, to enable the body mount effective immune response against the infections. Herd restructuring in sheep and goats through introduction of superior genes are only going to be effective under proper health management that ensures survival of superior breeds and their offspring. Vaccination is part and parcel of any successful production and reproduction livestock program aiming at enhancing food security and reducing poverty. There are over 300 million families relying on small ruminants livestock rearing distributed in over 70 countries Worldwide and the approximated sheep and goats population is 2.5billion. Hence, vaccination should target at least 2.1 billion (80%) animals for effectiveness (Felix Njeumi *et. al*., 2015).

2.2 PPR and SGP infectious diseases in sheep and goats and their control

2.2.1 Peste des Petits Ruminants (PPR)

2.2.1.1 General information

PPR disease, also known as goat plague, is an acute, fatal, small ruminant disease that is highly contagious with high morbidity rates of up to 100%. The disease is caused by *Peste des Petits Ruminants* Virus (PPRV) which belongs to the family Paramyxoviridae and *Morbilivirus* genus. This PPRV has four lineages that have been identified from affected animals and across the World; Lineage I-IV (Dhar *et al*., 2002). PPR disease is endemic in Sub-Saharan Africa, India, Pakistan and Middle East where disease outbreaks occur with the onset of heavy rainfall. The disease is more severe with high mortality rates in goats

compared to sheep (Ayalet *et. al*., 2012). It is a notifiable disease on the list of the World Organization for Animal Health (WOAH formerly referred to as OIE).

2.2.1.2 Pathogenesis of PPR

The *Peste des Petits Ruminants* virus (PPRV) has an incubation period of six days post infection. The major point of entry is the nasopharynx, where aerosols containing the infectious virus are inhaled. The primary multiplication of the PPRV occurs in the pharyngeal and mandibular lymph nodes after which viraemia occurs and virus spreads to other lymphoid tissues, the respiratory and digestive tract mucosae (Parida *et. al*., 2015). During disease development, in acute phase, affected animals develop fever, depression and finally anorexia. The disease causes formation of erosive lesions in the mucosa of the digestive and respiratory tracts and affected animals shed the virus in all their secretions and excretions. This virus has high affinity for leucocytes; they attack and destroy leucocytic cells resulting in leucopenia and low body immunity that predisposes affected animals further to secondary infections. Affected animals later develop bronchopneumonia which is a result of secondary bacterial infections mostly due to *Pasteurella* or *Mannheimia* pathogens in the respiratory system. This microorganism is a normal microbiota and takes advantage of immunosuppressed animals (Markey *et. al*., 2013). In some scenarios of severe infection, pregnant animals abort. *Peste des Petits Ruminants* is fatal and mortalities are usually reported within ten days post infection and death rates could be as high as 70-80%. Goats are usually worst affected by PPR while sheep tend to develop sub-acute form with fever, mucosal erosions, intermittent diarrhea and nasal catarrh (Markey *et al*., 2013).

2.2.1.3 Laboratory Diagnosis of PPR

Peste des Petits Ruminants disease is best diagnosed during the acute phase of the pathogenesis and the appropriate samples include; ocular and nasal swabs, scrapings of buccal

and rectal mucosa and blood are critical in this viraemic stage. Other useful samples are tissues from the spleen, lungs, mesenteric and bronchial lymph nodes and tracheal and intestinal mucosa which are collected at post mortem (Markey *et al*., 2013). There are rapid antigen detection techniques available such as the enzyme linked immunosorbent assays (ELISA) (Libeau *et al*., 1994), Agar gel Immunoelectrophoresis and Haemagglutination assay (Wosu, 1991) for detection of *PPR* viral elements from the samples. Detection of antibodies in serum can be done using competitive ELISA or via Virus neutralization (Libeau *et al*., 1995). There are specific primers are available for use in Reverse Transcriptase Polymerase Chain Reactions (RT-PCR) for detection of virus.

2.2.1.4 Control of PPR

There are two major control strategies recommended for PPR disease: (1) Test and slaughter methods in countries that are free from PPR where policies exist for compensation of affected farmers and (2) quarantine and mass vaccination in regions where the disease is endemic and compensation is impractical, like Kenya and in other emerging countries. In order to break the epidemic of PPR virus, vaccinations should at least target 70-80% of the sheep and goat populations (Singh R.P, 2011).

2.2.2 Sheeppox and Goatpox [Sheep and Goat pox (SGP)]

2.2.2.1 General information

Sheeppox and Goatpox are diseases caused by two distinct viruses referred to as sheeppox and goatpox viruses respectively and the two viruses belong to the family Poxviridae and genus *Capripoxvirus*. The diseases caused by sheeppox and goatpox viruses are similar in characteristics and there is sufficient evidence that co-infections and cross infections occur between sheep and goats (Gershon *et al*., 1989). Capripox virus strains are known to cause

severe illnesses in both sheep and goats even though the two are genetically distinct (Tulman *et al*., 2002). The diseases are endemic in Africa, India and Middle East countries.

2.2.2.2 Pathogenesis of SGP

The viruses usually replicate primarily on the skin or lungs depending on entry route and later spread to the rest of the body through regional lymph nodes where successive viral replication progresses. In disease development, the clinical signs and pathognomonic skin lesions appear after about a week post infection with macules as initial lesions that expand into papules that later become necrotic and ulcerate. The necrotized papules after some time form scabs that may eventually fall off leaving depressed scars (Markey *et al*., 2013).

The viruses sometimes invade the digestive and respiratory systems and cause internal lesions in the tongue, esophagus, rumen, abomasum, large intestines, tracheal mucosa and hemorrhages in the lungs. When lesions appear in the mouth and rest of digestive system, they lead to anorexia, negative metabolic energy balance and weight loss. On the other hand, lesions in the respiratory tract result in respiratory distress and other health complications of affected animals that result in mortalities especially in young immune-compromised animals.

2.2.2.3 Laboratory diagnosis of SGP

There are various laboratory diagnostic approaches for confirmation of SGP which include; histology of acute skin lesions which demonstrate large cellular infiltration, vasiculitis, edema and presence of eosinophilic intracellular inclusion bodies. When using electron microscope, the morphology of *Capripoxviruses* can be differentiated from *Parapoxviruses*. The Sheep and Goat Pox virus can be cultivated using lamb testes and kidney cell cultures, where the virus gives characteristic cytopathic effects (inclusion bodies).

Detection of the highly antigenic P32 *Capripoxvirus* structural protein is used in antigen trapping ELISA, whereas PCR uses various *Capripoxvirus* specific primers to detect viral

DNA in tissue biopsies and cultures. Serological tests can also be used to detect antibodies to *Capripoxviruses*. These include; Neutralization test, Indirect Fluorescent antibody test, (Chand *et al*., 1994) and Indirect ELISA (Carn *et al*., 1994, Heine *et al*., 1999).

2.2.2.4 Control of Sheeppox and Goatpox diseases in sheep and goats

For endemic areas, the best control strategy is annual vaccination of sheep and goats. The *Capripoxvirus* shares major neutralization sites that help to generate and mount strong protection against all strains of *capripoxviruses* prevalent in the field. In Kenya, there are several modified live vaccines available on the markets; such as: the Kenyan sheeppox strain useful in sheep and goats, the Mysore strain for goats and Romanial strain for sheep. There are also sub-unit vaccines available and useful for the control of sheeppox and goatpox infections (Carn *et al*., 1994).

2.3 Combined PPR/SGP vaccine successes

There have been successful attempts by the Global Alliance for Livestock Veterinary Medicine (GALVmed) to produce a combined vaccine for PPR and SGP in Morocco through the Moroccan animal health company (MCI). The MCI is the only known World-wide current manufacturer of combined PPR and SGP vaccine that is registered in Morocco. This combined vaccine was successfully tried and proven in Karamoja, Uganda; a PPR hot spot and the good impacts of the vaccine in controlling the diseases was appreciated by the livestock keepers that benefited from the trial (Ayebazibwe, 2022).

The combined PPR/SGP vaccine from the MCI, referred to as LYOPOX-PPRTM, is currently available in Morocco, pending authorization and marketing to other countries (International development Research center). The PPR/SGP bivalent vaccine was proven to be effective both in sheep and goats in Morocco (Fakri *et. al*., 2015). Kenya on the other hand has the two vaccines against PPR and SGP available in separate formulations; Pestevax® and S&G vax®

[Kenya veterinary vaccines production institute (KEVEVAPI)]. There have been no attempts in Kenya to manufacture a combined vaccine encompassing PPR and SGP; something this project aimed to stimulate and join Morocco on the same journey. The project was undertaken in the natural usual environment where the goats and sheep are found to enable assessment of the efficacy of the combined vaccine when other environmental factors are at play concurrently, as it usually happens with natural disease outbreaks.

2.4 Livestock keepers' resistance against vaccinations

There is a common trend among livestock keepers living in rural areas among vulnerable communities to resist vaccinations due to the adverse reactions always associated with employed methods and resultant animal reactions. These communities are much emotionally attached to their animals; their resource banks and livelihood sources; hence repel any vaccinations linked to trauma and negative effects. These repulsions hinder the coverage of the desired livestock percentages (80%) during vaccination (Personal observation, 2021).

During massive vaccination campaigns, most of the livestock keepers prefer their animals to be given single injections; thus posing a great challenge in scenarios where several vaccines are scheduled to be given at the same time, administered multiple separate jabs. For sheep and goats, there are no current vaccine combinations for any two or three of the following diseases; PPR, SGP and CCPP. Therefore, in the absence of combined (bivalent, trivalent, tetravalent etc), it makes it mandatory to administer three injections for goats (PPR, SGP and CCPP) and two injections for sheep (PPR and SGP) during annual immunization programs. In a previous study conducted in Narok County in Kenya, it was found that some farmers resisted multiple vaccinations undertaken on different time frames on their animals citing adverse reactions and inconveniences. The resisting farmers (25.5%), requested for vaccine combinations across all the livestock vaccines (S.W Kairu *et al*., 2013).

2.5 Animal welfare and freedoms, and livestock keepers' concerns

Animal welfare is defined through broad philosophical-based approaches depending on understanding of various people in a society; it balances culture, use and value of animals. There are, therefore, various elements emphasized on animal welfare by different communities and societies. The first emphasis is on physical health in relation to traumatic injuries and biological functions of the animal (Fraser, 2008). Animals are expected to be well treated to avert pain, hunger and distress and be provided with conducive, environment to express normal behavior.

Some vaccination protocols sometimes infringe into animals' rights and freedoms by causing traumatic injuries and pain, something that the communities and animal welfare crusaders are against. It has always been advised that health service delivery to animals be friendly and also convenient for the owner of the animal. The owners play crucial role as guardian to the animal owing to the mutual benefits between them.

In some instances, where four vaccines are administered in cattle, against diseases such as; Anthrax, Black Quarter (BQ), Haemorrhagic Septicemia (HS) and Contagious Bovine Pleuropneumonia (CBPP) in the annual vaccination disease control program, the animals are usually subjected to four injections at the same time, in various parts of the body. For sheep (PPR and SGP) and goats (PPR, SGP and CCPP), the animals have to endure two and three injections respectively for vaccinations against the respective diseases. These numerous injections on animals cause distress, leading to stampede and inflammatory reactions on the various injection sites; something that, by extension, causes distress to the livestock owners.

There are five animal rights and freedoms that, when adhered to, helps mitigate against pain, distress on animals and promotes growth, production, community cooperation and posterity of the society (Corrado *et. al*., 2009). The animal freedoms are guidelines on how animals

should be handled and managed, as expressed by the Farm Animal Welfare Council (FAWC). These animal freedoms are critical and binding and they are designed to guide all undertakings that involve animals (Farm Animal Welfare Council, 2009) and (Canali and Keeling, 2009). They include: freedom from hunger and thirst where animals are entitled to diet and water for the purposes of health and vigor; freedom from discomfort as animals need a conducive and comfortable resting shelter; freedom from pain, injury or disease through appropriate diagnosis in order to elucidate causes of disease for timely treatment that restores good health to the animal and prevent prolonged suffering. The other freedoms are; freedom to express normal behavior where animals are to be provided with adequate space and enabling environment to express their normal behavior; freedom from fear and distress.

There are other guidelines which state that animals should never be subjected to induced pain emanating from inappropriate management when carrying out any procedures on them (Welfare Quality, 2009). Therefore, administration of several vaccines by injections and with each individual vaccine injected separately on the same animal on different sites of the body, leads to traumatic lesions, pain and fear on the animals; this infringes on the freedom against fear, distress and discomfort. As a result, animals respond through stampede, further self-injuries and injury to other animals, especially young ones, in scenarios where animal restraining structures are not permanently mounted, which is common in the rural areas. The numerous injection sites subjected to the animals sometimes develop into swellings due to trauma and this seems not to auger well with livestock keepers who are emotionally attached to the animals (Personal observation, 2021).

These injections and the vaccines are themselves a form of stress. Sheep and goats are very sensitive and such stresses could lead to lowered immunity, giving way to opportunistic diseases such as febrile fever and *Pasteurellosis* (Temple and Manteca, 2020).

2.6 Mixing of vaccines and vaccination

There are many serious diseases in animals that need to be addressed through vaccination; in most cases several vaccines being administered at the same time, during vaccination campaign days. In order to prevent multiple injections when giving vaccines, several vaccines can be mixed and injected as a single injection. Vaccine combination saves time, reduces efforts necessary to administer the same vaccines as individual separate injections, and poses no health risks (are safe to the target species) (Chaudhary *et. al*., 2009).

The immune system is a complex system which evolved to effectively respond to the various antigens exposed to it (Siegrist, 2018); some of them at the same time in one site, equivalent to one concoction of vaccine mixture. During multiple simultaneous vaccine administration, the body elicits an effective immune response against the antigens/vaccines in the mixture individually, similar to when the vaccines are administered separately (Ayalet *et. al*., 2012). Simultaneous multiple administration of vaccine mixtures, therefore, is not expected to overwhelm or adversely affect the immune system's ability to respond to the individual vaccines/ diseases. Individuals immunized using vaccine mixtures, at the same prescribed doses, are expected to generate immune responses, against all the agents in the combinations, which are as strong as in cases when the respective vaccines are administered separately.

In a study that was conducted in Ethiopia by Ayalet *et. al*.:(2012), it was shown that combination of SGP and PPR vaccines, given together as one bolus in sheep and goats was effective. Vaccine mixtures were arrived at from the individual prescribed doses for the individual vaccines as recommended by manufacturers to enable right dosage rates and enable the body to mount desired immune responses against the given vaccines (Ayalet *et al*., 2012). Good immune response, monitored by seroconversion, was observed when SGP and PPR vaccines were injected as a mixture, in one injection; there was no interference between the

two vaccines in terms of immunogenicity and efficacy. A similar finding in India indicated that *Pestes des Petits Ruminants* did not interfere with immunogenicity of other unrelated vaccine antigens as shown by Rajak *et al*.:(2005). Nonetheless, in some research findings it was observed that, concurrent vaccination involving *Peste des Petits Ruminants* (PPR) and sheep and goat pox (SGP) vaccines had a synergistic positive effects on enhancing antibody production for PPR vaccine and inhibitory effects on SGP vaccine humoral immune response. However, there was adequate cell mediated immune responses, induced by the SGP vaccine that was not affected by the simultaneous vaccination (Zhang *et. al*., 2021).

