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RISK ASSESSMENT AND MANAGEMENT FOR PESTE DES PETITES RUMINANTS (PPR) IN SUDAN

تقويم مخاطر طاعون المجترات الصغيرة في السودان

By

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DEDICATION

TO ALL COLLEAGUES WORKING IN

ANIMAL DISEASES CONTROL FIELDS

AND

FOR THE PROSPERITY OF SMALL

RUMINANTS PRODUCTION SECTOR

IN MY GREAT COUNTRY SUDAN

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LIST OF ACRONYMS

Abs	Antibodies
AHEDC	General Directorate of Animal Health and Epizootic Diseases Control
AGID	Agar Gel Immunodiffusion
AGPT	Agar Gel Precipitation Test
ALOP	Appropriate Level of Potection
BB	Blocking Buffer
BDSL	Biological Diagnostic Supplies Limited
BP	Base pair
BPS	Buffered Physiological Saline
CBS	Central Bureau of Statistics
CC	Conjugate Control
cDNA	Complementary Deoxyribonucleic Acid
CDV	Canine Distemper Virus
cELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CIEP	Counter immunoelectrophoresis
CIRAD	The International Cooperation Centre in Agronomic Research for Development, France
Defra	Department of Environment, Food and Rural Affair Agency
Df	Degree of Freedom
DIVA	Differentiation of Infected from Vaccinated Animals
DMV	Dolphin Morbilli Virus
DNA	Deoxyribonucleic Acid
EMPRES	Emergency prevention System for Trasboundary Animal and Plant Pests and Diseases Programme
F protein	The Fusion protein
FAO	Food and Agriculture Organization of the United Nations
GATT	General Agreement on Tariffs and Trade
GDP	Gross Domestic Product
GIS	Geographical Information System
H protein	The Haemagglutinin protein
H_2O_2	Hydrogen Peroxide
HA	Hemagglutination Test

HRPO	Horseradish Peroxidase Conjugate
IAEA	International Atomic Energy Agency
IcELISA	Immunocapture Enzyme Linked Immuno-Sorbent Assay
IGAD	Intergovernmental Authority on Development
INFs	Interferons
IPPC	International Plant Protection Convention
L protein	Large Protein (Polymerase)
M	Medium
MAb	Monoclonal Antibody
Ml	Microliter
MoLFR	Ministry of Livestock, Fisheries and Rangelands
M protein	Matrix protein
mRNA	Messenger Ribonucleic Acid
MV	Measles Virus of Humans
Nm	Nano meter
No.	Number
NP	Nucleoprotein
N protein	The Nucleocapsid Protein
NS	Normal saline
ºC	Degree Centigrade
OD	Optical Density
OIE	The International Organization for Animal Heath
OPD	Ortho-Phenylenediamine
OR	Odds Ratio
P protein	Phosphoprotein
PAGE	Electrophoretic Profile in Polyacrylamide Gel
PBS	Phosphate Buffered Saline
PDV	Propoise Distemper Virus
PCR	Polymerase Chain Reaction
PD	Phosphate diluents
pH	Measure of the Acidity or Basicity
PI	Percentage of Inhibition
PMV	Phocine Morbilli Virus

PPR	Peste Des Petits Ruminants
PPRV	Peste Des Petits Ruminants Virus
RNA	Ribo-nucleic Acid
RNP	Ribonucleo-Protein
RPV	Rinderpest Virus
RT	Reverse Transcriptase Enzyme
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SP	Strong Positive
SPS	Sanitary and Phyto-Sanitary
SPSS	The Statistical Package for Social Sciences for Windows
SVRI	Soba Veterinary Research Institute
TADs	Transboundary Animal Diseases
Taq	Thermostable DNA Polymerase
TB	Tuberculosis
TCID	Tissue Culture Infective Dose
$TCID_{50}$	50% Tissue Culture Infective Dose
TCRV	The Tissue Culture Rinderpest Vaccine
UK	United Kingdom
UN	The United Nations
UNEP	United Nation Environmental Programme
USD	United States Dollar
Vero	African Green Monkey Kidney Cell
VNT	Virus Neutralization Test
WHO	World Health Organization
WTO	World Trade Organization
WP	Weak Positive

الملخص

مرض طاعون المجترات الصغيرة من الامراض الفيروسية السارية التي بجانب اصابتها للضأن و الماعز و بعض فصائل المجترات الصغيرة البرية، تم اثبات و جود الفيروس المسبب للمرض في فصائل اخرى مثل الجمال. و هذا المرض يعد من الامراض ذات الاهمية الاقتصادية في السودان. في هذه الرسالة تم التقصي عن بعض عوامل الخطورة المرتبطة بحدوث و انتشار المرض في السودان باستخدام الدراسات الوبائية، بالاضافة لاجراء تقييم اولي نوعي لمخاطر انتشار فيروس طاعون المجترات الصغيرة في سلسلة صادر الضأن من السودان بغرض تحديد سياسات للمكافحة مستندة لدرجة الخطورة.

تشتمل الرسالة على ثلاث دراسات، اولا دراسة معرفة معدل الانتشار المصلى لمرض طاعون المجترات الصغيرة **(PPR)** فى الضأن والماعز و تحليل العوامل التى تساهم فى حدوث و انتشار المرض. جمعت 710 عينة مصل من حيوانات غير مطعمة و تم جمع بيانات باستخدام استمارات تقصى عوامل الخطورة للقطعان المحتلفة من ولايات سنار، القضارف، نهر النيل و شمال كردفان خلال الشهور مايو– يونيو– اكتوبر 2012م و فبراير 2013م على التوالى.
تم تحليل 480 عينة سيرم بواسطة الاليزا فكانت العينات الموجبة كالتالى؛ سنار **57.2%**، القضارف **46.2%**، نهر النيل **34.9%** و كذلك شمال كردفان **39.8%**. و كان معدل الانتشار المصلى الكلى 45.6% (219/480). ادخلت بيانات عوامل الخطورة و هى اربعة عشر عاملا خاصة بالحيوانات و اسلوب التربية و كذلك العوامل المناخية للولايات محور الدراسة فى برنامج التحليل الاحصائى **SPSS** و اجري عليها تحليل احادى المتغيرات باستخدام اختبار **Chi– Square**. اظهر التحليل ان تسعة عوامل لها تأثير على معدل الانتشار المصلى للمرض بدلالة احصائية ضمن مستوى ثقة 95% و **P–value 0.05**؛ وهى العامل الجغرافى للولاية و المحلية حيث كان اعلى معدل انتشار فى ولاية سنار تليها القضارف و فى محليتى ابو زبد بشمال كردفان **91.7%** و بربر في ولاية نهر النيل. اما فى يخص اسلوب التربية و الرعى وجد ان الحيوانات التى تتبع لنظام المرعى المفتوح حول القرى وتحفظ فى حظائر مسورة بالشجيرات الشوكية لها معدل انتشار اعلى. اما العوامل الخاصة بالحيوان فوجد ان المرض اكثر انتشارا في سلالة الكواهلة للضأن، كما ان الاناث اكثر اصابة من الذكور و الحيوانات ذات الاعمار اعلى من 12 شهر لها معدل انتشار اعلى. من بين العوامل المناخية الاربعة و هى درجة الحرارة، معدل الامطار، الرطوبة وسرعة الرياح التى خضعت للتحليل اظهرت النتائج ان معدل الانتشار الاعلى سجل فى الحيوانات الموجودة فى الولايات ذات معدلات الامطار العالية و سرعات الرياح الشديدة خلال فترة الدراسة. خضعت عوامل الخطورة التسعة ذات الدلالة الاحصائية لتحليل متعدد المتغيرات باستخدام **Logistic Regression** و وجد ان خمسة عوامل لها ارتباط احصائي بمعدل الانتشار المصلى لمرض طاعون المجترات الصغيرة ضمن مستوى ثقة 95% و **P–value 0.05** و هى العامل الجغرافى للولاية والمحلية، نظام التربية، الجنس و العمر.
خلصت نتائج الفصل الثاني الى انه يجب مراعاة عوامل الخطورة التى تساهم فى زيادة معدل الانتشار المصلى لمرض طاعون المجترات الصغيرة عند وضع سياسات مكافحة المرض و اختيار زمن تطبيقها خاصة عوامل الخطورة المناخية،

ونوصى بان يتم التطعيم ضد المرض قبل بداية هطول الامطار لجميع الولايات و مراقبة حركة الحيوانات بين الولايات وذلك لاختلاط القطعان المختلفة فى المراعى و نقاط المياه.