2.7 Immune generation from vaccination

2.7.1 General information

Vaccines are antigens that are introduced into the body for the immune system to respond actively by producing specific antibodies and lymphocytes towards them; thus, making the body resistant to the specific disease(s). After vaccination, the body develops memory cells that get localized in lymphoid tissues and organs. The memory cells from vaccination produce immunoglobulins whenever stimulated by disease-causing organisms (Quinn *et. al*., 2011).

Most viral antigens/vaccines promote both humoral and cell mediated immune development by triggering B and T-cell stimulation. This happens in a balanced fashion and there is a synergistic response between them, with respect to the particular disease (Siegrist, 2018).

Since vaccines are comprised of treated microorganisms or antigenic elements that do not cause disease, the immune system is able to produce antibodies that remain in circulation after vaccination without actual disease development. These antibodies are specific to the disease(s) vaccinated against. The presence and persistence of memory cells in the lymphoid tissues generate anamnestic immune response whenever the body is exposed to subsequent disease challenges; the responses are specific to the vaccine(s), without altering other body

functions. Once vaccinated, sheep and goats show adequate seroconversion after 7 days and 10 days against SGP and PPR vaccines respectively. Therefore, protective antibody levels for both vaccines (PPR and SGP) could be comfortably detected fourteen days post vaccination (F. Fakri *et. al*., 2015). Animals vaccinated against PPR and SGP retain immunity for about 3 years as observed by Martrenchar *et. al*.:(1999), Sreenvasa *et. al*.:(2000) and Panday (2004).

2.7.2 Vaccine Safety

Vaccines are immunogenic substances produced and released for use after certification for safety and freedom from hazards both to humans, animals and the environment. The *Peste des Petits Ruminants* vaccine has been used widely across various continents of the World with no particular history of causing adverse reactions on humans, animals and environment. Studies that were conducted in India for vaccine safety, immunogenicity and potency showed no adverse reactions on sheep and goats that had been vaccinated with PPR vaccine. The study showed sero-conversion by both sheep and goats, building adequate antibody levels that shielded them against PPR viral exposure, unlike unvaccinated groups which came down with the disease. The vaccinated lots of sheep and goats did not develop any complications during vaccination and even after the PPR virus challenge, the animals maintained their vital parameters and health (Jaykumar *et. al*., 2020).

Vaccination against sheep and goat pox (SGP) viruses has always remained the ideal method in controlling sheep and goat pox in shoats in endemic areas. The inactivated *Capripoxvirus* vaccine has been used widely offering cross protections over all the field strains of sheep and goat pox originating in Africa and Asia (Kitching & Taylor:1985, Kitching:1986). There are also specific strains of Capripoxvirus vaccine such as Romanian & RM-65 and Mysore specific for goats and sheep respectively (Davies and Mbugwa, 1985). In Kenya however, the KSGP:0240 strain is used commonly and offers good sero-conversion to both sheep and

goats. Vaccination with KSGP:0240 strain offers immunity to sheep and goats for one year while Romanian strain offers immunity for up to 30 months (Tuppurainen *et. al*., 2014). Therefore, disease control by vaccination is safe approach and should be highly encouraged.

2.7.3 Antibody and leucocyte production from vaccination

The body responds to antigens in vaccines after vaccination through production of antibodies and leukocytes detectable in blood and serum. Adequate antibodies in serum are detected after a latent period of 10-14 days, post vaccination. The initial response to an antigen is normally the primary response when vaccines are given to naïve individuals, and usually succeeded by a secondary immune response, initiated by exposure to a natural disease or by subsequent vaccine boosters. Immunological memory also develops and leucocyte memory cells remain in circulation offering protection for years against the specific disease(s) (Siegrist, 2018).

2.7.4 Vaccine Interaction

There is increased need for combined vaccines that have multiple antigens in disease control in both humans and animals, in order to reduce economic costs in administering the vaccines. The immune responses induced by combined vaccines, is expected to be more or less similar to the immunity realized by individual vaccines when given independently. There are both physical and chemical factors that influence vaccine responses when combined and are always taken into consideration when constituting combined vaccines (Zimmermann *et al*., 2019). One of the factors is antigen competition; however, PPR and SGP vaccine combinations do not result in depression of immune responses to the individual vaccine components as shown by Chaudhary *et. al*.: (2009). The second factor is carrier-induced epitope expression associated with hapten polysaccharide vaccines interfering with antibody production and hence need to be inhibited prior to vaccination (Dagan *et al*., 1998). The third factor is due to induced interferon, which is common with live vaccines; this results in

stimulation of interferon production and inhibition of antigen multiplication in live vaccines, which lowers immune development (Pichichero, 2013). It has been shown under laboratory set up that, when PPR and SGP vaccines are mixed together and given as a single bolus, the vaccine mixture is safe and effective, eliciting desirable immune responses in both sheep and goats equivalent to immunity developed when SGP and PPR were given individually in separate injections (Fakri *et al*., 2015).

2.8 Methods of analyzing immunity in vaccinated animals

2.8.1 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay uses antibodies, coated with enzymes such as alkaline phosphatases or peroxidases, which are used to pick specific antigens. The test has a sensitivity of 90.8% and specificity of 95.1% (Balamurugan *et al*. 2007). The enzymes usually use PNPP (p-Nitrophenyl Phosphatase, Disodium Salt) as their substrate and therefore, through assaying the enzyme activities on antibody-antigen complexes, positive cases can be detected. This substrate is normally added at the end of reaction to confirm if antibody-antigen complex has formed, and the coated enzyme acts on the substrate resulting in colour change which is detected using an ELISA reader. The ELISA test can be used directly or indirectly to detect antigens or antibodies in serum or body fluids (Carn *et al*. 1994; Heine *et al*. 1999).

2.8.2 Complement fixation test (CFT)

Formation of antibody-antigen (Ab-Ag) complex activates the complement system, which comprises 11 proteins; the proteins attach to the complex and cause opsonisation. Complement fixation test: tests for presence of the Ab-Ag complex. In positive cases (presence of Ab-Ag complex) the complement gets fixed, hence not available for any other complex. So, when an indicator system (sensitized sheep red blood cells, in this case) is added

to the test solution, there will be no hemolysis, However, in negative cases, where there is no antibody specific to the particular antigen, Ab-Ag complex will not be formed, and complement will not be fixed. The free complement will then cause lysis of the sensitized sheep red blood cells, added as indicator system. Thus, this reaction is used to determine individuals that have immunity and those that lack immunity to a particular disease/antigen (Quinn *et. al*., 2011).

2.8.3 Neutralization test

Antibodies neutralize antigens by forming antigen-antibody complexes. Also, some antigens are cytopathic; that is: they are able to grow and cause destruction to cells. This destructive effect is usually prevented by antibodies and therefore cell destruction is usually absent wherever antigens are bound by corresponding antibodies (Quinn *et. al*., 2011). Neutralization test has a sensitivity of up to 80% and a specificity of 100% (Balamurugan *et al*. 2006).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was undertaken in Kwale County, Kenya (Figure 3.1). Agriculture is one of the main economic activities carried out in Kwale County with 85% of farmers practicing subsistence farming. The agricultural sector plays a crucial role in guaranteeing food security, poverty reduction and employment creation in the county; most of the farmers practice mixed farming. In spite of the importance of agriculture, food insecurity is still a challenge in the county. Kwale being one of the counties located in the ASAL, the community livelihoods are also majorly dependent on livestock. From the recent country (Kenya) census conducted in 2019, it is documented that: the county had approximately 334,013 and 43,034 heads of goats and sheep respectively which were mainly found in the ASAL of Kinango sub-county (184,996 goats and 37,507 sheep). These small stocks are usually reared under free range grazing in the vast arable land that is suitable for livestock. There are a number of ranches in Kinango sub-county that contribute immensely to the economy of Kwale County at large. The ranches offer employment opportunities and also avail improved breeds for the small scale livestock keepers such as the meat traits (Galla goats) that have spread widely in Kwale County among farmers, a role played by the ranches.

Despite the livestock wealth and particularly the sheep and goats that are well adapted to the ASAL climate, PPR and SGP diseases in sheep and goats, as well as Contagious Caprine Pleuropneumonia (CCPP), are greatest challenges to small ruminants production. Due to vaccination costs and complexity of administering vaccines to address these three diseases of economic significance, most government and Non-governmental Organization (NGO) funded vaccination programs for sheep and goats have only dwelt on addressing CCPP mostly and partly SGP. PPR: is a neglected disease in Kwale County; there has been no vaccination

campaign against the disease, thus precipitating a great risk to the small ruminants production (Personal observation, 2021). Most large scale keepers move freely in the free range hence risking spread of disease within the range lands when their livestock are not adequately protected through vaccination. Hence the selection of Kinango sub-county for this study was crucial in order to prepare a ground for implementation of the findings upon completion of the study.

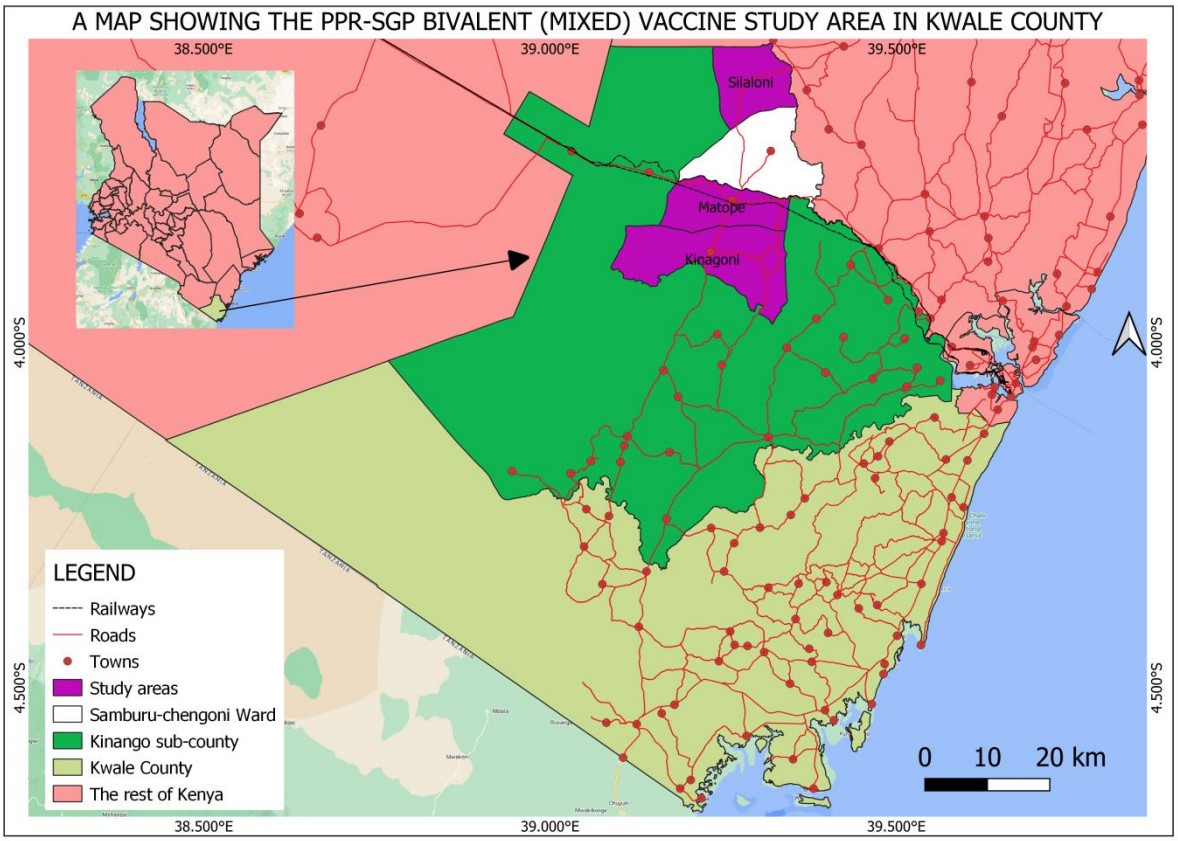


Figure 3.1: Map of Kwale County showing areas where the study was conducted

3.2 Animal welfare and Ethical clearance

The study was conducted in line with recommendations of the ethical committee of the University of Nairobi in manner that ensured integrity and quality research as indicated in the

letter of approval **REF: FVM BAUEC/2022/393** (Appendix 1). Individuals' verbal consent, were obtained from farmers who willingly accepted to participate in the study.

3.3 Sample size calculation

The resource equation approach in one-way ANOVA by Arifin *et. al.* (2017) was applied; E= (total number of experimental units) - (number of treatment groups) and E value was between 10 and 20. E is the degree of freedom of ANOVA also denoted as DF (between-subject error). For one-way ANOVA, DF was calculated as: DF = N – k = kn – k = k(n – 1), where N = total number of subjects, k = number of groups, and n = number of subjects per group. Hence, n = DF/k + 1, given that DF has a minimum (10) and maximum (20); Minimum n = 10/k + 1 Maximum n = 20/k + 1. Total sample size (N); Minimum N = Minimum n x k; Maximum N = Maximum n x k (Arifin *et. al*., 2017). This project had four treatment groups; one for testing efficacy of PPR and SGP vaccine combinations, second as a positive control for PPR vaccine, third as positive control for SGP vaccine and fourth as a negative control, given buffer. Using Maximum values for E, N = (E+k), E=20 and k=4 hence, N=24. E=k(n-1), n=E/k + 1, n=20/4 +1= 6, a minimum of 16 and a maximum of 24 animals was to be recruited from each target species, with each species grouped into four groups that were comprised of between 4-6 animals per group.

The field trials however, recruited more animals to take care of eventualities associated with sale of animals and theft among others when animals are in an uncontrolled environment.

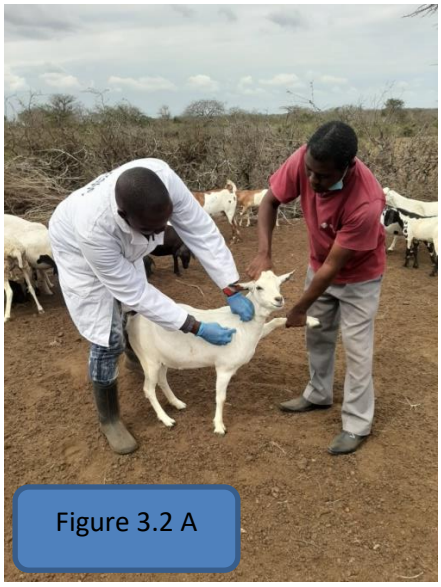
3.4 Vaccine Preparation

The PPR and SGP vaccines used were prepared using Nigerian 75/1 strain and Kenyan Sheep and Goat Pox strains KSGP:0240 respectively, from Kenya veterinary vaccine production

institute (KEVEVAPI); both of which were live attenuated vaccines. The two vaccines were reconstituted separately adhering to manufacturer's guidelines. Vaccine combination was done during vaccination by drawing respective doses; one milliliter of each vaccine type into one syringe to form the mixed vaccine (2ml) under test trials.

3.5 Field Trial

A total of 41 sheep and 42 goats that were clinically healthy, were selected from willing farmers, ear tagged with serial numbers. Before start of the research, the herds were dewormed appropriately; serum collected from each animal and screened for presence of PPR and SGP virus specific antibodies. Only animal herds found negative for the diseases were selected and grouped into four groups and vaccinated as follows; first group using combined PPR/SGP, second and third groups given single PPR and SGP vaccines respectively and fourth group given normal saline (physiological buffer). The groups were then clinically monitored regularly by daily observations of body temperatures, appetite for feed and water, swellings and any other abnormalities involving the study animals, for 14 days. Figures 3.2 A and B represent one such vaccination exercise.



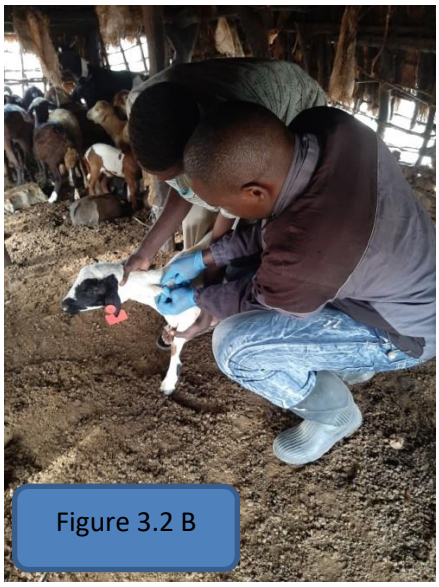


Figure 3.2: Photos of selected goats (A) and sheep (B) during vaccination, in Kwale County.

Blood samples, for serum, were then collected at day 14 post vaccination to determine respective sero-conversion. Figures 3.3 A and B below, show the experimental goats and tubes containing blood samples.

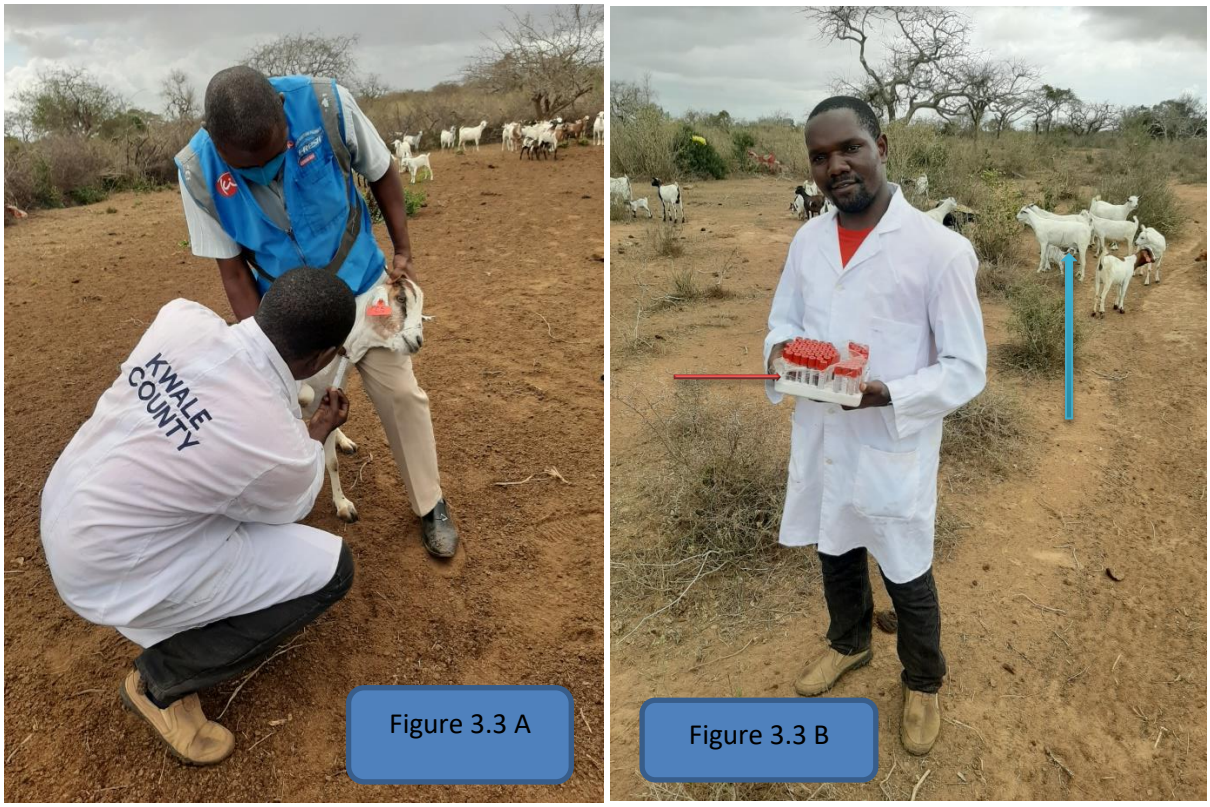


Figure 3.3: Photos of healthy (A) and recently kidded goats (B) in the background (blue arrow) 14 days post vaccination, during serum sample collection in Kwale County; and tubes containing collected blood samples (B; red arrow)

3.6 Serological tests used

Two serological tests were used: Competitive ELISA and Double antigen ELISA

3.6.1 Procedure for Competitive ELISA for detection of PPR antibodies

A *Peste des Petits Ruminants* antibody test competitive ELISA kit was obtained from ID.vet Innovation and Diagnostic company for the research. All the test reagents and samples were allowed to come to room temperatures (21℃ ± 5℃) and homogenized using vortex prior to use. The microplate was inoculated with 25µl Dilution Buffer 13 in all microwells, 25µl Positive Control solution into 2A1 and 2B1 microwells, 25µl Negative Control solution to

2C1 and 2D1 microwells and finally 25µl of serum sample to the remaining microwells according to labels allocated. The plate was then incubated at 37℃ (±3℃) for 45 minutes ± 4 minutes.

After incubation the microwells were washed three times using 300µl of wash solution, dropped into each well by a multi-channel micropipettor with disposable tips. After each washing, the microptate was blot dried; it was then injected with conjugate 1X solution; prepared from conjugate 10X that was diluted with Diluting Buffer 4 solution in the ratio 1:10. The conjugate 1X was injected into each microwell and the microplate was incubated at 21℃ (±5℃) for 30 minutes (±3 minutes). Following the incubation, the microplate was washed again 3 times by inoculating 300µl of wash solution into each microwell followed by blot drying after every washing. Thereafter, 100µl of substrate solution was added into each microwell and the microplate incubated at 21℃ (±5℃) for 15 minutes (±2). After lapse of the 15 minutes incubation period, 100µl of Stop Solution was added into each microwell in order to stop further reactions, the microplate read using ELISA reader at 450nm optical density and results recorded.

3.6.2 Procedure for Double Antigen ELISA for detection of Sheep and Goat Pox antibodies

An Capripox multispecies double antigen antibody test ELISA kit was obtained from ID.vet Innovation and Diagnostic company for the research. All the test reagents and samples were allowed to come to room temperatures (21℃ ± 5℃) and homogenized using vortex prior to use. The microplate was inoculated with 25µl Dilution Buffer 13 in all microwells, 25µl Positive Control solution into 2A1 and 2B1 microwells, 25µl Negative Control solution to 2C1 and 2D1 microwells and finally 25µl of serum sample to the remaining microwells

according to labels allocated. The plate was then incubated at 37℃ (±3℃) for 45 minutes ± 4 minutes.

After incubation, the microwells were washed three times using 300µl of wash solution dropped into each well by a multi-channel micropipettor with disposable tips. After each washing, the microptate was blot dried; it was then injected with conjugate 1X solution; prepared from conjugate 10X that was diluted with Diluting Buffer 4 solution in the ratio 1:10. The conjugate 1X was injected into each microwell and the microplate was incubated at 21℃ (±5℃) for 30 minutes (±3 minutes).

Following the incubation, the microplate was washed again 3 times by inoculating 300µl of wash solution into each microwell, followed by blot drying after every washing. Thereafter, 100µl of substrate solution was added into each microwell and the microplate incubated at 21℃ (±5℃) for 15 minutes (±2). After lapse of the 15 minutes incubation period, 100µl of Stop Solution was added into each microwell in order to stop further reactions, the microplate read using ELISA reader at 450nm optical density and results recorded.

3.6.3 Validation of the ELISA test results

a. Mean value of negative controls optical densities (OD_{NC}) greater than 0.7 ($OD_{NC}>0.7$).
b. Mean value of positive control (OD_{PC}) less than 30% of the OD_{NC} ($OD_{PC}/OD_{NC}<0.3$).

3.7 Data handling and processing

The serological comparison response data for both the combined PPR-SGP bivalent vaccines and the respective PPR and SGP monovalent vaccines were entered into a data base using Microsoft excel that enabled getting reciprocal values to generate antibody titres and graphs. Further statistical analysis for serological comparisons response to both monovalent and combined vaccines was entered into a data base using SPSS 20.0 for windows (SPSS Inc., Chicago USA). The independent sample's t test was used for continuous variable. The

differences were considered significant if the P-vale was found to be less than 0.05 (Fakri *et. al.*, 2015).

CHAPTER FOUR: RESULTS

4.1 Animal body reactions to vaccination

Throughout the study period of 14 days post vaccination, the animals maintained their body temperatures within the normal ranges; between 37.5℃ and 38.5℃ for both sheep and goats cohorts. The study animals expressed good appetite and behavior while at their grazing fields during the day and mating was also noted in some reproductive active animals throughout the study. Animals that were in their late third trimester of gestation: kidded and lambed normally to sound kids and lambs respectively without complications of dystocia. Some animals bled mildly immediately upon injection, however, the bleeding did not persist and there were no swellings or abscess formation on the injection sites throughout the study. At day seven post vaccination, all the injection marks had resolved and animals' skins were healthy and intact. The animals continued to exhibit their normal behaviors during the study period and looked aesthetically better following the deworming and vaccination. There were no clinical abnormalities observed on the study animals throughout the experiment.

4.2 Results on screening of sera from targeted goat and sheep herds for presence of respective antibodies, before start of experiment

A total of 47 sheep and 45 goats were first screened for PPR and SGP antibodies, to rule out previously vaccinated/infected animals. The animals were also screened for any suspected underlying conditions associated with the disease and whether they had previous history of infections involving the two diseases. All of them were found to be negative for both PPR and SGP antibodies; also the respective temperatures taken were found to be within the normal range. Details of the respective screening results are given in Tables 4.1 and 4.2. Graphical presentations of the immune reactions for PPR reactors for the screened sheep and goats, are

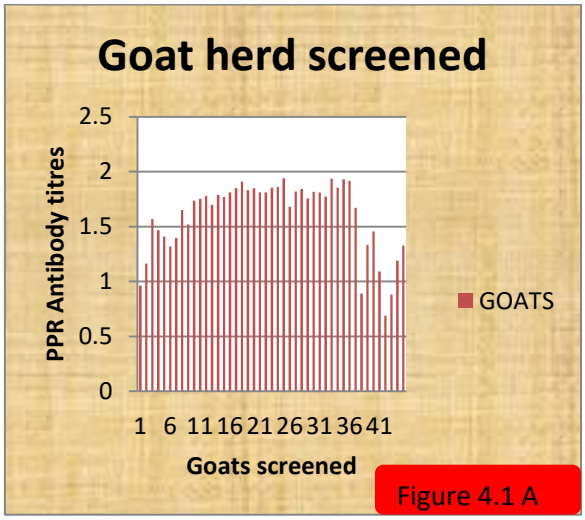
given in Figure 4.1, while the graphical presentations of the immune reactions for SGP reactors for the screened sheep and goats, are given in Figure 4.2.

Table 4.1: Competitive ELISA Screening results against *PPR* disease in sheep and goats; using optical densities.

OPTICAL DENSITIES FROM IDVET COMPETITION ELISA FOR PPR-GOATS AND SHEEP SERA												
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.062	1.29	1.415	1.419	1.182	1.119	0.872	0.962	1.52	1.849	1.939	1.935
1B	C++ 0.089	1.408	0.867	1.367	1.514	0.931	1.464	1.165	1.735	1.91	1.68	1.855
1C	C- 1.805	1.491	1.2	1.118	1.539	0.786	0.988	1.568	1.752	1.83	1.821	1.932
1D	C- 1.021	1.293	1.154	1.382	0.903	1	1.09	1.468	1.779	1.849	1.844	1.917
1E	1.426	1.401	1.388	1.41	0.845	1.28	0.69	1.409	1.698	1.812	1.755	1.671
1F	1.635	1.341	1.473	1.214	1.023	0.827	0.88	1.317	1.788	1.81	1.818	0.891
1G	1.378	1.767	1.526	1.553	1.491	1.485	1.191	1.398	1.771	1.854	1.812	1.334
1H	1.467	1.63	1.022	1.185	1.61	0.983	1.328	1.652	1.812	1.861	1.774	1.456
	KEY NOTES											
	1. C++, Average Positive Control 0.0755											
	2. C-, Average Negative Control 1.413											
	3. SHP - Sheep samples starts from 1E1 through 1C7											
	4. GT - Goat samples starts from 1D7 through 1H12											

Table 4.2: Double-antigen ELISA Screening results against sheep and goat pox disease in sheep and goats; using optical densities.