ثانيا دارسة حالات (**cases**) و شواهد (**controls**) للتقصي عن بعض عوامل الخطورة البيئية و الوقائية المتعلقة بمرض طاعون المجترات الصغيرة في السودان للفترة من 2008 الى 2012م. شملت الدراسة 114 محلية من 14 ولاية في السودان. قسمت المحليات الي حالات و عددها 47 محلية و شواهد عددها 67 محلية و ذلك حسب بلاغات المرض الواردة لادارة صحة الحيوان ومكافحة الاوبئة بالخرطوم ، حيث ان الحالات تمثل المحليات التي تم بها تسجيل بلاغات خلال سنوات الدراسة الخمس بينما الشواهد هي المحليات التي لم ترد منها بلاغات. جمعت بيانات لسبعة من العوامل الخاصة بالولايات التي تقع فيها المحليات موضع الدراسة. تم رصد و تحليل البيانات با ستخدام نظام الحزمة الاحصائية للعلوم الاجتماعية **SPSS** و العوامل هي؛ النطاق البيئي ، معدل هطول الامطار السنوي، كثافة توزيع المجترات البرية، موقع الولاية على الحدود الدولية، تعداد الضأن و الماعز بالولاية، مساحة الولاية و نسبة تغطية التطعيم ضد طاعون المجترات الصغيرة خلال سنوات الدراسة. عند اجراء التحليل احادي المتغير univariate تم حساب نسبة الارجحية Odds Ratios(OR) باستخدام اختبار Mantel Haenszel و نتج ان ثلاثة عوامل لديها ارتباط احصائي بالمرض و هي و جود المحلية في ولاية تقع على الحدود الدولية (OR = 2.942, *p*–value= .019)، و جود المحلية في ولاية ضمن نطاق السافنا منخفضة الامطار البيئي (OR = 2.134, *P*–value=.052) و التعداد الكبير للضان و الماعز بالولاية (OR = 1.591, *p*– value=.251). ادخلت هذه العوامل الثلاث في تحليل متعدد المتغير multivariate با ستخدام الانحدار اللوجستي logistic regression الذي نتج عنه عامل واحد مرتبط احصائيا بحدوث المرض و هو وجود المحلية ضمن ولاية تقع على الحدود الدولية (*P*– value = .027).

ثالثا دراسة تحليل و تقييم اولي نوعي لمخاطر انتشار فيروس طاعون المجترات الصغيرة في سلسلة الضأن المعد للتصدير من السودان باستخدام منهج المنظمة العالمية لصحة الحيوان لتحليل مخاطر الاستيراد، بعد اضافة بعض التعديلات ليتلائم مع طبيعة المرض المستوطن. و ذلك بالتناغم مع تحليل سلسلة صادر الضأن من السودان من المنتج و حتى التصدير باستخدام الدليل المعد لذلك بواسطة منظمة الامم المتحدة للاغذية و الزراعة الخاص بالسيطرة على امراض الحيوان بطرق مستندة على تقييم المخاطر. خلصت الدراسة الى ان التقييم الكلي لانتشار فيروس مرض طاعون المجترات الصغيرة في صادر الضأن من السودان، يعتبر ذو احتمالية ضعيفة. حيث ان خطورة اطلاق الفيروس في الضأن المعد للتصدير قبل شحنه و دخوله للمحجر النهائي في سواكن قيمت بانها ذات احتمالية متوسطة، مما يعني امكانية انتشار الفيروس خلال السنوات الثلاث المقبلة. و خطورة التعرض للفيروس اثناء شحنه الى و تواجده في المحجر النهائي في سواكن و شحنه الى الدولة المستوردة قيمت بانها ذات احتمالية ضعيفة جدا، مما يعني انه نادر و لا يمكن حدوثه الا في ظروف استثنائية.

و اخيرا تمت صياغة مستخلصات البحث و توصيات مكافحة مرض طاعون المجترات الصغيرة استنادا على نتائج فصول البحث المختلفة.

SUMMARY

Peste des Petits Ruminants (PPR) is a highly contagious viral disease of small ruminants with a confirmed circulation in other species such as camels. PPR is one of the most important economical diseases in Sudan. In the present research some of the potential risk factors associated with PPR were investigated using a cross- sectional and case- control studies, and then a preliminary qualitative assessment for PPR risk in exported sheep was carried out to determine a risk- based control measures.

The thesis is composed of three studies, the first one is a seroprevalence and risk factors of Peste des Petits Ruminants (PPR) were studied in unvaccinated sheep and goats in Sudan. A total of 480 sera samples were collected from the sheep (n=261) and goats (n=219) of Sennar, Gedarif, River Nile, and North Kordofan states during May, June, and October 2012 and February 2013, respectively. The sera were tested for the presence of antibodies against PPR using competitive Enzyme Linked Immunosorbent Assay. The overall seroprevalence of PPR was recorded as 45.6% (n=219/480); whereas, 57.2% in Sennar, 46.2% in Gedarif, 34.9% in River Nile and 39.8% in North Kordofan. A total of 14 risk factors were investigated using structured questionnaire, of which 9 were found to be associated with PPR seroprevalence ($p \leq 0.05$). Among the localities, Abozabad located in North Kordofan had the highest prevalence (91.7%) of PPR followed by Barbar in River Nile. PPR seroprevalence was higher in pastoralists, animals housed in scarp fences, females, and Kwahla sheep. In addition, PPR was higher in the states that had high rainfall and wind-speed. The associated 9 factors were further analyzed multivariably by logistic regression, and finally 5 of them (states, localities, husbandry system, gender, and age) were found to be associated with PPR seroprevalence ($p \leq 0.05$).

The second is a case- control study for PPR outbreaks to investigates some environmental and management risk factors in Sudan in the period from 2008 to 2012. One hundred and fourteen Localities from 14 states out of 15 were divided into cases (n= 47) and controls (n= 67) according to the PPR outbreak history; cases are localities with PPR outbreaks through the five years of the study while controls are the localities which haven't reported any outbreak during the study period. Data about seven risk factors were collected and analysed using SPSS software, the factors are; ecological zone, annual rainfall, wildlife density, location at border with a foreign country, vaccination coverage against PPR, Sheep and goats population and State area. In the univariate analysis Odds Ratios (OR) were calculated using mantel Haenszel test and three factors were found to have a significant association with the occurrence of PPR outbreaks; being a locality in a state at the country borders (OR = 2.942, p-value= .019), or locality in Low rainfall ecological zone (OR = 2.134, P-value=.052) and the factor of having large size population of small ruminant (OR = 1.591, p- value=.251). These potential risk factors were entered into a multivariate analysis using logistic regression and only the factor of being at the border with a foreign country was found to be significantly associated with PPR occurrence (P- value = .027).

The third study is a preliminary qualitative risk assessment for PPRV spread among sheep exports value chain using the OIE frame work for import risk analysis with some adjustments as to fit with an assessment for an endemic disease, together with value chain analysis designed by FAO for disease management. The overall estimated risk for PPR spread in sheep exports value chain was found to be Low. The PPR release risk in sheep value chain,

which represent the probabilities of PPRV existence in sheep herds prior to send to livestock markets, within markets, in addition to the virus probabilities to spread in the internal quarantine, and was found to be Medium, which means that the risky event is likely to occur more than once in the next three years. The exposure risk which represent the probability of PPRV to spread among the sheep herds selected for exportation and it is depending on the contact with an infected sheep or fomites within the transporting trucks to the terminal quarantine or / and in the terminal quarantine or / and in the fomites of the transporting ship to the importing country. In this study the exposure risk was assessed to be very low (V Low), that means the risk of PPRV spread is rare (the risky event may occur in exceptional circumstances).

Finally research conclusions were summarized in the end of the chapters and according to the findings of chapter four and the studied risk factors; control and prevention measures were recommended.

INTRODUCTION

Peste Des Petites Ruminants (PPR) is an acute or subacute viral disease of goats and sheep characterized by fever, erosive stomatitis, congunctivitis, gastroenteritis and pneumonia. Goats are usually more severly affected than sheep. PPR was first described in Cote d`Ivoire in West Africa where it used to be named as kata, pseudo- rinderpest, pneumoenteritis complex and stomatitis pneumoenteritis syndrome (Gopilo, 2005), and it has been reported in Sudan since 1971(Saeed et al, 2004), recently the outbreaks occurrence have increased in different areas in many states in Sudan (Saeed et al, 2010).

PPR is considered as one of the priorities of FAO- Emergency Prevention System for Transboundary Animal and Plant Pests and diseases Programme (EMPRES), which addressed that the total population at risk for PPR represents about 63% of the global small ruminant's population according to the known geographical distribution of the disease (EMRES TADs, Bulletin 34).In eastern Africa list of priority animal diseases, PPR comes first (FAO-ECTAD, Nairobi, 2010).

The epidemiology of PPR in Eastern Africa is less clearly understood, Also the link between PPR patterns and factors that could influence the disease dynamic including socio-cultural and economic factors such as nomadism, transhumance, livestock trade has yet to be fully established (Kihu et al, 2010).Sero-prevalence studies were conducted in four states in Sudan, to explore PPR Abs status in unvaccinated sheep and goats and to investigate risk factors that have an association with PPR occurrence. Many studies of sero-prevalence for antibodies against PPR virus and PPR virus isolation were conducted in Sudan by scientific researchers and veterinary authorities, but there are few ones were done to study the epidemiological and environmental aspects of PPR outbreaks in Sudanese small ruminants and to investigate the risk factors which are contributing to PPR outbreaks occurrence and spread.

Outbreaks of Peste des Petits Ruminants (PPR) occurred annually in Sudan; although most cases are underreported (Saeed *et al.*, 2010). Among all reported outbreaks to the General Directorate of Animal Health and Epizootic Disease Control, PPR outbreaks were taking the first or the second class among the highest number of reported outbreaks during the last 5 years (Anonymous, AHEDC, 2008- 2012).

Infectious disease exhibits classic time- space clustering where case arise at similar time in similar places because of the contagious nature of the disease. This clustering may provide clues to the causes of the disease process and may assist in formulating disease prevention

and control programs (Ward and Carpenter, 2000). There are considerable differences in the epidemiological pattern of the PPR disease in different ecological systems and geographical areas (Gopilo, 2005). According to ecological zones the PPRV will survive longer in dry regions and might thereby engender genetic resistance to infection (Lefevre and Diallo, 1990). Also there are often a number of risk factors that contribute to the overall risk of PPR transmission in a particular community, these factors are quite attributes of the sub-population such as the amount of movement, exchange of animals, distance from services and inter-species contact or interaction with wildlife (Elsawalhy *et al.*, 2005). Concerning seasonal effect on PPR in Africa; the more important epidemics of disease occur in the beginning and the end of the wet season among the settled farmers to the South of the Sahel and these outbreaks are perhaps explained by the variation in the susceptibility of different sheep and goat breeds and by the migration pattern of the Fulani pastoralists (Grenfell and Dobson, 1998). On the other side higher incidence of PPR were observed during the dry months of December and January in West Africa (Okoli, 2003).

The morbidity rate of PPR increases with environmental stress such as confinement of animals during winter and rainy seasons. However the effects of environment on PPR occurrence are solely based on the nature of animal husbandry conditions and socio-economic status of the owner (Munir, 2013).

Sudan is an exporting country for livestock and livestock products. The livestock sector in Sudan is an important contributor to the national economy, accounting for 25% of the gross domestic product (GDP) and employing 40% of the country population. Livestock exports represent the second generating source of foreign exchange currency after oil. The majority of sustained Sudanese exports of live sheep and sheep meat are to The Kingdom of Saudi Arabia. Although Sudan has the advantage of being near the Gulf market for sheep and sheep meat, it faces competition from Australia and other countries in terms of price, reliability of regular supply and terms of promotion and trade (ElDirani *et al*, 2009).

Since 1995 Sudan is an observer and still on procedure to gain World trade Organization (WTO) ` membership. Being a WTO` member may assist Sudanese livestock exporting sector to find new markets internationally, hence the meat of Sudanese livestock is of high quality and its production is depending on natural rangelands. But before joining WTO the country needs to improve the livestock production, local marketing systems, animal health, quarantine systems and infrastructures. National sanitary and phytosanitary measures (SPS) measures also need to be revised, updated and based on scientific risk analysis to ensure high

quality for exports and to protect national human, animal and plant health and life against the imported commodities. This preparedness comprises beside the financial resources, it comprises a technical and scientific support by national expertise for livestock production, animal health, risk analysis and trade facilities (Salih, 2007).

The WTO` agreements are the legal foundation for the international trading system that is used by the bulk of worlds trading nations, and they were generated from the 1986-1994 Uruguay round of world trade negotiations held under the auspices of what was then the GATT (The General Agreement on Tariffs and Trade)(WTO, 2010). The WTO` agreement on the application of sanitary and phytosanitary measures (SPS) aims to help trade flow and ensure that the measures established by member`s government to protect human, animal and plant health or life; are consistent with obligations prohibiting arbitrary and discrimination on trade between countries where same conditions are prevailed. WTO SPS agreement has chosen the standards of three organizations to be adopted by WTO members as international standards for trade. The organizations are FAO/WHO Codex Alimentarius Commission (CODEX), International Organization of Animal Health (OIE) and The International Plant Protection Convention (IPPC). OIE has recommended the import risk analysis as the formal tool for determining the Appropriate Level of Protection (ALOP) for the member state (Salih, 2007).

Risk analysis is a tool intended to provide decision- makers with more complete information in order to make informed decisions and to assess decision impacts regarding international trade (Miller *et al.*, 1993).The risk is defined as a measure of the likelihood and magnitude of an adverse event that caused by a hazard as the entry, establishment and spread of a disease agent through the importation of a commodity (Morley, 1993).

A formal framework for risk analysis in veterinary science was developed specifically to provide an objective method for making decision on inter-country trade, but it is also applicable to other areas of animal disease control and more widely to other risky decision contexts. The recommended risk analysis by OIE consists of four components: Hazard Identification, Risk assessment, risk management and risk communication (FAO, 2011).

Risk analysis is very important in veterinary sector since OIE has recommended it as the tool for assessing imports of animal and animal origin, as described in the terrestrial animal health

code, chapter (2). On the national level, risk analysis constructs a basis for transparency leading to scientific evidence- based strategies for diseases control, and constitutes a major role in the national early warning system for TADs and other emerging diseases. Risk analysis is equally applicable to other areas of decision making than importation, such as those affecting disease surveillance or control programs (MacDiarmid and Pharo, 2003). Hence, using of risk assessment is very crucial for exporting countries because it provides a tool for the analysis and characteristics for regionalization (Miller, 1993). Regionalization now known as zoning and compartmentalization which are procedures implemented by a member country under the provision of the OIE code, while zoning applies to an animal subpopulation defined primarily on geographical basis(using natural, artificial or legal boundaries), and compartmentalization applies to an animal subpopulation defined primarily by management and husbandry practices related to Biosecurity (OIE, 2014).

Movement of livestock and their products in different value chains is an important means of disease spread. Value chain is defined as a group of people linked by an activity to supply a specific commodity such as livestock and its products. Livestock value chain analysis is aiming to:

- identify the main groups and organizations work in livestock from input supplier to, producer, trader, processor, retailer through to final consumer,
- mapping the different routes to market and assess how well the market chain is working.

Risk analysis when combined to value chain analysis will help in understanding these movements which must be taken into account in setting management strategies (FAO, 2011).

Many studies of sero-prevalence for antibodies against PPR virus and PPR virus isolation were conducted in Sudan by scientific researchers and veterinary authorities, but few studies were done to study the epidemiological and environmental aspects of PPR outbreaks in Sudanese small ruminants. So this research is aiming to study the risk factors that are associated with the outbreaks occurrence, and to assess the risks of PPR in the different value chains of sheep and goats so as to suggest and address a scientific risk- based control measures to prevent and mitigate the burden of PPR outbreaks among the vulnerable producers and sheep and goats owners.

Research objectives:

The research was aiming to assess the PPR risks and suggesting the most effective control measures for PPR in Sudan, by achieving the fallowing objectives:

-Studying some of the risk factors that are contributing to PPR occurrence and spread in Sudan, by carrying out a seroprevalence study and a case-control study to investigate some of the ecological, environmental and management potential risk factors that might be associated with the occurrence of PPR outbreaks in different localities in Sudan states.

- Developing a qualitative assessment for PPR risks, analyzing risk factors and determining the risk hotspots in sheep production value chains, which may lead to the exposure and spread of PPR Virus among the exported herds,

- Suggesting evidence- based risk management measures that may ensure the health status of Sudanese exports of live sheep, and could be used as national SPS measures in contribution to the current PPR control efforts.

CHAPTER ONE:

LITERATURE REVIEW

1.1 Epidemiology of PPR

1.1.2 History and geographical distribution

Peste des petites ruminants (PPR) was first described in Cote d` lvoire, West Africa by Gargadenne and Lalanne in 1942 where it used to be named pseudorinderpest, Kata, stomitis-pneumoenteritis syndrome, (Shuaib, 2011). Then it was confirmed in Nigeria, sengal and Ghana. For many years PPR was restricted to West Africa until it was discovered in Sudan (Gopilo, 2005). In 1980-1982 PPR was reported in the east of African continent, in Sudan. In 1987 it spread to India and Abu Dhabi. During the last years the disease was reported in the Middle east and in Arabian Peninsula, and there is serological prove of the disease in Syria and Turkey (Kaukarbayevich, 2009).

Global distribution

The development of trade relation, transport, tourism and migration of wild animals susceptible to PPR contribute to the spread of the disease. In the present time PPR is reported in almost all countries of Central, Middle and South Asia, in the countries of Middle East and African continent such as Nigeria, Benin, Togo, Chana, Senegal, Sudan, India, Abu Dhabi, Mali, Guinea, Liberia, Cote De Voir, Cameroon, Ethiopia, Yemen, Oman, Turkey, Iran, Afghanistan, Pakistan, Saudi Arabia, Chad, Democratic Republic of Congo and Central African Republic (Kaukarbayevich, 2009).

Distribution of PPR of disease in Sudan

The first outbreaks of PPR in sheep and goats that had occurred in Sudan were reported as Rinderpest (RP) in three areas; in Southern Gedarif state (Eastern Sudan) in 1971, then in Goats in Central Sudan during 1971-1972 (Elhassan *et al*., 1994). Since then, PPR outbreaks continued to be reported in Darfur, Central Sudan and Khartoum state (Saeed *et al*., 2004).
Also PPR was detected and isolated from the states of Gezira, White Nile, Khartoum, North Kordofan and River Nile during 2000-2002 (Saeed et al. 2010).

PPR outbreaks were investigated in Gezira, White Nile, Khartoum, Kordofan and River Nile states during the period 1999-2001, to study the morbidity and mortality rates of PPR as shown in Table (1) (Saeed et al. 2004).

Table (1): Occurrence of PPR outbreaks in Sudan, location, date, morbidity and mortality rates during the years 1999 and 2000.

State	Area	Date	Morbidity%	Mortality%
Gezira	Azaza	Feb1999	24	11.5
White Nile	Gitaina	Apr 2000	22	15
Khartoum	Abudelaig	Jun 2000	30	17.2
Khartoum	CVL	Jul 2000	33.3	13.3
khartoum	Soba	Aug 2000	23	10
North Kordofan	Goaz hamad	Sep 2000	27	12
Khartoum	Kuku	Feb 2001	27	19
River Nile	Eldamar	Mar 2001	31.5	21
Mean%			27.2	14.9

In 2001, a study was carried out to estimate the prevalence of antibodies (Abs) against PPR virus in nine different states of Sudan, by collecting 1005 serum samples and using competitive ELISA to detect antibodies to PPR as shown in Table (2)(Mohammed, 2008).