OPTICAL DENSITIES FROM IDVET DOUBLE ANTIGEN ELISA FOR SGP-GOATS AND SHEEP SERA												
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.308	1.335	1.474	1.512	1.414	1.338	1.202	1.226	1.213	1.248	1.323	1.252
1B	C++ 0.215	1.558	1.570	1.591	1.630	1.640	1.587	1.605	1.481	1.642	1.687	1.656
1C	C- 1.519	1.558	1.608	1.616	1.616	1.652	1.641	1.597	1.619	1.685	1.714	1.694
1D	C- 1.554	1.569	1.594	1.612	1.660	1.694	1.708	1.656	1.691	1.744	1.761	1.755
1E	1.602	1.557	1.528	1.522	1.614	1.594	1.702	1.747	1.761	1.808	1.855	1.949
1F	1.651	1.609	1.639	1.604	1.662	1.740	1.842	1.826	1.862	1.885	1.878	1.944
1G	1.622	1.637	1.673	1.669	1.790	1.754	1.891	1.855	1.948	1.905	1.943	1.928
1H	1.580	1.566	1.641	1.664	1.675	1.698	1.835	1.792	1.842	1.891	1.891	1.823
	KEY NOTES											
	1. C++, Average Positive Control 0.2615											
	2. C-, Average Negative Control 1.5365											
	3. SHP - Sheep samples started at 1E1 through 1C7											
	4. GT - Goat samples started at 1D7 through 1H12											



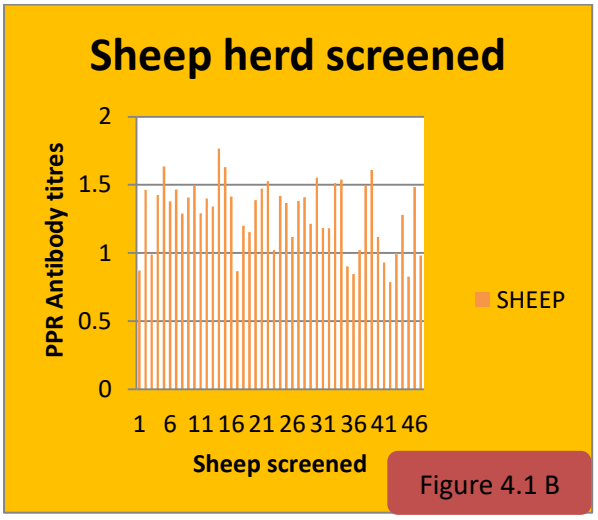
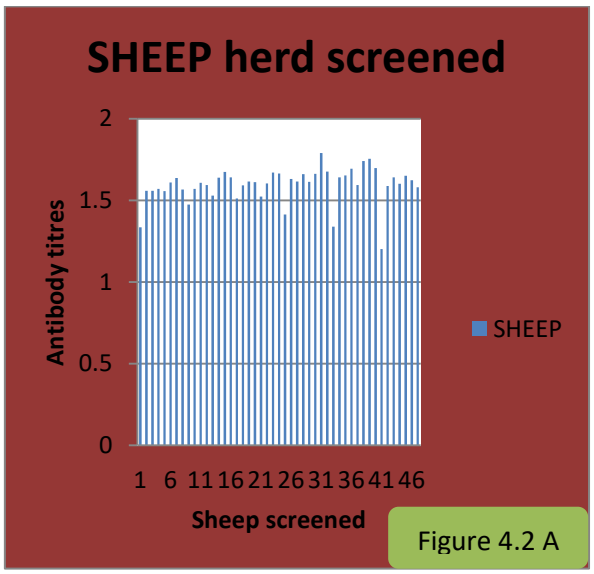


Figure 4.1: *PPR* antibody titres in screened goats (A) and sheep (B) before vaccination.



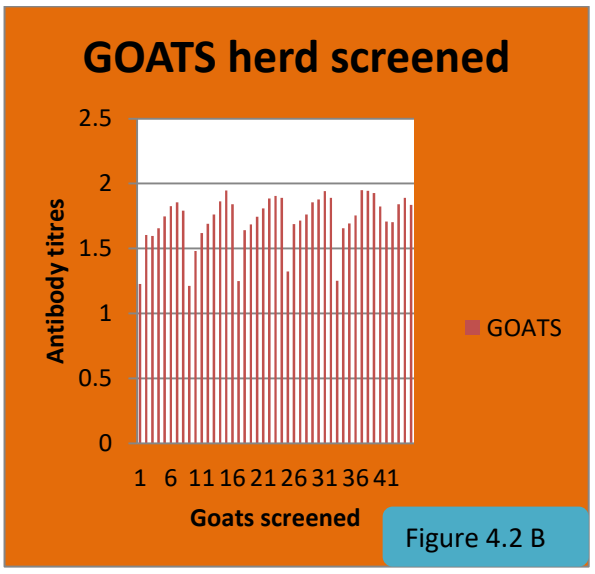


Figure 4.2: SGP antibody titres in screened sheep (A) and goats (B) before vaccination.

4.3 Results validity for *PPR* competitive ELISA (cELISA) and SGP Double antigen ELISA (daELISA).

The validities of the ELISA tests were tested following the manufacturer's criteria. For all PPR cELISA tests carried-out, the mean value of negative controls was 1.413, which was greater than 0.7, and the mean value of positive controls was 5.3% of the mean value of negative control, which was less than 30%, hence; the results were valid. For Sheep and Goat Pox, double antigen ELISA tests carried-out, the mean value of negative control was 1.5365, which was greater than 0.7, and the mean value of positive control was 17% of the mean value of negative control which was less than 30%, hence the results were valid. Details of the respective results validity are given in Tables 4.3, 4.4, 4.5, 4.6, 4.7 and 4.8.

Table 4.3: Validity results for *PPR* competitive ELISA, with respect to screened sheep and goats; using competition percentage (S/N%) values.

COMPETITION PERCENTAGES (S/N%)												
Date: **30/03/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.062	91	100	100	84	79	62	68	108	131	137	137
1B	C++ 0.089	100	61	97	107	66	104	82	123	135	119	131
1C	C- 1.805	106	85	79	109	56	70	111	124	130	129	137
1D	C- 1.021	92	82	98	64	71	77	104	126	131	131	136
1E	101	99	98	100	60	91	56	100	120	128	124	118
1F	116	95	104	86	72	59	62	93	127	128	129	63
1G	98	125	108	110	106	105	84	99	125	131	128	94
1H	104	115	72	84	114	70	94	115	128	132	126	103
	KEY NOTES											
	1. C++ Positive Control											
	2. C- Negative Control											
	3. SHP - Sheep samples started at 1E1 through 1C7											
	4. GT - Goat samples started at 1D7 through 1H12											
	5. S – Sample Value											
	6. N – Mean value of Negative control											

Table 4.4: Validity results for SGP Double Antigen ELISA, with respect to screened sheep and goats; using Percentage inhibition (S/P%) values.

Percentage Inhibition (S/P%) values												
Date: **30/03/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.062	15.8	4.9	1.2	9.6	15.6	18.4	24.4	25.4	22.7	16.8	22.4
1B	C++ 0.089	3.5	2.6	4.2	7.3	8.1	3.9	5.3	4.4	8.2	11.8	9.3
1C	C- 1.805	3.5	5.6	6.2	6.2	9.0	8.2	4.7	6.4	11.6	13.6	12.3
1D	C- 1.921	2.5	4.5	5.9	9.6	12.3	13.4	9.3	12.1	16.2	17.6	17.1
1E	5.1	3.4	0.7	1.2	6.0	4.5	13.0	16.5	17.6	21.2	24.9	32.3
1F	8.9	8.0	8.0	5.1	9.8	15.9	23.9	22.7	25.5	27.3	26.7	31.9
1G	6.7	7.8	10.7	10.4	19.8	17.0	27.8	24.9	32.2	28.9	31.8	30.7
1H	3.4	2.3	8.2	10.0	10.8	12.6	23.4	20.0	23.9	27.8	27.8	21.6
	KEY NOTES											
	1. C++ Positive Control											
	2. C- Negative Control											
	3. SHP - Sheep samples started at 1E1 through 1C7											
	4. GT - Goat samples started at 1D7 through 1H12											
	5. S – Sample value											
	6. P – Mean value of positive control											

Table 4.5: Validity results for *PPR* competitive ELISA, with respect to vaccinated sheep and goats; using optical densities.

OPTICAL DENSITIES FROM IDVET COMPETITION ELISA FOR PPR-GOATS AND SHEEP SERA									
Date **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength **450nm**	
	1	2	3	4	5	6	7	8	9
2A	C++ 0.363	0.325	1.123	0.377	0.208	0.859	0.312	0.252	0.416
2B	C++ 0.328	0.35	1.518	0.452	0.295	1.633	0.341	0.272	0.372
2C	C- 1.818	0.324	1.407	0.382	1.606	1.627	0.298	0.268	
2D	C- 1.877	0.342	1.4	0.386	1.31	0.891	0.3	0.305	
2E	0.338	0.321	0.891	0.32	0.817	1.4	0.315	0.293	
2F	0.358	0.402	1.562	0.343	0.818	0.343	0.305	0.29	
2G	0.333	1.084	0.836	0.333	0.724	0.316	0.306	0.281	
2H	0.367	1.308	0.34	0.387	0.89	0.34	0.302	0.264	

	KEY NOTES
	1. C++ Positive Control
	2. C- Negative Control
	3. Goat samples begin from 2E1 through 2F2 and from 2H3 through 2E6
	4. Sheep samples begin at G2 through G3, and from F6 through B9

Table 4.6: Validity results for *PPR* competitive ELISA, with respect to vaccinated sheep and goats; using competition percentage (S/N%) values.

COMPETITION PERCENTAGES (S/N%)									
Date **20/05/2022**		Plate No. **Plate 4**		Animal Species; **Ovine and Caprine**				Wavelength **450nm**	
	1	2	3	4	5	6	7	8	9
2A	C++ 0.303	31.03	107.21	35.99	19.86	82	29.79	24.06	39.71
2B	C++ 0.328	33.41	144.92	43.15	28.16	155.89	32.55	25.97	35.51
2C	C- 1.018	30.93	134.32	36.47	153.32	155.32	28.45	25.58	
2D	C- 1.077	32.65	133.65	36.85	125.06	85.05	28.64	29.12	
2E	32.27	30.64	85.06	30.55	77.99	133.65	30.07	27.97	
2F	34.18	38.38	149.12	32.74	78.09	32.74	29.12	27.68	
2G	31.79	103.48	79.81	31.79	69.12	30.17	29.21	26.83	
2H	35.04	124.87	32.46	36.95	84.96	32.46	28.83	25.2	
	KEY NOTES								
	1. Average OD C++ Positive Control = 0.3155								
	2. Average OD C- Negative Control = 1.0475								
	3. S – Sample value								
	4. N – Mean value of Negative control								
	5. Goat samples begin from 2E1 through 2F2 and from 2H3 through 2E6								
	6. Sheep samples begin at G2 through G3, and from F6 through B9								

Table 4.7: Validity results for SGP Double Antigen ELISA, with respect to vaccinated sheep and goats; using optical densities.

OPTICAL DENSITIES FROM IDVET DOUBLE ANTIGEN ELISA FOR SGP-GOATS AND SHEEP SERA									
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**	
	1	2	3	4	5	6	7	8	9
2A	C++ 0.284	0.266	0.248	0.234	1.582	0.245	0.270	1.722	1.596
2B	C++ 0.210	0.211	0.179	1.537	1.536	0.172	0.148	1.627	1.284
2C	C- 1.500	0.227	0.209	1.527	1.548	0.179	0.183	1.662	
2D	C- 1.587	0.196	0.168	1.550	0.163	0.174	0.167	1.606	
2E	0.179	0.154	0.131	1.530	0.142	0.159	0.152	1.659	
2F	0.228	0.198	0.175	1.629	0.172	0.192	0.170	1.708	
2G	0.226	0.180	0.160	1.606	0.136	0.150	0.155	1.765	
2H	0.222	0.193	0.191	1.611	0.166	0.153	1.719	1.766	
	KEY NOTES								
	1. C++ Positive Control, Average positive control 0.247								
	2. C- Negative Control, Average Negative control 1.544								
	3. Sheep samples begin at 2E1 through 2C5								
	4. Goat samples begin from 2D5 through 2B9								

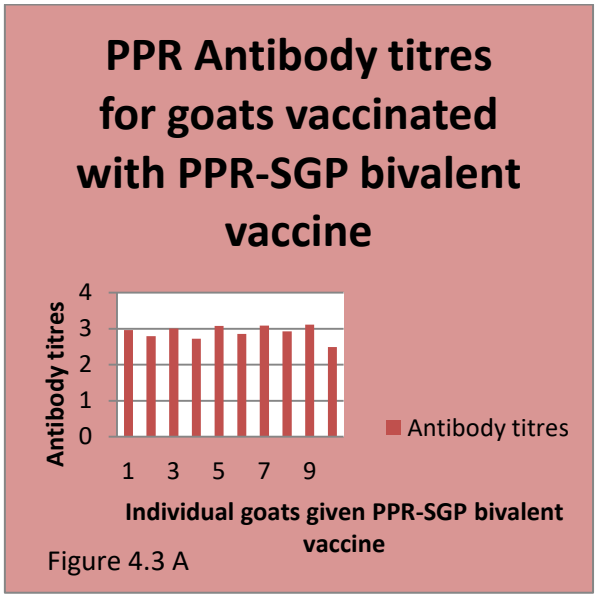
Table 4.8: Validity results for SGP Double Antigen ELISA, with respect to vaccinated sheep and goats; using percentage inhibition (S/P%) values.

Percentage inhibition (S/P%)									
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**	
	1	2	3	4	5	6	7	8	9
2A	C++ 0.303	98.5	99.9	101.0	2.9	100.2	98.2	13.7	4.0
2B	C++ 0.328	102.8	97.5	0.5	0.6	105.8	107.6	6.4	20.0
2C	C- 1.018	101.5	102.9	1.3	0.3	105.2	104.9	9.1	
2D	C- 1.077	103.9	106.1	0.5	106.5	105.6	106.2	4.9	
2E	105.2	107.2	108.9	1.2	108.1	106.8	107.3	8.9	
2F	101.5	103.8	105.5	6.6	105.8	104.2	105.9	12.6	
2G	101.6	105.2	106.7	12.5	108.6	107.5	107.1	17.0	
2H	101.9	104.2	104.3	5.2	106.2	107.2	13.5	17.1	
	KEY NOTES								
	1. C++ Positive Control, Average positive control 0.247								
	2. C- Negative Control, Average Negative control 1.544								
	3. S - Sample value								
	4. P – Mean value of positive control								
	5. Sheep samples begin at 2E1 through 2C5								
	6. Goat samples begin from 2D5 through 2B9								

4.4 Immune responses to mixed (PPR-SGP bivalent) vaccine

When the goats and sheep were inoculated with the mixed vaccine (containing both PPR and SGP vaccines) and immune responses to PPR and SGP separately tested after 14 days, results for the two (goats and sheep) were as given in Figures 4.3 and 4.4.

Both sheep and goats mounted adequate immunity, following vaccination using the PPR-SGP bivalent vaccine with 100% success rate recorded fourteen days after vaccination. The animals expressed good levels of antibodies for both PPR and SGP viruses in their sera. The competitive percentages for PPR antibody levels in the mixed vaccines ranged from 30.64 to 38.38% and 28.45 to 32.74% in goats and sheep respectively. For the competitive ELISA test for PPR antibodies, the animals were considered positive if their competitive percentages were below 50%, hence the PPR-SGP bivalent vaccine reacted positively for PPR antibodies in both sheep and goats. The PPR-SGP bivalent vaccine had a percentage inhibition (S/P%) ranging from 98.5 to 107.2% and 100.2 to 108.6% in sheep and goat cohorts respectively. In the double antigen ELISA test for SGP antibodies, animals were considered positive if their S/P percentages were >50%, therefore, the PPR-SGP bivalent vaccine responded well with positive results in both sheep and goats (Libeau *et. al*, 1995 and Mehmood *et. al*, 2009).