Table (2): Seroprevalence of PPR in nine different states of Sudan during 2001

State	Ovine				Caprine			
	No. samples	(+) ve	(-) ve	(+) %	No. samples	(+) ve	(-) ve	(+) %
West Kordofan	80	47	33	59	20	8	12	40
South Kordofan	115	71	44	62	65	17	48	26
Sinnar	80	47	33	59	20	5	15	25
White Nile	80	40	40	50	20	8	12	40
Khartoum	150	93	57	62	50	23	27	46
River Nile	66	41	25	62	64	0	34	0
Kassala	75	49	26	65	25	12	13	48
Red Sea	80	59	21	74	20	2	18	10
West Bahar Algazal	25	13	12	52	-	-	-	-
Total	751	460	291	61	254	75	179	30

Another study was conducted to investigate the seroprevalence of PPR, by collecting 519 serum samples from sheep and goats during 2001-2003 in six different states in Sudan, as shown in Table (3)(Osman et al. 2009).

Table (3): Prevalence of PPRV antibodies in sheep and goats sera tested by c-ELISA, 2001-2003.

State	No of samples	Positive %	Negative %
River Nile	53	33.96	66.04
Darfur	63	49.21	50.79
Blue Nile	81	60.49	39.51
Khartoum	136	55.88	44.12
Southern	106	52.83	47.17
Kordofan	80	41.25	58.75

The situation of PPR in Sudan was investigated during suspected outbreak in sheep in 2008. A total of 1198 serum samples and 61tissue samples were collected from sheep, goats and

camels, then the sera were examined for PPR antibodies using C-ELISA, and the tissue samples were examined for PPR antigen detection using IcELISA as shown in table (4), (5) and (6)[Saeed et al. 2010].

Table (4): Detection of PPR antigen in tissue samples in Sudan during 2008 using IcELISA.

State	Total tested	No. of positives	%	No. of negatives	%
Khartoum	23	5	21	18	79
River Nile	1	1	100	0	0
Gezira	11	3	27	8	73
Gedarif & Kassala	2	2	100	0	0
Kordofan & Darfur	24	15	62	9	38
Total	61	26	42	35	58

Table (5): Seroprevalence of PPR in sheep and goats sera in Sudan during 2008 using cELISA

State	Ovine				Caprine				Total	
	tested	(+) ve	(-) ve	(+)%	Tested	(+) ve	(-) ve	(+) %	Tested	(+) %
Khartoum	16	15	1	93.8	160	90	70	56.3	176	59.7
River Nile	60	32	28	53.5	12	5	7	41.7	72	51.4
Gezira	118	86	32	72.9	105	61	44	58.1	223	65.9
Gedarif & Kassala	55	50	5	90.9	7	0	7	0	62	80.6
Kordofan & Darfur	251	153	98	61	22	14	8	63.6	273	61.2
Total	500	336	164	67.2	306	170	136	55.6	806	62.2

Table (6): Detection of PPR antibodies in camel's sera in Sudan during 2008 using cELISA

State	Total tested	No of positives	%	No of negatives	%
Northern Sudan (River Nile)	11	0	0	11	100
Central Sudan(Tambool)	278	0	0	278	100
Eastern Sudan(PortSudan, Halfa Elgadida)	71	1	1.40	70	98.59
Western Sudan (Kordofan)	32	0	0	32	100
Total	392	1	0.25	391	99.74

The highest number of reported outbreaks of PPR during the period from 2008 to 2012 was received from Kassala state in the Eastern region of Sudan, followed by River Nile state, North and South kordofan and Northern states. Few outbreaks were reported by sates of Khartoum, Gezira, Sennar and White Nile in central and South Sudan. Relatively, very few outbreaks reported in Red Sea, Gedarif, Blue Nile and Darfur states (Anonymous, AHDEC, 2008- 2012).

1.1.2 Causative agent

PPR virus has been classified under family Paramyxoviridae, order Monoegavirales and genus Morbillivirus. It is an enveloped pleomorphic particle (Chauhan, 2009). The genus of Morbillivirus also includes other six viruses; Measles virus (MV), Rinderpest virus (RPV), Canine distemper (CDV), Phocine morbillivirus (PMV), Propoise distemper virus (PDV) and Dolphine morbillivirus (DMV) (Gopilo, 2005).

Under the electromicroscope, morbilliviruses display the typical structure of paramyxo viridae (Gopilo, 2005). PPRV virion varies in size from 150 to 700 nm. The virions contain a negative – stand RNA genome enclosed in a ribonucleoprotein (RNP) core. The genomic RNA is packaged by nucleoprotein (N) to form nucleocapsid along with phosphoprotein (P) and large protein (L) (Kumar et al., 2014). The virus structure shown in Figure 1. The Matrix protein (M) are basic membrane associated molecules that interact with surface glycoproteins in the lipid envelope as well as the virion RNP. F protein is a glycosylated protein in the

envelope, that constitute the peplomers or surface projections. H protein is responsible for attachment of the virus to the host cell. The L protein is the enzymatic component of the viral transcriptase and replicase (Gopilo, 2005).

PPRV is fragile and it cannot survive for long time outside the host. Its half life has been estimated to be 2.2 minutes at 56 C and 3.3 hours at 37 C (Chauhan, 2009) but the virus has a long survival time in chilled and frozen tissues. PPRV is stable between pH 4.0 and pH 10.0. The virus is killed by alcohol, ether and detergents as well as by most disinfectants; phenol and sodium hydroxide (Shuaib, 2011).

PPRV has four lineages, lineage I and II are restricted to western and central Africa; lineage III is common in Eastern Africa and the southern part of the Middle East. In Asia only viruses of lineage IV have been detected (Kwiatek et al., 2011).

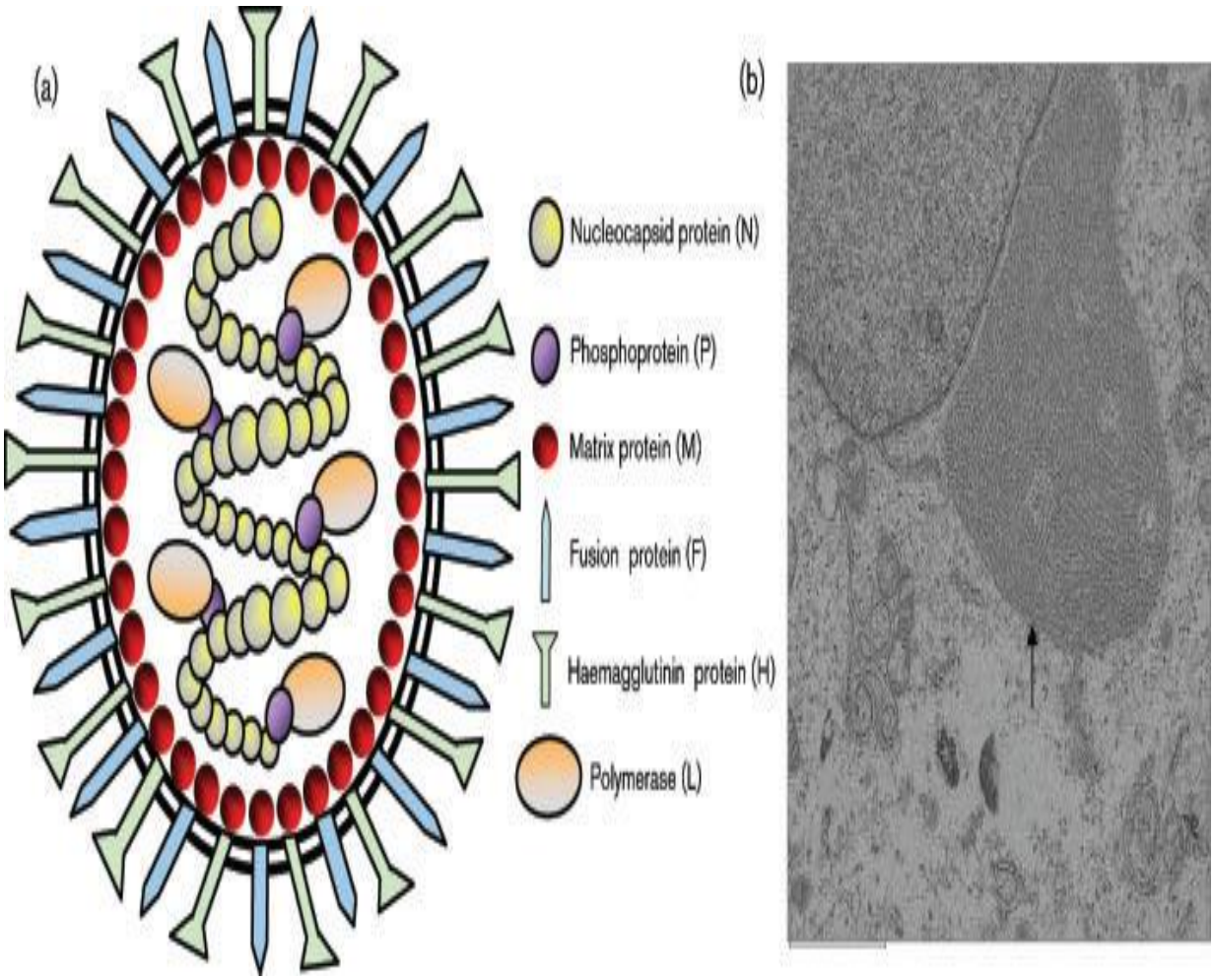

Figure 1: Structure of *Morbillivirus*. (a) A schematic diagram of morbillivirus virion structure. (b) Electron micrograph of viral ribonucleoprotein (RNP) present within an RPV-infected cell. The RNP is clearly seen as a typical 'herring-bone' structure (arrow) and is known to contain viral RNA and proteins within the cytoplasm. Bar, 2000 nm, ((Shuaib, 2011).