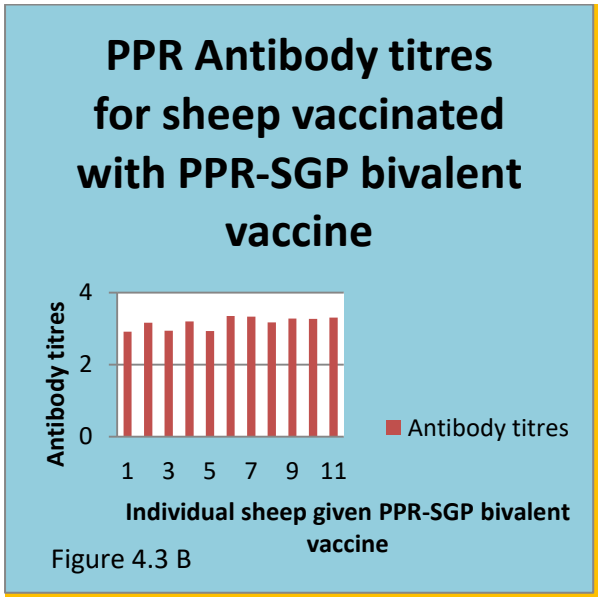
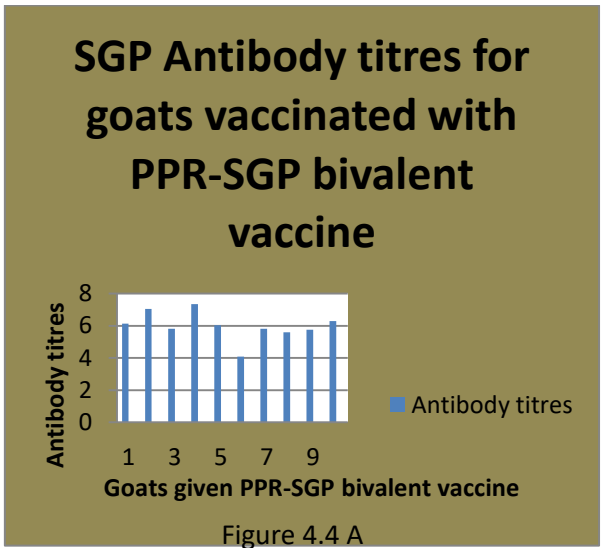


Figure 4.3: *PPR* antibody titres in goats (A) and sheep (B) vaccinated, using PPR-SGP bivalent vaccine.



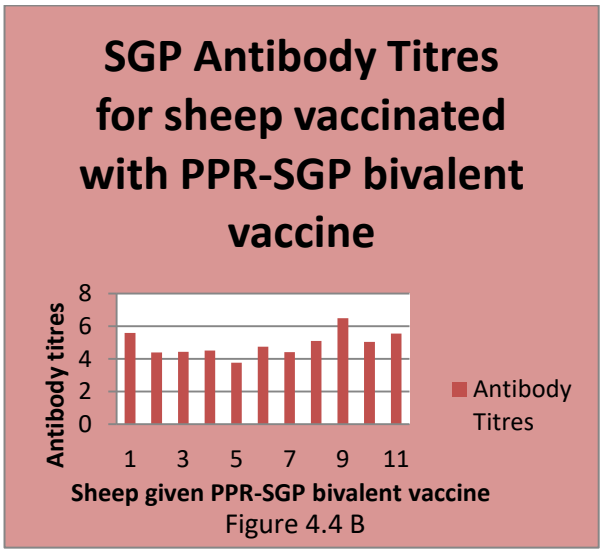


Figure 4.4: SGP antibody titres of goats (A) and sheep (B) vaccinated using PPR-SGP bivalent vaccine.

4.5 Immune responses to monovalent PPR and SGP vaccines

When the goats and sheep were inoculated with the monovalent vaccines for PPR and SGP, and respective antibody responses tested after 14 days, results for the two (goats and sheep) were as given in Figure 4.5 for PPR; and Figure 4.6 for SGP. For both PPR and SGP monovalent vaccines, their respective antibody titres exhibited by the vaccinated individuals were more than 2; the same picture was reflected by the respective animal species when vaccinated with the PPR-SGP bivalent vaccine, as depicted in Figures 4.3 and 4.4 above.

The monovalent PPR and SGP vaccines were used in their conventional form as recommended by the manufacturer (KEVEVAPI) and served as positive control. These vaccines elicited adequate antibody titres in the study animals (sheep and goats) and comparisons done between PPR in sheep and goats among cohorts given the monovalent vaccines (PPR and SGP) showed no significance differences in antibody induction with the 'p-value' being > 0.05 between sheep and goats as shown in Appendices 4, 5, 6, 7, 8 and 9.

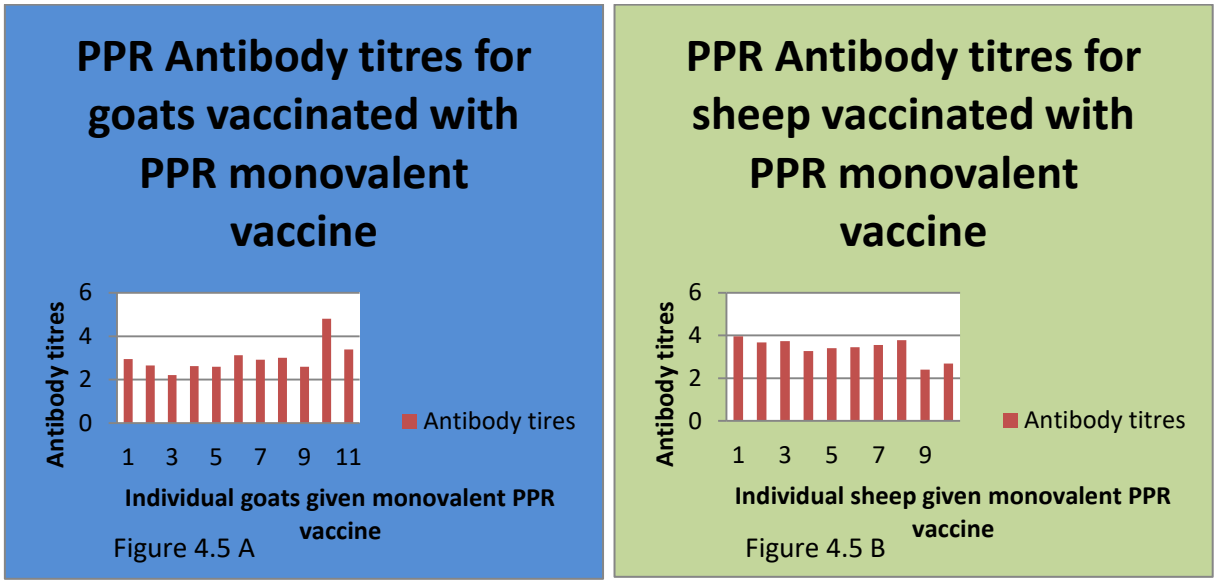
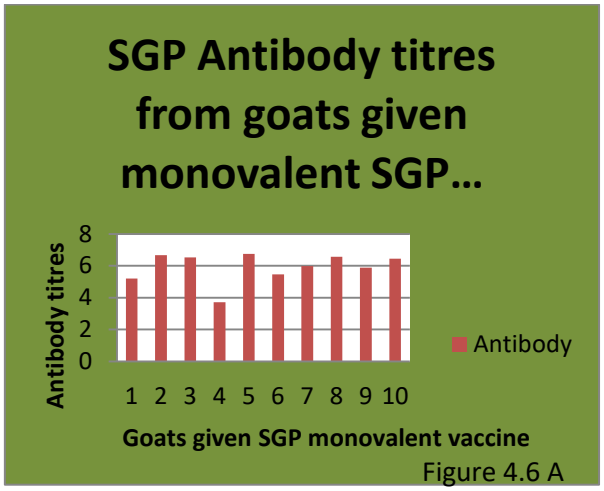


Figure 4.5: PPR antibody titres of goats (A) and sheep (B) vaccinated using PPR monovalent vaccine.



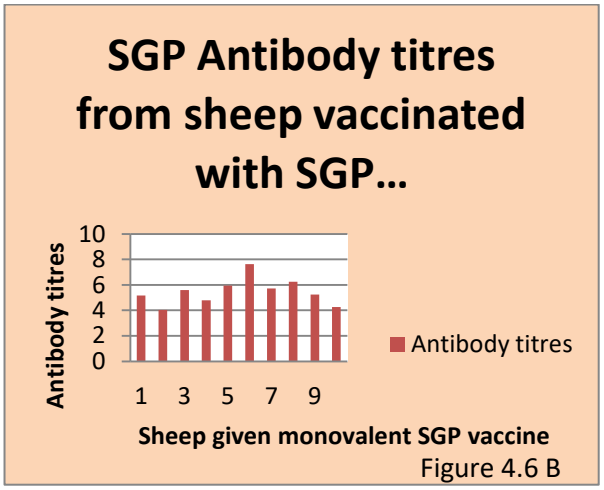


Figure 4.6: SGP antibody tires of goats (A) and sheep (B) vaccinated using SGP monovalent vaccine.

4.6 Comparisons of the average titres produced by the three vaccination modules [bivalent (PPR and SGP) vaccine, monovalent PPR vaccine and monovalent SGP vaccine, with respect to goats and sheep

Comparisons of the average titres produced by the three vaccination models, with respect to goats and sheep, are given in Tables 4.9, 4.10, 4.11 and 4.12, and Figures 4.7 and 4.8. Other details are given in Appendices 4, 5, 6, 7, 8 and 9.

The average PPR antibody titres at day 14 following vaccination, were recorded highest in sheep at 3.165 and 3.322 with PPR-SGP bivalent and PPR monovalent vaccines respectively. On the other hand the average titer levels recorded in goats were 2.89 and 2.873 for cohorts given the PPR-SGP bivalent and PPR monovalent respectively.

The average SGP titres were however, recorded highest in goats with 5.548 and 5.747 for the PPR-SGP bivalent and SGP monovalent vaccines respectively, against 4.807 and 5.291 for PPR-SGP bivalent and SGP monovalent vaccines respectively in sheep.

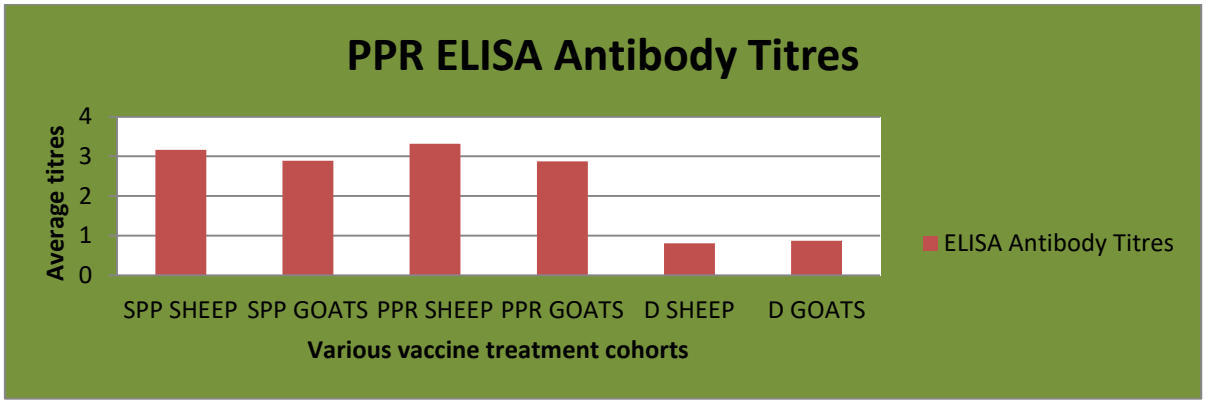


Figure 4.7: PPR Antibody titre trends for the various vaccine treatment groups in sheep and goats.

Key:

SPP means "mixed PPR and SGP vaccine"

PPR means "monovalent PPR vaccine"

D means "control" (saline inoculated)

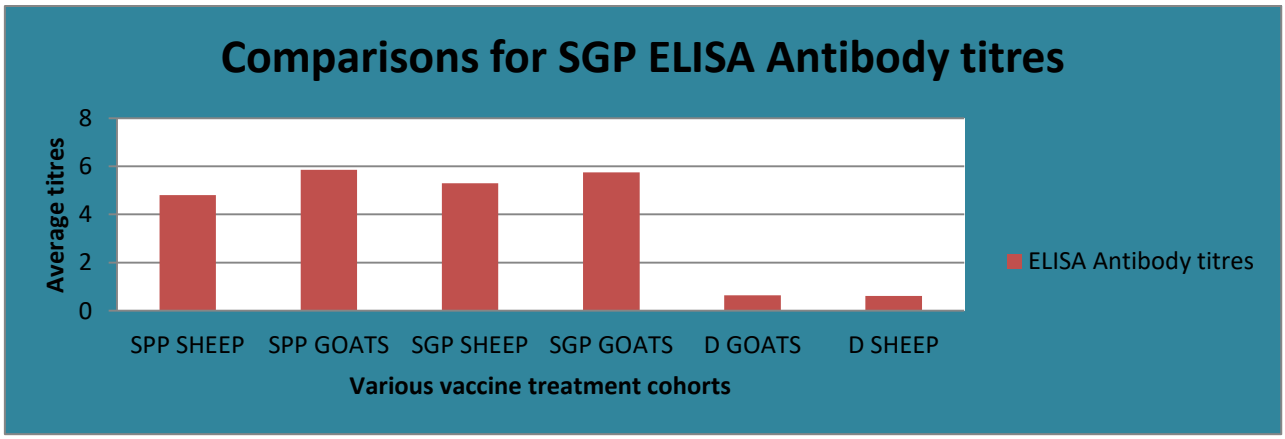


Figure 4.8: SGP Antibody titre trends for the various vaccine treatment groups in sheep and goats.

Key:

SPP means "mixed PPR and SGP vaccine"

SGP means "monovalent SGP vaccine"

D means "control" (saline inoculated)

4.7: Comparisons of the average titres produced by the bivalent (PPR and SGP) vaccine against the negative control (groups injected with diluent) with respect to PPR and SGP antibodies in goats and sheep

There was significant differences in the optic densities of groups on bivalent treatment and those given diluent for both sheep and goats cohorts. The "p-value" between sheep on mixed vaccine and those on diluent with respect to PPR antibodies was 4.60374E-10 (4.60374×10^{-10}) and that between goats on bivalent vaccine and those on diluent was 9.27568E-07 (9.27568×10^{-7}), lower than 0.05 (Fakri *et. al.*, 2015). The same trends were observed with respect to SGP where the "p-value" between sheep cohorts on bivalent vaccine and those on diluents was 8.38217E-27 (8.38217×10^{-27}) and those between goat cohorts on bivalent vaccine and diluent was 8.63415E-19 (8.63415×10^{-19}).

The average antibody titres for PPR in sheep and goats vaccinated using the bivalent (PPR-SGP) vaccine were; 3.1627 and 2.89 respectively while the titres for the same on sheep and goat cohorts that received diluent treatment was found to be 0.8087 and 0.8748 respectively as illustrated in figure 4.9 below.