1.1.3 Transmission

Transmission requires close contact between infected animals in febrile stage and susceptible animals because of the lability of the virus outside the living host. The discharges from eyes, nose and mouth, as well as the loose faeces, contain large amounts of the virus (Gopilo, 2005). Fine droplets from secretions and excretions especially when sick animals cough or sneeze, are infective for animals in close contact when inhaling these droplets. It is suspected that infectious materials can also contaminate water, feed troughs and bedding to be additional source of infection, however these sources are infective for short term since PPRV has a low resistance in the environment (Lefevre and Diallo, 1990).

1.1.4 Host range

PPR is a disease of small ruminant; sheep and goats (Lefevre and Diallo, 1990). PPR affect wildlife animals and it cause high mortality and sever disease in Dorcas gazelles (Gazalla dorcas), Nubian Ibex (Capra ibex nubiana), Laristan sheep (Ovis orientalis laristani), gemsbok (Oryx gazella) and other small wild ruminant species (Gopilo, 2005). PPRV was isolated in Sudan from camels during an outbeak in sheep and goats and camel in 2004 (Kwiatek et al., 2011). PPRV can cause high morbidity and mortality in buffalos and camels. Also a silent infection was observed in pigs that come in contact with infected goats, but pigs cannot transmit the virus. Cattle may be infected without showing any clinical signs on experimental inoculation (Shuaib, 2011).

1.1.5 Immunity

The surface glycoproteins haemagglutinin (H) and fusion protein (F) of Morbilliviruses are highly immunogenic and confer immunity. PPRV is antigenically closely- related to Rinderpest virus (RPV) and antibodies against PPRV are both cross- neutralizing and cross protective (Gopilo, 2005).

PPR infection in goats leads to a classic inflammatory response characterized by enhanced expression of cytokines such as Interferones (INFs). Morbilliviruses have been well known to inhibit IFN signling, during its infection severe immunosupression occurs with massive virus-specific immune response.

Attenuated Morbillivirus vaccines induce cell mediated immunity which may be important for protection. Passively acquired maternal antibodies against PPRV in kids are usually detected up to 6 months with gradual declining trend starting from the third month onwards. The protective titres are maintained until the fourth month. As maternal antibodies can interfere with vaccination, kids born from PPRV exposed or vaccinated mothers must be immunized after the third or fourth month of age (Kumar et al., 2014).

1.1.6 Clinical signs

The incubation period is 3- 4 days, during which the virus replicates in the draining lymph nodes of the oro-pharynx before spreading via blood and lymph to the tissues and organs including the lungs causing a primary viral pneumonia (Gopilo, 2005). Pre-acute signs are seen when PPRV first occur in naïve populations, and the signs are high fever, severe depression and death. In the acute form signs include sudden high fever of 40-41 C, depression and hair standing erect, redness in the mouth and eyes, epithelial necrosis causes small pin-point grayish areas on the gum, dental pad, palate, lips, inner surface of cheeks and upper surface of the tongue (Shuaib, 2011). Affected animals also exhibit dry muzzle and serous nasal discharge which become mucopurulent. Erosions on the mucous membrane of the buccal cavity accompanied by marked salivation. Conjuctivitis with ocular discharge is a feature of the disease. A profuse Diarroehea, which results in dehydration. Pregnant females may abort and signs of tracheitis and pneumonia are common, and there is a severe leucopenia which facilitates secondary bacterial infection such as pulmonary infection caused by Pasteurella species (Chauhan et al., 2011). The subacute form also lead to a fatal outcome 14-21 days after the onset of the febrile phase. A subclinical form of PPR which almost a symptomatic and appears especially in dry areas of Central Africa and it is supposed to be a predisposing factor for other lung affections (Seifert, 1996).

1.1.7 Pathology

Pathogenesis

The pathogenesis of PPRV is poorly understood and based on comparison with related Morbilliviruses. During PPR infection, the virus initially taken up by antigen presenting cells (APCs) present in the intraepithelial space and lamina propria of the respiratory mucosa (Kumar et al., 2014), from where it is transported to regional lymphoid tissues where the virus replicates then spreads via lymph and blood. PPRV is both lympho- and epithelio-

tropic (Gopilo, 2005). The infection usually results in conjunctivitis, rihnotracheitis, ulcerative stomitits, gastroenterititis and pneumonia. Also PPRV leads to extensive necrosis in lymphoid organs; Peyer`s patches, spleen, thymus and pulmonary lymph nodes, and hence infection with PPRV causes reduction in circulating peripheral blood leucocytes (leucopenia) (Kumar et al., 2014).

Histopathology

PPR virus causes epithelial necrosis of the mucosa of the respiratory and alimentary tracts marked by the presence of esinophilic intracytoplasmic and intraneuclear inclusion bodies (Gopilo, 2005). PPRV produces characteristic cytopathogenicity similar to other Morbilliviruses like a large number of multinucleated giant cells (synsytia) in the lymph nodes, splenic white pulp and gastrointestinal submucosal lymphoid tissues. The syncytia are followed by necrosis/ apoptosis. Squamous epithelial syncytia are also observed in digestive tract epithelium and tonsillar and facial tissues. Necrotic lesions in the intestinal lymph nodes probably lead to diarrhea (Kumar et al., 2014).

Postmortem findings

The carcass of an affected animal is usually emaciated, the hinder quarter soiled with soft/ watery faeces and the eye balls sunken. The eyes and nose contain dried up discharges. Nasal cavity is congested with clear or creamy yellow exudates and erosions. Lymph nodes associated with lung and intestines are soft and swollen (Chauhan et al., 2009). Large intestines have small haemorrhages along the fold of the lining results in the characteristic Zebra- striped appearance (Shuaib, 2011), and small intestine are congested with lining haemorrhages and some erosion (Chauhan et al., 2009).

1.1.8 PPR risk factors

There are often a number of risk factors that contribute to the overall risk of PPR disease transmission in a particular community, production system or value chain. These risk factors are often quite simple attributes of sub-population such as; the frequency of movement, trade in animals, distances from services and inter species contact or interaction of wild life. When the nature and distribution of risk factors for transmission and maintenance of an agent are known, it becomes possible to target surveillance and control measures to high risk settings. This maximizes the impact and minimizes the cost. The risk factors for transmission and

maintenance of PPR are partially understood, but more information on the interaction of wild life and livestock as well as on the role of specific production system/ activities would contribute to effective targeting (Elaswalhy et al, 2010).

The appearance of PPR may be associated with any of the following:

- History of recent movement or gathering together of sheep and goats of different ages with or without changes in housing and feeding,
- Introduction of recently purchased animals,
- Change in weather such as the onset of the rainy season (hot and humid) or dry cold periods (e.g. the HARMATTAN season in West Africa),
- Contact with trade or nomadic animals through shared grazing, water and or housing,
- A change in husbandry (e.g. towards increased intensification) and trading practices.

The development of trade relations, transport, tourism and migration of wild life animals susceptible to PPR contribute to the spread of the disease beyond the boundaries of Western Africa. Recently PPR was reported in almost all countries of central, middle and south Asia, in countries of Middle East and African countries.
PPR epizootic situation is characterized by cyclic recurrence with periods of 7 and14 years. The disease seasonality in all geographic zones is not clearly apparent. The increase of morbidity rates is mainly observed during the years with unfavorable weather conditions and poor fodder (Kaukarbayevich, 2009).

An extensive outbreak of PPR had been reported from different localities in Sudan during the period between 1989-1990. The outbreak occurred during the cold months of the year (autumn and winter). The change of humidity and ambient temperature might have contributed to the maintenance of the outbreak, also acute cases were found to harbor heavy parasitic infestation (Elhassan et al, 1994).

Some authors suggested that a more severe disease results from mixed infection of bacteria and viruses than a single infection. Nutritional and environmental factors have important effect on the appearance of PPR disease in a flock of animals, on the other hand Saliki (1998) previously reported that poor nutrition status, stress of movement and concurrent parasitic and bacterial infections enhance the severity of clinical signs(Osman et al, 2009).

The prevalence of PPR in states of Sudan under study, indicated wide spread of the disease in Northern, Southern, Western and central Sudan. It was observed that the prevalence of PPR antibodies was higher in states near the borders of the country. These would attribute to animal movement between Sudan and neighbouring countries (Osman et al, 2009). The geographical factor of the state or the province and of localities or counties within the same state; has a significant effect on PPR prevalence (Shuaib, 2011and Muse et al, 2012).

In Sudan due to the nomadic nature of most of animal herders the spread of infectious diseases depends on seasonality where during rainy seasons (July- October). Most of animals are sharing water sources leading to spread of infectious diseases (Saeed et al, 2010).
In endemic areas, most of the sick and dying animals are over 4 months and up to 18 to 24 months of age (Kaukarbayevich, 2009). PPR prevalence is found to be higher in lambs between 4 to 12 months, followed by sucklers (1 to 3 months), while the least prevalence found in animals more than one year (Sarker and Islam, 2011). Zahur et al (2009) has found similar association between PPR prevalence and age categories.

PPR seroprevalence was found to be higher in female animals than in males (Shuaib, 2011 and Abdalla et al., 2012) but Sarker and Islam (2011) have reported that PPR prevalence was significantly higher in males than in females, and goats are found to be more susceptible to PPR virus infection than sheep (Abd El-Rahim et al., 2010).

There is a significant association between PPR seroprevalence and the geographical location represented in states and localities (Shuaib, 2011).

Shuaib (2011) and Sarker and Islam (2011) have found a significant association between the different breeds of sheep and goats and the variations in PPR prevalence.

The stress of animal migration, coupled with low environmental temperature, and blostered by humidity and nutritional deficiency may contribute to the occurrence of PPR disease (Abd El-Rahim et al., 2010).

The seasonal variation is practically responsible for the occurrence of PPR, and the dusty dry winds that characterize winter season; has shown to enhance the spread of PPR(Sarker and

Islam, 2011). And the same finding was confirmed by Abdalla et al (2012). Grenfell and Dobson (1998) stated that widely spread epidemics of PPR occur in the beginning and end of the rainy season among the settled farmers.

1.2 Diagnosis

Clinical differential diagnosis is not possible as PPRV produces signs that are similar to those caused by other viruses in small ruminants, Therefore clinical diagnosis should be confirmed by laboratory analysis (Gopilo, 2005). A provisional diagnosis of PPR can be made from epidemiological and clinical features. The characteristic post mortem changes would further strengthen the provisional diagnosis (Shuaib, 2011).

PPR diagnosis is performed by; virus isolation, detection of viral antigene, nucleic acid sequencing and detection of specific antibodies in serum (Gopilo, 2005).

1.2.1 Virus Isolation:

Samples for virus isolation include heparinized blood, eye and nasal swabs (from live animals), tonsil, mesenteric lymph nodes, spleen, section of colon and lung. For successful isolation, samples must be collected during the hyperthermic phase (Gopilo, 2005). The most widely used cell culture systems are primary lamb kidney, ovine skin and Vero cells (Shuaib, 2011).

Once isolated in cell culture, a candidate PPRV may be identified by one of the three procedures:

• animal inoculation: PPR causes clinical disease in goats and sheep but not in cattle ,

• reciprocal cross neutralization (differential neutralization): PPRV is neutralized by both PPR and RPV reference sera, but is neutralized at greater titre with the homologous serum,

• molecular techniques: cDNA probe, electrophoretic profile in polyacrylamide gel (PAGE) and PCR (Gopilo, 2005).

1.2.2 Antigen detection techniques:

Agar Gel Immuno-diffusion Test:

AGID is relatively simple, fast, cheap, and can be performed in any laboratory and even in the field. Standard antiserum is made by immunizing sheep with 5 ml of PPR virus with a titer of 10^4 $TCID_{50}$ (50% tissue culture infective dose) per ml, given at weekly intervals for 4

weeks. The animals are bled 5-7 days after the last injection. Standard RP hyperimmune antiserum is also effective in detecting PPR antigen. One of the important advantages of this test is that it is highly specific (92%) (Shuaib, 2011).

Hyperimmune serum:

Conventional serological techniques and virus isolation are normally used to diagnose morbillivirus infection in samples submitted for laboratory diagnosis. However, such techniques are not suitable for use on decomposed tissue samples, the polymerase chain reaction (PCR), has proved invaluable for analysis of such poorly preserved field samples (Gopilo, 2005).

Counter immunoelectrophoresis:

CIEP is the most rapid test for viral antigen detection. The test is carried out on the same principle as the AGID, using the same reagents, except that the gel is electrically charged to improve the sensitivity of the test (Shuaib, 2011).

ELISA for antigen detection:

A monoclonal antibody-based sandwich ELISA was found to be highly sensitive in detection of antigen in tissues and secretions of infected goats. The main advantages of this assay are:

• Rapidity, it can be performed in a precoated plate in less than 2 hours;
• Specificity;
• Robustness, it can be carried out on samples which have not been kept under ideal conditions and where no viable virus is present;
• Simplicity.

The immunocapture ELISA is suitable for routine diagnosis of rinderpest and PPR from field samples such as ocular and nasal swabs (Gopilo, 2005).

cDNA probes:

It could differentiate between the two viruses without need for virus isolation. cDNA directed against the matrix protein, fusion protein and phosphoprotein gene were found to cross-hybridize to a much greater extent and were not suitable for use as discriminating probes. Unfortunately, this hybridization cannot be used widely because it requires fresh specimens

and in addition to the short half life of [P^{32}] there are constraints with the handling of isotopes (Shuaib, 2011).

Reverse transcription polymerase chain reaction (RT-PCR):

The PCR technique has been the most popular and highly sensitive tool so far for diagnosis of PPR. Conventional serological techniques and virus isolation are normally used to diagnose morbillivirus infection in samples submitted for laboratory diagnosis (Shuaib, 2011).
The method consists of repetitive cycles of DNA denaturation, primer annealing and extension by a DNA polymerase effectively doubling the target with each cycle leading, theoretically, to an exponential rise in DNA product. The replacement of the polymerase Klenow fragment by thermostable polymerase derived from Thermus aquaticus (Taq) has greatly improved the usefulness of PCR. Using this system, a rate of amplification up to 107 to 109 times has been reported. The efficiency achieved can vary enormously, however, since it is dependent on factors such as the number of cycles, the quantity of the starting material, the length of the target DNA, the temperature conditions of annealing and priming, and the polymerase used. When the starting material is DNA, high purification of the nucleic acid is not necessary so the procedure is greatly simplified. These qualities have made the PCR one of the essential techniques in molecular biology today and it is starting to have a wide use in laboratory disease diagnosis (Gopilo, 2005).

1.2.3 Serology:

Virus neutralisation:

The virus neutralisation test (VNT) is sensitive and specific, but time-consuming and expensive. The standard neutralisation test is carried out in roller-tube cultures of primary lamb kidney cells or Vero cells when primary cells are not available. VNT is the most reliable test for detection of morbillivirus antibodies. Serum against either PPR or RP may neutralise both viruses, but would neutralize the homologous virus at a higher titre than the heterologous virus. Therefore for differentiation purpose reciprocal cross neutralization is used (Gopilo, 2005).

cELISA:

Competitive and blocking ELISA based on monoclonal antibodies specific for N-protein (Libeauet al., 1995) and H-protein (Gopilo, 2005). These tests either used gradient purified

virus or expressed antigens. In the N-protein cELISA, the serum antibodies and the MAb compete on specific epitope on nucleoprotein obtained from recombinant baculovirus. Though no cross reaction in N-protein cELISA was reported,

A high level of competition up to 45% was observed among the negative (Libeau et al., 1995). Despite the fact that neutralizing antibodiesare not directed against the N-protein, but the H-protein (Gopilo, 2005), a correlation of 0.94 between VNT and cELISA was observed suggesting that the former was more sensitive (Libeau et al., 1995). The relative sensitivity of this cELISA to VNT was 94.5, while the specificity was 99.4%. Both blocking ELISA and cELISA detecting anti-H antibodies are based on competition between an anti-H monoclonal antibody (MAb) and serum antibodies, but in case of blocking ELISA the test sera are preincubated with antigen and then incubated with the MAb. The sensitivity and specificity of the H-blocking ELISA were found to be 90.4% and 98.9% respectively. PPR cELISA using MAb directed against the H-protein cross reacted to some extent with rinderpest, while RP cELISA is specific, therefore an animal was assumed to have experienced RP if it is positive in both PPR and RP ELISA. The protocol of cELISA. The absorbance in PPR ELISA is converted to percentage of inhibition (PI) using the formula: PI=100-(absorbance of the test wells/ absorbance of the MAb control wells) x 100. Sera showing PI greater than 50% are scored positive. The overall specificity of c-ELISA test was 98.4% with a sensitivity of 92.2% when compared with VNT. The diagnostic efficacy of the assay in terms of sensitivity and specificity was calculated using two-sided contingency table (Gopilo, 2005).

1.3 Treatment of PPR

There is no treatment for the PPR as a viral disease but broad spectrum antibodies and sulphanomides can be used to control the secondary infections of enteritis and bronchopneumonia in order to influence the course of disease favourably (Seifert, 1996).

1.4 Prevention and Control of PPR

Currently PPR is one of the priorities subsequent to Rinder pest for international organizations like FAO, OIE and IAEA to control and finally eradicate it (Kumar et al., 2014). Controlling of PPR may seem to be relatively easy compared to other economically viral diseases, such as foot and mouth disease and blue tongue. This may be attributed to high antigenic stability, single serotype of the virus and the induction of a lifelong immune response after vaccination (Singh, 2011).

RPV vaccine has been used for PPRV control in the Sudan for many years in the past. However, RPV (rinderpest) vaccination campaigns were recently stopped in the course of

affirming African countries as RPV free. Concurrently with the rinderpest campaign, vaccination against PPRV using a homologous vaccine produced locally in the Soba Veterinary Research Institute (SVRI) was established in 2002. A plan to control PPRV was established, but organized vaccination campaigns are not yet practiced (Shuaib, 2011).