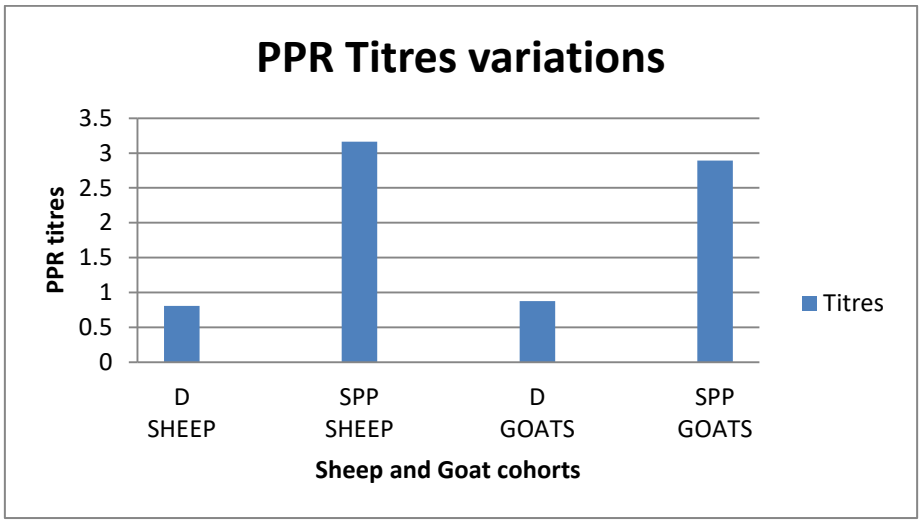


Figure 4.9: PPR titre variations between cohorts given bivalent (SPP) vaccine and diluent (D) treatments in sheep and goats.

Comparisons of the average competition percentages among the various cohorts, the cohorts on bivalent treatment had 30.18% and 33.03% for the sheep and goats, whereas cohorts on diluent treatment had 118.04% and 109.13% for the sheep and goats respectively. The cohorts on diluent had their immune titres above 50% mark for competition percentage (S/N%) hence were unprotected (Libeau *et. al*, 1995).

For the SGP antibody titres on the other hand, the same trends was observed with sheep and goats cohorts on bivalent vaccine exhibiting 4.8098 and 5.8548 titres levels and those on diluent treatment had 0.6387 and 0.6073 titres for the sheep and goats respectively as shown in below in figure 4.10.

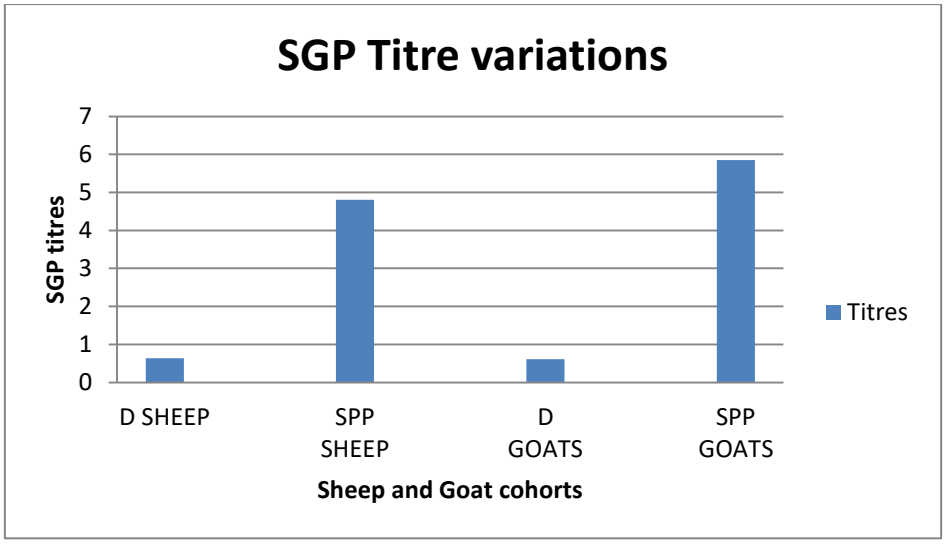


Figure 4.10: SGP titre variations between cohorts given bivalent (SPP) vaccine and diluent (D) treatments in sheep and goats.

Comparisons of the average percentage inhibition among the various cohorts, the cohorts on bivalent treatment had 103.01% and 105.88% for the sheep and goats, whereas cohorts on diluent treatment had 1.67% and 5.37% for the sheep and goats respectively. The cohorts on diluent had their immune titres below 50% mark of percentage inhibition (S/P%) and hence, lacked protective antibodies (Libeau *et. al*, 1995 and Mehmood *et. al*, 2009).

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The study confirmed that the PPR and SGP vaccines, whether used singularly (as monovalent) or as mixed (bivalent) were safe on the sheep and goats. All animal across the study cohorts expressed normal physiological behaviors allaying any fears related to vaccine safety among rural livestock keepers, who are always skeptic on vaccinating their animals. The observation that no animals developed swellings at injection sites during and after vaccination: improved the confidence of the society on vaccination as a tool to disease control. Also a lot was learnt from these findings by the researcher, for example: the fact that, swelling developments, clinical disease development after vaccination and even mortalities were not associated with the PPR and SGP vaccine, whether given as monovalent or as combined. A large vaccination campaign conducted in Morocco using bivalent PPR-SGP vaccine showed good safety in sheep and goats (Fakri, *et al*., 2015). Similar findings were also observed by other researchers such as: Hosamani *et. al*.:(2006) and Singh *et. al*.:(2004).

Results of this research showed that no interaction existed between PPR and SGP vaccines, when mixed together and injected as a single bolus. The PPR and SGP monovalent vaccines produced by the Kenya Veterinary Vaccines production institute (KEVEVAPI) are usually freeze-dried-live-attenuated vaccines, managed at -20℃ before reconstitution with diluents. It is crucial to note that the PPR and SGP vaccine types available as produced by KEVEVAPI utilize the same diluent type for reconstitution into an injectable liquid vaccine and the combined pre-mixed vaccine was miscible. From the results, all the 11 sheep and 10 goats that were given the mixed (PPR-SGP bivalent) vaccine under test were able to mount a robust immunological response against PPR and SGP antigens. In these findings, there were no vaccine interferences between PPR and SGP vaccines, both *in vitro* and *in vivo*. These

observations are coherent with similar research which was conducted in Ethiopia involving controlled laboratory sheep and goats, who were vaccinated using a mixed vaccine of PPR and SGP and later challenged with active disease antigens; through mixing vaccinated with infected animals who had both PPR and SGP disease in the same herd for some times. All the animals vaccinated using the PPR-SGP bivalent vaccine mounted adequate immunity and were protected against respective infection challenges exposed to them (Ayalet *et. al*., 2012). Also in this research, there were no differences in the immunological responses across the age groups given the mixed vaccine and the monovalent PPR and SGP vaccines, indicating suitability for the test product for any growing herd, which mostly are comprised of all animal ages, especially so in a society set up as usually happens during routine vaccinations in the communities. Both sheep and goats responded adequately to the mixed vaccine without any feasible variations in their immune titres. There was 100% induction of immune response, with respect to PPR, and SGP, in the two respective groups of goats and sheep given monovalent and mixed (PPR-SGP bivalent) vaccines. This observation was similar to findings of a controlled experiment done in Ethiopia involving a PPR-SGP bivalent vaccine in goats; initially sero-conversion was at had 75% response rate but later-on scaled-up to 100% response rate upon exposure to PPR disease virus. On the SGP vaccine, there was 100% and 99.2% sero-conversion in goats and sheep respectively from the PPR-SGP bivalent vaccine. Out of 128 sheep vaccinated using the PPR-SGP bivalent vaccine, one developed classical pox lesions upon deliberate exposure to SGP disease virus. For the goats given PPR-SGP bivalent vaccine, none developed PPR clinical disease nor succumbed after exposure to a PPR disease virus, affirming the effectiveness of the PPR-SGP combined vaccine (Ayalet, *et al*., 2012). Apart from SGP, the PPR vaccine has been shown to be compatible with other vaccines such as foot and mouth disease (FMD). A research undertaken in 2017, PPR vaccine

injected separately or concurrently with a trivalent FMD (Type A, Type O and Asian 1) vaccine, showed good sero-conversion for both PPR and FMD in the animals, at 100% rate; indicating that neither PPR nor FMD interfered with each other's immunogenicity (Mansoor *et. al*., 2017). The PPR vaccine has not been shown to interfere with other vaccine antigens given together on same animals simultaneously (Elbayoumy *et. al*., 2013).

The monovalent PPR and SGP vaccines were tested for *in vivo* viability and for comparison with the mixed (PPR-SGP-bivalent vaccine) when given under same field conditions. The outcome showed 100% viability for both PPR and SGP vaccines used which acted as positive controls for the research. There were no significant differences in the immune responses mounted by the mixed (PPR-SGP bivalent) vaccine and the respective PPR and SGP monovalent vaccines given separately, as depicted by their titre levels. This observation was similar to that observed in Morocco where a bivalent vaccine of PPR-SGP combination was produced and tested in various African countries and after 14 days post vaccination, the target sheep and goats mounted a robust immune response towards PPR and SGP vaccine antigens. The immunity generated from the PPR-SGP vaccine combination, did not vary from the immunity produced by the individual SGP and PPR vaccines when given separately (Fakri *et. al*., 2015). Thus, using a mixed vaccine could significantly reduce vaccination cost and achieve a desirable disease control outcome, in addition to better animal welfare by reducing of injection sites. Both of them being freeze dried, live attenuated, sharing a common diluent, and given the scientific proof for compatibility *in vivo*, there is room for more research and possibilities of coming up with a PPR-SGP bivalent vaccine which will help in the eradication efforts against PPR and SGP diseases.

There was better animal welfare consideration by significant reduction in animal injection sites on the group that was given the single shot of PPR-SGP bivalent vaccine. While using

the combined vaccine a double benefit was realized by the animals (sheep and goats) hence, creating potential for controlling PPR and SGP at minimal costs and less suffering in future. It was also noted that, even with the successful production of a PPR-SGP bivalent vaccine in Morocco, there was no mass production for the same product and hence PPR-SGP bivalent vaccine was not found in the market for consumption and utilization in the fight against the two diseases, as observed by Fakri *et. al*.: (2015). The PPR-SGP bivalent vaccine was proven to offer protection against both PPR and SGP diseases, in sheep and goats, over a period of twelve months post vaccination (Amanova *et. al*., 2021).

In Morocco, a veterinary pharmaceutical laboratory called MCI Santé animale, registered a combined PPR-SGP vaccine which they are yet to produce in large quantities and avail to the rest of the World for use. Hence, an opportunity for the production of a PPR-SGP bivalent vaccine existed Worldwide, for manufacturers to invest in. The institution is currently working on the possibilities of combining PPR, SGP and Rift Valley Fever (RVF), to come up with a trivalent vaccine. Rift Valley Fever is a significant disease in sheep and goats and vulnerability index for the RVF virus is high in sheep - a study conducted in Mozambique showed higher sero-prevalence of RVF in sheep (Blomström *et al*., 2016). Therefore, a trivalent vaccine with PPR, SGP and RVF would be economically beneficial for small ruminant herds' transformation. Since the year 2017, there have been efforts by the global alliance for livestock veterinary medicines (GALVmed) through their partnership with VetAid to come up with a PPR-SGP bivalent vaccine for the small ruminants. The alliance, GALVmed, had targeted to vaccinate over 2,000,000 of sheep and goats by end of 2017 with both PPR and SGP in Narok County, Kenya, but the cost of administering the two vaccines separately had a huge bearing on cost. They hence, sought some breakthroughs for a

possibility of a bivalent vaccine that would administer the two vaccines (PPR and SGP) in a single shot.

Vaccine combinations have been common in humans over the past 70 years, dating way back in 1948 AD, when diphtheria, tetanus, and pertussis (DTP) vaccines were combined into a single shot for infants and children. This has become a cornerstone to both pediatric and adult immunization protocols (Skibinski *et. al*., 2011). The United States recommends immunization for children against over sixteen diseases in their first two years of age, owing to the numerous endemic diseases. Therefore, in order to comply with the recommendations, on a single day, a child would receive over six separate injections, enduring severe pain. It is the trauma children passed through, that necessitated need for innovations to combining several vaccines into one syringe, reducing number of vaccine shots and simplifying vaccine administration. The combination of vaccines: made it easier for people to comply to vaccination protocol which became less painful and reduced number of visits to vaccination centres. There are numerous advantages attached to vaccine combinations such as: reducing number of injections, reduction on individuals' trauma, increasing compliance to vaccination recommendations, reduced vaccination costs and better target coverage, timely achievements of vaccine targets, reduced storage costs and gives an opportunity for incorporation of other vaccines into the same vaccination program based on prevailing demands and objectives (Skibinski *et. al*., 2011). Therefore, vaccine combinations should be procured judiciously to address prevailing diseases of economic and public health significance and should be region and/or disease(s) target specific.

5.2 Conclusions

- The live attenuated PPR and SGP vaccines are compatible and safe on small ruminants when combined and injected as a single shot without adverse reactions on animals. This gives room for facts collection and further deliberations on creation of a PPR-SGP bivalent vaccine that would be vital and boost efforts in eradicating PPR and SGP diseases.
- It was proven scientifically in these research findings that: mixing *Peste des Petits Ruminants* (PPR) vaccine and Sheep and Goat Pox (SGP) vaccine together and giving the combination to sheep and goats has no disadvantages or deleterious effects on the respective immune responses in the respective animal's body, compared to responses towards the two vaccines administered separately.
- The combined PPR-SGP bivalent vaccine, given in one shot can be used in controlling the diseases at low costs in areas where the diseases are endemic and with no proper infrastructures coupled with haphazard breeding of sheep and goats in regions such as Eastern Africa, West Africa and Southern Asia. These findings are therefore, a step ahead in the right direction in Kenya where there is infrastructure and presence of a running KEVEVAPI that could borrow the findings and work towards realization and mass production of PPR-SGP bivalent vaccines that would help control as well as help eradicate PPR and SGP in Eastern Africa and scale the same across West Africa and Southern Asia.

5.3 Recommendations

- The research findings need to be shared with the Kenya Veterinary Board, Kenya Directorate of Veterinary services and subsequently to the Kenya Veterinary Vaccine production institute among other stakeholders such as the International Livestock Research Institute (ILRI), Washington State University (WSU), African Network for Animal Welfare (ANAW), Food and Agricultural Organization of the United Nations (UN-FAO), the PPR Global Eradication Plan (GEP) implementing task force, the World Food Forum (WFF).
- There is need for composition of a special taskforce to look into the findings and recommend further action points to be followed here in Kenya to empower and task KEVEVAPI to embark on production of PPR-SGP bivalent vaccine.
- The study was expected to stimulate multi stakeholder efforts in coming up with bivalent vaccine products that encompass PPR and SGP combinations that would improve farmers' cooperation and help minimize vaccination costs. The PPR and SGP vaccine combination could be a useful tool in the strategy towards PPR and/or SGP eradication.
- In the event of successful production of PPR-SGP bivalent vaccine, it should be used as the vaccine of choice in the vaccination plans under the PPR Global Eradication Plans (PPR-GEP) blue print launched on the 4th of November 2022.
- There is also need for continuous awareness campaigns on the importance of vaccinating sheep and goats against the two diseases.