Vaccination is considered as the most effective way of controlling PPR (Kumar et al., 2014). Currently used vaccines require effective cold chains and hence high costs are required to conduct vaccination campaign. To reduce the costs of vaccination, it would be advisable to not only use a thermo-resistant vaccine but also a polyvalent vaccine for the control of other important disease together with PPRV. The thermo-stability of the current PPRV homologous vaccine has been dramatically improved by a new freeze-drying process and addition of stabilizing agents (Shuaib, 2011).

A single dose of PPR vaccine containing ~$10^3 TCID_{50}$ of vero cell attenuated PPRV, is believed to provide protective immunity in sheep and goats for about 4 years (Kumar et al., 2014).

The approach to controlling PPR relies on animal movement control combined with vaccination (Chauhan et al., 2009). Vaccination can be divided into three inter- dependent stages, based on prioritizing available resources. These stages are; 1) Reducing disease intensity through vaccinating targeted populations, 2) Controlling PPR by intensive vaccination, 3) Implementing mass vaccination campaigns that provide high levels of vaccination coverage. In case of eradication, it is important and preferable to use marker vaccines or chimeric vaccine for differentiating infected from vaccinated animals (DIVA) (Singh, 2011).

Eradication of PPR could be achieved and there are several aspects that assist in eradication such as; there is only one serotype of PPRV and it is believed that perfect cross protection appears to exist within strains from different lineages. Also the virus does not survive for a long period of time outside the host, as it is readily destroyed by heat and sunlight and hence needs continuous source of susceptible animals for survival. It is very important in the eradication process to consider and understand the role of other ruminants -whether wild or domestic - in the maintenance of PPRV (Kumar et al., 2014).

1.5 PPR economy

PPRV is currently considered as one of the main animal trans-boundary pathogens that constitute a significant threat to livestock production in developing countries. In those areas affected by the disease, PPR is considered a major limiting factor in the development of the small ruminant industry. This is especially evident in many countries in Africa and Asia where sheep and goats play an integral role in sustainable agriculture and employment (Shuaib, 2011). The PPR epidemics can cause mortality rates of 50–80% in naive sheep and goats populations. Due to the confusion with other diseases, the economic impacts of PPR are probably underestimated, but it is believed that PPR is one of the major constraints of small ruminant farming in the tropic (Gopilo, 2005).

1.6 Risk analysis and Value Chain analysis

Risk analysis is very important in veterinary sector; hence OIE has recommended it as a tool for assessing imports of animal and animal products, as described in the terrestrial animal health code, chapter (2). On the national level, risk analysis constructs a basis for transparency leading to scientific evidence- based strategies for diseases control, and constitutes a major component in the national early warning system for TADs and other emerging diseases. Risk analysis is equally applicable to other areas of decision making beside importation, such as those affecting disease surveillance or control programs (MacDiarmid and Pharo, 2003). Hence, use of risk assessment is very crucial for exporting countries because it provides a tool for the analysis and characteristics for regionalization (Miller, 1993).

Control of Transboundary Animal Diseases (TADs) demands strategic planning, aimed at targeting disease control measures where they will have most impact relative to the cost. Planning for disease prevention and control should be risk- based and people centered.

This strategic planning must be based on knowledge of the pathogen, the disease it causes and risk factors, the livestock populations in which it is active and the people who manage and own these animals. Understanding and reduction of disease risks require Risk Analysis (Taylor, 2009).

Risk analysis is a tool intended to provide decision makers with an objective, and it comprises

hazards identification, risk assessment, risk management and risk communication (MacDiarmid and Pharo, 2003).

Hazard Identification is a process of identifying all the potential hazards in a given situation, while the hazard is an agent that can cause harm or damage to people, animals, plants or environment (e.g. a virus). It is a necessary first step, a hazard being something potentially harmful to animals, human, plant and environment (Anonymous, FAO, 2011). This step is defined as the process of identifying any pathogenic agent which could potentially be introduced in the commodity considered for importation, and since risk analysis is equally applicable for other areas such as disease surveillance and control programme; so hazard identification is merely a step towards identifying what is that might go wrong in whatever activity is being considered (MacDiarmid and Pharo, 2003).

Risk assessment

Risk assessment is a process of estimating as objectively as possible the probability that importation would result in entry of an exotic disease agent and that local livestock would be exposed to that agent (MacDiarmid and Pharo, 2003).

Also it is defined as a formal systematic process of evaluating the risk resulting from the hazard, and describes the risk in terms of both likelihood (probability) and the impact (consequences) of unwanted outcome (e.g. an epidemic).

Risk pathway is a graphical depiction of the biological pathways to provide a useful frame work "mind map" in a simple and transparent manner to provide the following:

- Identify pathways and variables
- Identify information requirements
- Ensure a logical chain of events in space and time
- Provide a framework for the development of a mathematical model
- Ensure the appropriate estimate is calculated
- Clarify ideas and understanding of problems and
- Assist with communicating the model structure.

Scenario trees are the most appropriate and effective way in depicting biological pathways (MacDiarmid and Pharo, 2003). The risk pathway is a series of conditions that must be met, or events that have to occur in order for the unwanted outcome to occur. And the analysis of pathway is the main tool used in risk assessment (Anonymous, FAO, 2011).

Risk management utilizes risk assessment results in a judgment process to balance potential benefits against assessed risk and to formulate risk reduction measures.

It is composed of: risk appraisal, option appraisal, implementation of risk reduction measures and monitoring and evaluation (OIE, 2014).

Risk communication an open information exchange between all those affected by the both the risk in question and the decision taken (the stakeholders), before the final policy decisions are taken (Anonymous, FAO, 2011).

To identify the opportunities for disease transmission and the factors that are affect the probability and amount of disease transmission in the value chains, Approach to preliminary risk analysis should be carried out by answering the following key questions:

- What are the risks for the disease transmission within the local livestock population?
- What are the risks for the disease maintenance within the local livestock population?
- What factors affect the magnitude of disease risks?
- What are the risk pathways involve in the above?
- How are the risk pathways related to the different production system (value chains)?
- Who are the people involved in risk pathways?
- What can be done to reduce risks and help to control diseases?

Factors (risk factors) should be further subdivided according whether factors affect transmission mainly via:

- General /background factors: Include things like status of neighbours, cross border trade (official and unofficial), border regulation, immunity, vaccination, surveillance etc.
- Live animals;

  consider movements of live animals (volume, type, seasonality, destination and use) supply breeding stock and young stock as well as slaughter stock movements, handling by intermediaries and markets, regulations, checks and enforcement, border and internal inspection posts, drivers of movements, ability to police borders, exposure of susceptible livestock and biosecurity precautions.
- Animal products; consider their movements and exposure of re-products livestock contact and biosecurity precautions for re- animal product contacts.

- Fomites; Consider all people and vehicles that have direct or indirect contact with livestock, journey structures, regulations, biosecurity precautions for re-fomites contacts (Taylor, 2009).

Value Chain analysis

Value chains are groups of people linked by an activity to provide, process, produce, transport and supply a specific commodity. Value chain analysis is the study of the chains that link production system, markets and consumers to determine risk hotspots and to suggest an effective risk reduction intervention.

The main objectives of value chain analysis are to: - identify the main people, groups and organizations in livestock value chain from the input supplier to the producer, trader, processor, and retailer and through to the final consumer.

-Mapping the different routes to market the livestock and livestock products, which could be what currently exists and what potentially is available or could be developed.

-Assess how well the marketing chain is working.

Value chain analysis for disease management should be focused on the opportunities for disease transmission, practices aimed in risk reduction and resources and capability of people in value chain to react to disease challenge (Anonymous, FAO, 2011).

CHAPTER TWO:

MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Sudan, Which is located in the north eastern part of Africa, with an 853 km (530 mi) coastline bordering the Red Sea. The total area of Sudan is 1,886,068 km^2 (728,215 sq mi), and it is the third largest country in the continent (after Algeria and Democratic Republic of the Congo) and the sixteenth largest country in the world. Sudan lies between latitudes 8° and 23°N. The country is bordering seven countries; Egypt from North, South Sudan from South, In the East Ethiopia and Eritrea and in the West Libya, Chad and central Africa, with the Red Sea coast in Northeast of the country (Anonymous, Ministry of Cabinet, 2014).

Sudan has Human population of 33,979,594. The livestock population estimate over 104,278,000Head from cattle, sheep, goats and camels. The small ruminants' population is 69,945,000 Head (Anonymous, MoLFR, 2011-2012).

Sudan has different ecological zones; desert, semi- desert and low rainfall wood land savanna.

One third of the total land area being desert, about 40% suitable for grazing and less than one- quarter potentially arable. Livestock sector in Sudan is an important contributor to the national economy, accounting for 25% of the GDP and employing 40% of the country`s population (ElDirani et al, 2009). During the time of study Sudan was divided into 15 states.

2.2 Study population:

The target Population for the seroprevalence study is the unvaccinated sheep and goats in the localities of Sinnar, Gedarif, River Nile and North Kordofan states. In the preliminary qualitative risk assessment the target population was the exported sheep to the kingdom of Saudi Arabia during the year 2012.

2.3 Data collection:

Climatic data: were collected from the Sudanese Meteorology Authority. The data include records of Rainfall, day temperature, Wind speed and relative humidity for the four sampled states during the sampling period for each state.