REFERENCES

Abid Mehmood, Qurban Ali, Javaid Ali Gadahi , Salman Akbar Malik , and Syed Imam Shah 2009. Detection of PPR virus antibodies in sheep and goat populations of the North West Frontier Province of Pakistan. Veterinary World, Vol.2(9):333-336.

Amanova, Z.; Zhugunissov, K.; Barakbayev, K.; Kondybaeva, Z.; Sametova, Z.; Shayakhmetov, Y.; Kaissenov, D.; Dzhekebekov, K.; Zhunushov, A.; Abduraimov, Y.; 2021. Duration of Protective Immunity in Sheep Vaccinated with a Combined Vaccine against Peste des Petits Ruminants and Sheep Pox. Vaccines, 9, 912.

Anne-Lie Blomstrom, PhD, Associate Professor, Isabelle Scharin, DVM, Hedvig Stenberg, DVM, Jaquline Figueiredo, DVM, Ofe´ lia Nhambirre, DVM, MSc, Ana Abilio, BSc, Master Phil, Mikael Berg, PhD, Professo1 and Jose´ Fafetine, DVM, MSc, PhD (2016). Seroprevalence of Rift Valley fever virus in sheep and goats in Zambe´ zia, Mozambique. Infection Ecology and Epidemiology, 6: 31343 -

António Monteiro, José Manuel Costa and Maria João Lima (2017). Goat System Productions: Advantages and Disadvantages to the Animal, Environment and Farmer.

Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci*. 2017;**24(5)**:101–105.

Ayebazibwe, C., Akwongo, C.J., Waiswa, J., Ssemakula, O., Akandinda, A., Barasa, M., Nkamwesiga, J., Lule, P., Mabirizi, A., Lule, P., Roesel, K. and Kiara, H. 2022. Peste des petits ruminants in Uganda: Assessment of animal health systems and coordination mechanisms. ILRI Research Report 105. Nairobi, Kenya: ILRI.

Balamurugan V, Sen A, Saravanan P, Singh RP, Singh RK, Rasool TJ, Bandyopadhyay SK. One-step multiplex RT-PCR assay for the detection of *Peste-des-petits-ruminants* virus in clinical samples. *Vet Res Commun.* 2006;30:655–666.

Balamurugan V, Singh RP, Saravanan P, Sen A, Sarkar J, Sahay B, Rasool TJ, Singh RK. Development of an indirect ELISA for the detection of antibodies against Peste-des-petits-ruminants virus in small ruminants. Vet Res Commun. 2007 Apr;31(3):355-64. doi: 10.1007/s11259-006-3442-x. Epub 2006 Dec 23. PMID: 17216310.

Balamurugan, V., Hemadri, D., Gajendragad, M. R., Singh, R. K., & Rahman, H. (2014). Diagnosis and control of peste des petits ruminants: a comprehensive review. Virus Disease, Vol. 25, pp. 39–56. https://doi.org/10.1007/s13337-013-0188-2

Bergevoet, R., A. van Engelen, (2014). The Kenyan meat sector Opportunities for Dutch agribusiness; . Wageningen, LEI Wageningen UR (University & Research centre), LEI Report 2014-032. 64 pp.; 4 fig.; 9 tab.; 30 ref.

Boshra H, Truong T, Babiuk S, Hemida MG (2015) Seroprevalence of Sheep and Goat Pox, Peste Des Petits Ruminants and Rift Valley Fever in Saudi Arabia. PLoS ONE 10(10): e0140328.doi:10.1371/journal.pone.014032

Bryan Markey, Finola Leonard, Marie Archambault, Ann Cullinane, Dores Maguire (2013). Clinical Veterinary Microbiology (Second edition), page 648.

Canali, E. and Keeling, L. (2009) Welfare Quality project: from scientific research to on farm assessment of animal welfare, Italian Journal of Animal Sciences, 8(2S), page. 900–903.

Carn, V. M. , Kitching R. P., Hammond J. M., and Chand P., 1994: Use of a recombinant antigen in an indirect ELISA for detecting bovine antibody to capripoxvirus. *J. Virol. Methods* 49, 285–294.

Chand P., Kitching R.P. & black D.N. (1994). Western blot analysis of virus-specific antibody responses to capripoxvirus and contagious pustular dermatitis infections in sheep. Epidemiol. Infect., 113, 377–385.

Chaudhary SS, Pandey KD, Singh RP, Verma PC, Gupta PK (2009). A Vero cell derived combined vaccine against sheep pox and Peste des petits ruminants for sheep. Vaccine 27:2548-2553.

Coetzer, J.A.W.; Tustin, R.C. *Infectious Diseases of Livestock*; Oxford University Press: New York, NY, USA, 2004; pp. 1–2352. [**Google Scholar**]

Corrado & Marina (2009) Animal welfare: review of the scientific concept and definition, Italian Journal of Animal Science, 8:sup1, 21-30, DOI: 10.4081/ ijas.2009.s1.21. To link to this article: https://doi.org/10.4081/ijas.2009.s1.21

Dagan, R., Eskola, J., Leclerc, C., Leroy, O., 1998. Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants, Infection and Immunity, 66, 2093-8.

Davies F.G. & Mbugwa G. (1985). The alterations in pathogenicity and immunogenicity of a kenya sheep and goat pox virus on serial passage in bovine foetal muscle cell cultures. J. Comp. Pathol., 95, 565–576.

ElArbi AS, Kane Y, Metras R, Hammami P, Ciss M, Beye A, Lancelot R, Diallo A and Apolloni A (2019). PPR Control in a Sahelian Setting: What Vaccination Strategy for Mauritania? Front. Vet. Sci. 6:242. doi: 10.3389/fvets.2019.00242

Elbayoumy, M. K., Ghattas,W. M., Allam, A. M. and Mahmoud, M. A., (2013). Immunological studies for using of combined inactivated respiratory virus vaccine (pneumo-3) and sheep pox vaccine in goats. Academic Journal of Animal Diseases, 2, 01–06

Fakri F., Ghzal S., Daoism A., Elarkam L.,. Douieb Y., Zephier K., Tadlaoui O., Fassi-Fihri (2015). Development and field application of a new combined vaccine against Peste des Petits Ruminants and Sheep Pox. Trials in Vaccinology Vol 4, 33-37

Farm Animal Welfare Council (2009) Animal Welfare in Great Britain: Past, Present and Future. FAWC, 17 Smith Square, London. http://www.fawc.org.uk

Felix Njeumi, Giancarlo Ferrari, Eran Raizman, Adama Diallo, Joseph Domenech, Nadège Leboucq, Susanne Munstermann (2015). Global Strategy for the Control and Eradication of PPR. ISBN 978-92-5-108733-6

Fraser, D. Understanding animal welfare. *Acta Vet Scand* **50** (Suppl 1), S1 (2008). https://doi.org/10.1186/1751-0147-50-S1-S1

Gelagay Ayalet 1, Nigussu Fasil 2, Shiferaw Jembere 1, Gedlu Mekonen 3, Teshale Sori 2 and Haileleul Negussie 2 (2012). Study on immunogenicity of combined sheep and goat pox and peste des petitis ruminants vaccines in small ruminants in Ethiopia. African Journal of Microbiology Research Vol. 6(44), pp. 7212-7217, 20 November, 2012. Available online at http://www.academicjournals.org/AJMR. DOI: 10.5897/AJMR12.1531. ISSN 1996-0808 ©2012 Academic Journals

Gelagay Ayalet[1]*, Nigussu Fasil[2], Shiferaw Jembere[1], Gedlu Mekonen[3], Teshale Sori[2] and Haileleul Negussie[2] (2012). Study on immunogenicity of combined sheep and goat pox and peste des petitis ruminants vaccines in small ruminants in Ethiopia. African Journal of Microbiology Research Vol. 6(44), pp. 7212-7217, 20 November, 2012. Available online at: http://www.academicjournals.org/AJMR. DOI: 10.5897/AJMR12.1531. ISSN 1996-0808 ©2012 Academic Journals

Gershon, P. D. and Black, D. N. (1989). A comparison of the genome of capripox virus isolates of sheep, goats and cattle. Virology, 1G4 : 341-349

Heine H.G., Stevens M.P., Foord A.J. & Boyle D.B. (1999). A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32, the homolog of the vaccinia virus H3L gene. J. Immunol. Methods, 227, 187– 196.

Hosamani M., Singh S.K., Mondal B., Sen A., Bhanuprakash V., Bandyopadhyay S.K., Yadav M.P., Singh R.K. A bivalent vaccine against goat pox and Peste des Petits ruminants induces protective immune response in goats. *Vaccine.* 2006;**24**:6058–6064.

Jaykumar Satav, KVN Rathnakar Reddi, Gopu Akhila, Dahiphale Hanumant, TVS Rao, GS Reddy (2020). Evaluation of Safety, Immunogenicity and Potency of PPR Vaccine. DOI: 10.36347/sjavs.2020.v07i12.001. Key Title: Sch J Agric Vet Sci ISSN 2348–8883.

Kairu-Wanyoike, H. Kiara, C. Heffernan, S. Kaitibie, G.K. Gitau, D. McKeever, N.M. Taylor,Control of contagious bovine pleuropneumonia: Knowledge, attitudes, perceptions and practices in Narok district of Kenya, Preventive Veterinary Medicine, Volume 115, Issues 3â€“4, 2014, Pages 143-156, ISSN 0167-5877.

Kenya National Bureau of Statistics Real Towers, Upper Hill P.O. Box 30266,00100 Nairobi, Tel: +254-20-3317583/6/8. Email:directorgeneral@knbs.or.keinfo@knbs.or.ke. Website: https://knbs.or.ke

Kihu, S.M., Gitao, G.C., Bebora, L.C. *et al.* Economic losses associated with Peste des petits ruminants in Turkana County Kenya. *Pastoralism* **5**, 9 (2015). https://doi.org/10.1186/s13570-015-0029-6

Kihu, S., Njagi, L., Njogu, G., Kamande, J., & Gitao, C. (2012). *Peste des petits ruminants in Kenya; Pastoralist knowledge of the disease in goats in Samburu and Baringo Counties*

Kitching R.P. and Taylor W.P., 1985. Clinical and antigenic relationship between isolates of sheep and goat pox viruses. Tropical Animal Health and Production, 17, 64-74.

Kitching R.P. and Taylor W.P., 1985. Transmission of capripoxvirus. Research in Veterinary Science, 39, 196-199.

Kitching R.P., 1986. Passive protection of sheep against capripoxvirus. Research in Veterinary Science, 41, 247-250

Libeau G, Diallo A, Colas F, Guerre L (1994) Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. Vet Rec 134:300–304

Libeau G., Prehaud C., Lancelot R., Colas F., Guerre L., Bishop D.H.L. & Diallo A. (1995). Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. Res. Vet. Sci., 58, 50–55.

Mabirizi, A., Nkamwesiga, J., Lumu, P., Roesel, K., Kato, C.D. and Kiara, H. 2022. The effect of concurrent vaccine administration for peste des petits ruminants, capripox and contagious caprine pleuropneumonia on immune response of goats and sheep. Poster presented at the Boosting Uganda's Investment in Livestock Development (BUILD) project annual planning meeting, Kampala, Uganda, 20–22 September 2022. Nairobi, Kenya: ILRI.

Martrenchar A, Zoyem N, Njoya A, Ngo Tama AC, Bouchel D, Diallo A 1999: Field study of a homologous vaccine against Peste des Petits Ruminants in northern Cameroon. Small Ruminant Res. **31** 277–280.

Muhammad Khalid Mansoor & Abdullmajeed Hamood Al-Rawahi &Hatim Ali El-Tahir & Badar Al-Faraei & Muhammad Hammad Hussain & Muhammad Nadeem Asi & Ibrahim Al-Hussani & Safwat Sabar (2017). Concurrent vaccination of goats with foot

and mouth disease (FMD) and peste des petits ruminants (PPR) booster vaccines. DOI 10.1007/s11250-017-1391-8

OIE (2008a). Peste Des Petits Ruminants. Terrestrial Manual. pp 1036- 1046

OIE (2008b). Sheep Pox and Goat Pox. Terrestrial Manual. pp. 1058- 1068

OIE. Chapter 3.7.9. Peste des petits ruminants (infection with peste des petits ruminants virus: In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals; Office International des Epizooties: Paris, France. 2019

Quinn P.J, B K Markey, F C Leonard, E S FitzPatrick, S Fanning and P J Hartigan (2011). Veterinary Microbiology and Microbial Disease Second edition: (Vaccine and vaccination; page 236-270).

Pandey KD 2004: *Sheep pox vaccine production and prospects of eradication of the disease.* In: Compendium of Proceedings and Abstracts of the National Symposium on Control of Economically Important Viral Disease of Animal 45.

Parida S, Muniraju M, Mahapatra M, Muthuchelvan D, Buczkowski H, Banyard AC. Peste des petits ruminants. Vet Microbiol. 2015 Dec 14;181(1-2):90-106. doi: 10.1016/j.vetmic.2015.08.009. Epub 2015 Sep 5. PMID: 26443889; PMCID: PMC4655833.

Parray, O. R., Yatoo, M. I., Muheet, Ahmed Bhat, R., Ullah Malik, H., Bashir, S. T., & Nabi Magray, S. (2019). Seroepidemiology and risk factor analysis of contagious caprine pleuropneumonia in Himalayan Pashmina Goats. *Small Ruminant Research*, *171*, 23–36. https://doi.org/10.1016/j.smallrumres.2018.12.004

Pichichero ME, Casey JR and Almudevar A. Nonprotective responses to pediatric vaccines occur in children who are otitis prone. Pediatr Infect Dis J 2013; 32: 1163–1168.

Pronab Dhar [1], B P Sreenivasa, Thomas Barrett, Mandy Corteyn, R P Singh, S K Bandyopadhyay (2002). Recent epidemiology of peste des petits ruminants virus (PPRV). https://pubmed.ncbi.nlm.nih.gov/12135634/

Rajak K K, Sreenivasa Bp, Madhusudan Hosamani, R. P. Singh, S.K. Singh, R K Singh, S.K. Bandyopadhyay (2005). Comparative Immunology, Microbiology and Infectious Diseases 28(4):287-96. DOI:10.1016/j.cimid.2005.08.002. Source: PubMed

Siegrist CA. Vaccine immunology. In: Plotkin SA, Orenstein W A, Offit PA, Edwards KM, eds. Plotkin's vaccines. 7th ed. Philadelphia, PA: Elsevier; 2018.

Singh RP. Control strategies for peste des petits ruminants in small ruminants of India. Rev Sci Tech. 2011 Dec;30(3):879-87. doi: 10.20506/rst.30.3.2079. PMID: 22435198.

Singh RP, SreenivasaBP, Dhar P, Bandyopadhyay SK (2004). A sandwich- ELISA for the diagnosis of peste des petits ruminants infection in small ruminants using anti-nucleocapsid protein monoclonal antibody. Archives of Virology. 149:2155-2157.

Skibinski DA, Baudner BC, Singh M, O'Hagan DT. Combination vaccines. J Glob Infect Dis. 2011 Jan;3(1):63-72. doi: 10.4103/0974-777X.77298. PMID: 21572611; PMCID: PMC3068581

Sreenivasa BP, Dhar P, Singh RP, Bandyopadhyay SK 2000: Evaluation of an indigenously developed homologous live attenuated cell culture vaccine against peste des petits ruminants infection of small ruminants. In: Proceedings of the XX Annual Conference of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases and National Symposium on Trends in Vaccinology for Animal Diseases 84.

Temple D and Manteca X (2020). Animal Welfare in Extensive Production Systems Is Still an Area of Concern. Front. Sustain. Food Syst. 4:545902.doi: 10.3389/fsufs.2020.545902

Tulman, E. R., C. L. Afonso, Z. Lu, L. Zsak, J. H. Sur, N. T. Sandybaev, U. Z. Kerembekova, V. L. Zaitsev, G. F. Kutish, and D. L. Rock, (2002): The genomes of sheeppox and goatpox viruses. J. Virol. 76, 6054–6061.

Tuppurainen E.S.M., Pearson C.R., Bachanek-Bankowska K., Knowles N.J., Amareen S., Frost L., Henstock M.R., Lamien C.E., Diallo A. & Mertens P.P.C. (2014). Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antiviral Res., 109, 1–6.

Wosu LO (1991) Haemagglutination test for diagnosis of peste des petits ruminants disease in goats with samples from live animals. Small Ruminant Res 5:169–172. doi:10.1016/0921-4488(91)90041-N

Yune N, Abdela N (2017) Epidemiology and Economic Importance of Sheep and Goat Pox: A Review on Past and Current Aspects. J Vet Sci Technol 8: 430. doi: 10.4172/2157-7579.1000430

Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. Clin Microbiol Rev. 2019 Mar 13;32(2):e00084-18. doi: 10.1128/CMR.00084-18. PMID: 30867162; PMCID: PMC6431125.

Zhang, D., Yang, B., Zhang, T. *et al.* In vitro and in vivo analyses of co-infections with peste des petits ruminants and capripox vaccine strains. *Virol J* **18**, 69 (2021). https://doi.org/10.1186/s12985-021-01539-7

APPENDICES

Appendix 1: Ethical clearance from Biosafety, Animal use and Ethics committee.

UNIVERSITY OF NAIROBI
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REF: FVM BAUEC/2022/393

Raphael Chimweli Nyawa
Department of Pathology, Microbiology & Parasitology,
University of Nairobi.
04/07/2022

Dear Raphael,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

"Determining whether mixing *Peste Des petits* Ruminants and Sheep and Goat pox vaccines will affect their respective immune responses"

Raphael Chimweli J56/ 36783/2020

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 22nd June 2022. We have reviewed your application for ethical clearance for the study.

The number of sheep and goats, serological tests and protocols used to determine whether mixing *Peste Des petits* Ruminants and Sheep and Goat pox vaccines affects their respective immune responses meets the minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We also note that KVB registered veterinary surgeons will supervise the laboratory work

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine
University of Nairobi

Appendix 2: Ear tag identities of the various study groups for the *PPR* Competitive ELISA test in sheep and goats.

IDVET COMPETITION ELISA FOR PPR - GOAT AND SHEEP SERA RECORD SHEET									
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**	
	1	2	3	4	5	6	7	8	9
2A	C++	SPP5	D33	PPR22	PPR29	D36	SPP4	PPR21	PPR29
2B	C++	SPP6	D34	PPR23	PPR30	D37	SPP5	PPR22	PPR30
2C	C-	SPP7	D35	PPR24	D30	D38	SPP6	PPR23	
2D	C-	SPP8	D36	PPR25	D31	D39	SPP7	PPR24	
2E	SPP1	SPP9	D37	PPR26	D32	D40	SPP8	PPR25	
2F	SPP2	SPP10	D38	PPR27	D33	SPP1	SPP8A	PPR26	
2G	SPP3	D31	D40	PPR28	D34	SPP2	SPP9	PPR27	
2H	SPP4	D32	PPR21	PPR28A	D35	SPP3	SPP10	PPR28	
	KEY NOTES								
	1. C++ Positive Control								
	2. C- Negative Control								
	3. Goat samples begin from E1 through F2 and from H3 through E6								
	4. Sheep samples begin at G2 through G3, and from F6 through B9								
	5. SPP - Given mixed vaccine; Sheep & Goat Pox and PPR combined (Bivalent)								
	6. PPR – Given pure PPR vaccine (monovalent)								
	7. D - Given Diluent (Normal Saline) Only								

Appendix 3: Ear tag identities of the various study groups for the SGP Double Antigen ELISA test in sheep and goats.

IDVET COMPETITION ELISA FOR SGP - GOAT AND SHEEP SERA RECORD SHEET									
Date: **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**	
	1	2	3	4	5	6	7	8	9
A	C++	SPP5	SGP2	SGP10	D8	SPP6	SGP4	D2	D10
B	C++	SPP6	SGP3	D1	D9	SPP7	SGP5	D3	D11
C	C-	SPP7	SGP4	D2	D10	SPP8	SGP6	D4	
D	C-	SPP8	SGP5	D3	SPP1	SPP9	SGP7	D5	
E	SPP1	SPP8A	SGP6	D4	SPP2	SPP10	SGP8	D6	
F	SPP2	SPP9	SGP7	D5	SPP3	SGP1	SGP9	D7	
G	SPP3	SPP10	SGP8	D6	SPP4	SGP2	SGP10	D8	
H	SPP4	SGP1	SGP9	D7	SPP5	SGP3	D1	D9	
	KEY NOTES								
	1. C++ Positive Control								
	2. C- Negative Control								
	3. Sheep samples begin at 2E1 through 2C5								
	4. Goat samples begin from 2D5 through 2B9								
	5. SPP - Given mixed vaccine; Sheep & Goat Pox and PPR combined								
	6. D - Given Diluent (Normal Saline) Only								
	7. SGP - Given SGP vaccine alone								

Appendix 4: Comparisons for PPR immune titres, between PPR-SGP Bivalent (SPP) with monovalent PPR vaccine in sheep.

t-Test: Two-Sample Assuming Equal Variances		
	SPP-PPR-SH	***PPR-SH***
Mean	0.33436364	0.273909091
Variance	0.00348345	0.010709491
Observations	11.00000000	11
Pooled Variance	0.00709647	
Hypothesized Mean Difference	0.03630000	
Df	20.00000000	
t Stat	0.67244837	
P(T<=t) one-tail	0.25449671	
t Critical one-tail	1.72471824	
P(T<=t) two-tail	0.50899342	
t Critical two-tail	2.08596345	
p-value(0.509) >alpha(0.05)	hence we fail to reject the null hypothesis	

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-PPR sheep and PPR sheep.

Appendix 5: Comparisons for PPR immune titres between PPR-SGP Bivalent (SPP) with monovalent PPR vaccine in goats.

t-Test: Two-Sample Assuming Equal Variances

	SPP-PPR-G	**PPR-G**
Mean	0.314545455	0.347545455
Variance	0.011436873	0.003928273
Observations	11	11
Pooled Variance	0.007682573	
Hypothesized Mean Difference	0.0068	
Df	20	
	-	
t Stat	1.064905516	
P(T<=t) one-tail	0.149803458	
t Critical one-tail	1.724718243	
P(T<=t) two-tail	0.299606916	
t Critical two-tail	2.085963447	
	hence we fail to reject the null	
p-value(0.2996) > alpha(0.05)	hypothesis	

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-PPR goats and PPR goats.

Appendix 6: Comparisons for SGP immune titres between PPR-SGP Bivalent (SPP) with monovalent SGP vaccine in goats.

t-Test: Two-Sample Assuming Equal Variances		
	SPP-SGP-G	***SGP-G***
Mean	0.207909091	0.171636364
Variance	0.000943891	0.004327255
Observations	11	11
Pooled Variance	0.002635573	
Hypothesized Mean Difference	0.0363	
Df	20	
	-	
t Stat	0.001245869	
P(T<=t) one-tail	0.499509142	
t Critical one-tail	1.724718243	
P(T<=t) two-tail	0.999018284	
t Critical two-tail	2.085963447	
	hence we fail to reject the null	
p-value(0.999) >alpha(0.05)	hypothesis	

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-SGP goats and SGP goats.

Appendix 7: Comparisons for SGP immune titres between PPR-SGP Bivalent (SPP) with monovalent SGP vaccine in sheep.

t-Test: Two-Sample Assuming Equal Variances

	SPP-SGP-SH	***SGP-SH***
Mean	0.1708	0.174
Variance	0.000872178	0.001358222
Observations	10	10
Pooled Variance	0.0011152	
Hypothesized Mean Difference	0.0032	
Df	18	
t Stat	-0.428537267	
P(T<=t) one-tail	0.336672174	
t Critical one-tail	1.734063607	
P(T<=t) two-tail	0.673344348	
t Critical two-tail	2.10092204	
p-value(0.6733) > alpha(0.05)	hence we fail to reject the null hypothesis	

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-SGP sheep and SGP sheep.

Appendix 8: Comparisons for PPR immune titres between sheep and goats given PPR-SGP Bivalent (SPP) vaccine.

t-Test: Two-Sample Assuming Equal Variances		
	SPP-PPR-SH	***SPP-PPR-G***
Mean	0.316181818	0.314545455
Variance	0.000294364	0.011436873
Observations	11	11
Pooled Variance	0.005865618	
Hypothesized Mean Difference	0.0284	
Df	20	
t Stat	-0.819538896	
P(T<=t) one-tail	0.211069238	
t Critical one-tail	1.724718243	
P(T<=t) two-tail	0.422138476	
t Critical two-tail	2.085963447	

p-value(0.4221) >alpha(0.05)	hence we fail to reject the null hypothesis

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-PPR sheep and SPP-PPR goat.

Appendix 9: Comparisons for SGP immune titres between sheep and goats given PPR-SGP Bivalent (SPP) vaccine.

t-Test: Two-Sample Assuming Equal Variances		
	SPP-SGP-G	*SPP-SGP-SH*
Mean	0.207909091	0.155272727
Variance	0.000943891	0.003437018
Observations	11	11
Pooled Variance	0.002190455	
Hypothesized Mean Difference	0.0526	
Df	20	
t Stat	0.001822139	
P(T<=t) one-tail	0.499282098	
t Critical one-tail	1.724718243	
P(T<=t) two-tail	0.998564197	
t Critical two-tail	2.085963447	
p-value(0.9986) > alpha(0.05)	hence we fail to reject the null hypothesis	

Conclusion:

There is no significant difference in the means of ELISA Antibody titres for SPP-SGP sheep and SPP-SGP goats.

Appendix 10: Size of the targeted herds of sheep and goats for screening against *PPR* and SGP diseases.

IDVET COMPETITION ELISA FOR PPR - GOAT AND SHEEP SERA RECORD SHEET												
Date **30/03/2022**		Plate No. **Plate 4**		Animal Species; **Ovine and Caprine**				Wavelength **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	SHP5	SHP13	SHP21	SHP29	SHP37	SHP45	GT6	GT14	GT22	GT30	GT38
B	C++	SHP6	SHP14	SHP22	SHP30	SHP38	SHP46	GT7	GT15	GT23	GT31	GT39
C	C-	SHP7	SHP15	SHP23	SHP31	SHP39	SHP47	GT8	GT16	GT24	GT32	GT40
D	C-	SHP8	SHP16	SHP24	SHP32	SHP40	GT1	GT9	GT17	GT25	GT33	GT41
E	SHP1	SHP9	SHP17	SHP25	SHP33	SHP41	GT2	GT10	GT18	GT26	GT34	GT42
F	SHP2	SHP10	SHP18	SHP26	SHP34	SHP42	GT3	GT11	GT19	GT27	GT35	GT43
G	SHP3	SHP11	SHP19	SHP27	SHP35	SHP43	GT4	GT12	GT20	GT28	GT36	GT44
H	SHP4	SHP12	SHP20	SHP28	SHP36	SHP44	GT5	GT13	GT21	GT29	GT37	GT45
	KEY NOTES											
	1. C++ Positive Control											
	2. C- Negative Control											
	3. SHP - Sheep samples started at E1 through 1C7											
	4. GT - Goat samples started at D7 through 1H12											

Appendix 11: Interpretation of Results; for presence and absence of *PPR* antibodies, from the Competition percentages for the screened study animals.

ANALYSIS FOR POSITIVE AND NEGATIVE RESULTS												
Date **30/03/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.062	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1B	C++ 0.089	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1C	C- 1.805	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1D	C- 1.921	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1F	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1G	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1H	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	KEY NOTES											
	1. C++ Positive Control											
	2. C- Negative Control											
	3. SHP - Sheep samples started at 1E1 through 1C7											
	4. GT - Goat samples started at 1D7 through 1H12											

Appendix 12: Interpretation of Results; for presence and absence of SGP antibodies, from the S/P percentages for the screened study animals.

ANALYSIS FOR POSITIVE AND NEGATIVE RESULTS												
Date **30/03/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**				
	1	2	3	4	5	6	7	8	9	10	11	12
1A	C++ 0.308	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1B	C++ 0.215	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1C	C- 1.519	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1D	C- 1.554	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1F	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1G	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
1H	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	KEY NOTES											
	1. C++ Positive Control											
	2. C- Negative Control											
	3. SHP - Sheep samples started at 1E1 through 1C7											
	4. GT - Goat samples started at 1D7 through 1H12											

Appendix 13: Interpretation of Results; for presence and absence of *PPR* antibodies, from the Competition percentages for the various study groups.

ANALYSIS FOR POSITIVE AND NEGATIVE RESULTS									
Date **20/05/2022**		Plate No. **Plate 4**		Animal Species: **Ovine and Caprine**				Wavelength: **450nm**	
	1	2	3	4	5	6	7	8	9
2A	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
2B	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve
2C	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	
2D	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	
2E	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	
2F	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	
2G	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	
2H	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	

Appendix 14: Interpretation of Results; for presence and absence of SGP antibodies, from the S/P percentages for the various study groups.

ANALYSIS FOR POSITIVE AND NEGATIVE RESULTS									
Date **20/05/2022**		Plate No. **Plate 4**		Animal Species; **Ovine and Caprine**				Wavelength **450nm**	
	1	2	3	4	5	6	7	8	9
2A	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
2B	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
2C	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	
2D	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	
2E	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	
2F	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	
2G	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	
2H	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	
	KEY NOTES								
	1. 2A1 and 2B1 are Positive Controls								
	2. 2C1 and 2D1 are Negative Controls								
	3. Sheep samples begin from 2E1 through 2B5								
	4. Goat samples begin from 2C5 through 2B9								