Questionnaire for sample collections and data on risk factors investigations

A structured questionnaire was used to collect data about some risk factors that might have an association with the PPR sero-prevalence. General questions included in the questionnaire covered Location (State, Locality, area and Longitude and latitude), herd size, husbandry system, housing of animals, last PPR outbreak, date of last vaccination against PPR and (sex, age, breed and species) for each sampled animals. A template of the used questionnaire is shown in annex 1.

Data about PPR outbreaks, ecological zones, vaccination, sheep and goat population data and wildlife data were collected from the Reporting and Information Unit and Wildlife disease section in epidemiology unit in The General Directorate of Animal Health and Epizootics Control of The Ministry of Livestock and Rangelands.

Annual rainfall and states areas were collected from the Central Bureau of Statistics (CBS), Ministry of Cabient of the Republic of Sudan.

Data on PPR outbreaks, Animal census, vaccination and animal movement: were collected from the Monthly and annual reports of the General Directorate of Animal Health and Epizootics disease Control, from the General Administration of Animal Resources in the four sampled states.

Data for risk assessment:

Data about the numbers and sources of exported sheep and quarantine procedures were collected from the administration office of Swakin Quarantine in PortSudan city.

PPR outbreaks data were collected from the General Directorate of Animal Health and Epizootic Diseases control of the ministry of Animal Wealth, fisheries and rangelands.

Scientific information about PPR epidemiology and sheep production and marketing in Sudan were collected from scientific papers, studies and thesis published on open access e-journals and websites.

2.4 Methodology:

2.4.1 Methodology of the seroprevalence and risk factors study

The study was conducted in four states; Sinnar, Gedarif, River Nile and north kordofan.

2.4.1.1 Sample size:

1448 Samples were proposed to be collected from sheep and goats - As shown in Table (7) using the following sample size formula for each state: $N = (1.96)^2 \times P (1-P)/ L^2$

1.96: z value with confidence level 95%

P: guesstimate of the probable prevalence of reactors

L: allowable error (0.05)

The determined sample size couldn`t be collected in this study due to the following reasons:

1-Few animals were left unvaccinated after the mass PPR vaccination in different states,

2-Some herd owners didn't allow the study team to take samples and

3-for logistic reasons the study couldn't reach different areas in samplled localities

Table (7): Sample size supposed to be taken and the actual samples taken from the 4 states according to the mean of past PPR prevalence (P) in sheep and goats

State	2001(P) %	2003(P) %	2007(P) %	2008(P) %	2010(P) %	Mean of (P) %	Proposed Sample size	Samples taken
Sinnar	67.06	-	33.38	-	68.26	56.23	378	333
Gedarif	-	-	52.0	80.6	41.1	27.9	302	160
North Kordofan	-	41.2	-	61.2	50.49	50.9	384	118
River Nile	64.62	33.9	-	51.4	52.36	50.5	384	99

2.4.1.2 Sampling strategy

710 serum samples were collected from Sinnar, Gedarif, River Nile and North Kordofan states during May 2012, June 2012, October 2012 and Feb 2013 respectively.

1- Sinnar state: 333 Samples and questionnaire data were collected in 5 localities; Singa, Sinnar, East Sinnar, Abuhugar and Dindir but due to logestic reasons only selection of 138 samples from Abuhugar, East Sinnar and Dindir were tested for PPR Abs.

2- Gedarif state: 160 Samples and questionnaire data were collected from 4 localities; Elfashga, basonda, Elgorisha and Western Glabat. Selction of 143 samples from the 4 localities were tested for PPR Abs.

3- River Nile state: 118 Samples and questionnaire data were collected from 3 localities; Atbara, Barbar and Eladamar. 106 samples from the 3 localities were tested for PPR Abs.

4-North Kordofan: 99 Samples and questionnaire data were collected from 4 localities; Elkhiwai, Abuzabad, Umrwaba and Elrahad. 93 samples were tested for PPR Abs as showed in table (7) and illustrated in Figure (2).

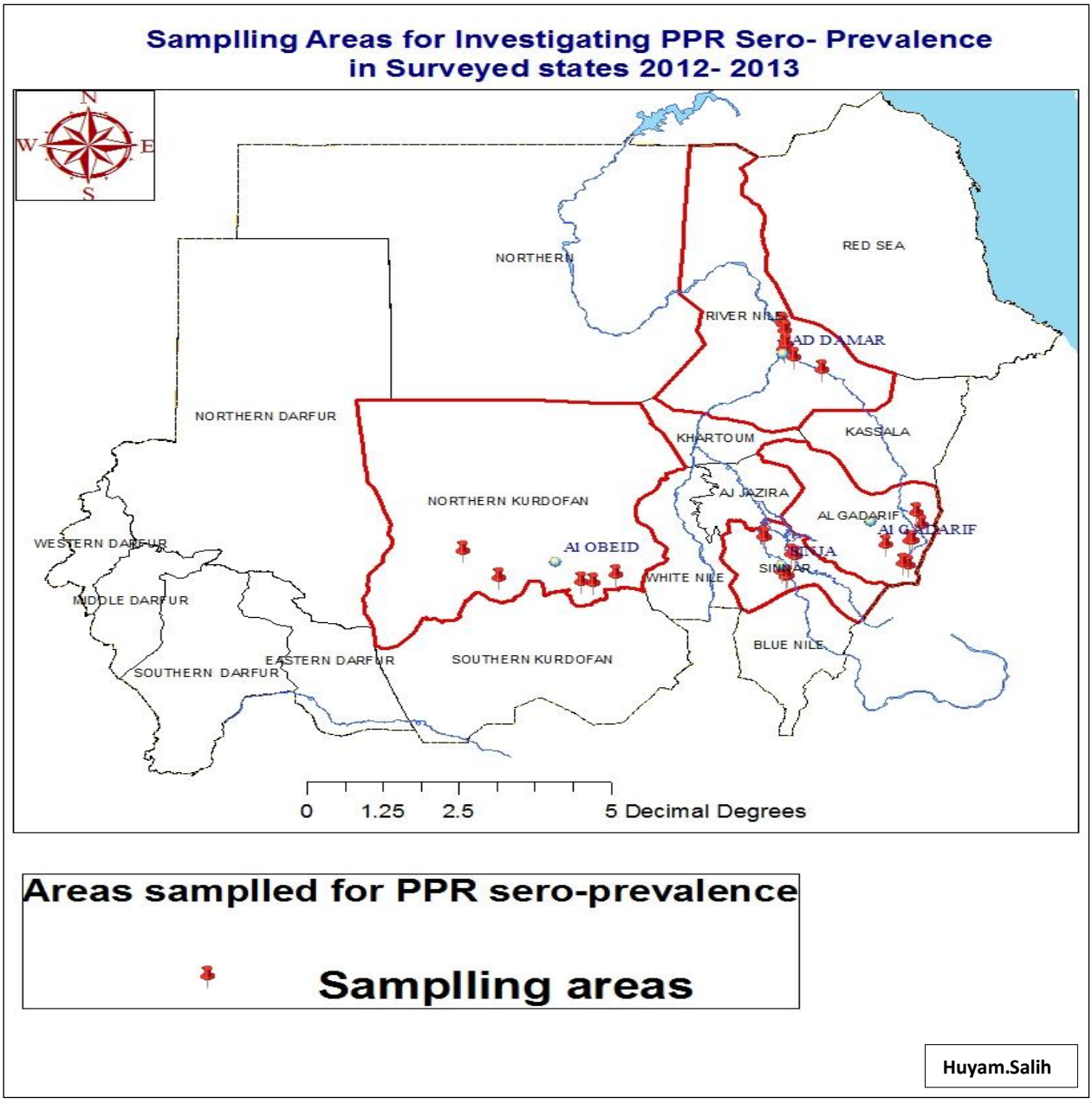
Samplling Areas for Investigating PPR Sero- Prevalence
in Surveyed states 2012- 2013
N
W
E
S
NORTHERN
RED SEA
RIVER NILE
AD DAMAR
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AJ JAZIRA
AL GADARIF
Al OBEID
WESTERN DARFUR
WHITE NILE
SINNAR
MIDDLE DARFUR
BLUE NILE
EASTERN DARFUR
SOUTHERN DARFUR
SOUTHERN KURDOFAN
0
1.25
2.5
5 Decimal Degrees
Areas samplled for PPR sero-prevalence
Samplling areas
Huyam.Salih

Figure (2)

2.4.1.3 Samples

Sera were extracted from the collected blood samples using electric centrifuge with speed of 5000 round/ Min in room temperature, and then harvested in Eppendorf tubes and frozen deeply in -20 C^{o} until the test time.

2.4.1.4 Competitive ELISA

PPRV antibody detection was carried out using PPR c-ELISA kits manufactured by the FAO Reference Laboratory (CIRAD EMVT; Montpellier, France), and obtained from BDSL, the distributing agent. distilled water (30 mL), PBS powder (Sigma, IL), Tween- 20 (100 mL), ELISA plates (Nunc, Maxisorp) anti mouse HRPO conjugate (2 mL) substrate, H_2O_2, OPD tablet (30 mg), antigen (1 mL), strong positive serum (1 mL), weak positive serum (1 mL), negative serum (1 mL) and monoclonal antibody. The c-ELISA test was carried out according to the kit protocol and the manual provided with it.

Description and Principle

The microwells were coated with purified recombinant PPR nucleoprotein (NP) and washed after antigen adsorption. The samples to be tested and the controls were added to the microwells. Anti-NP and monoclonal antibodies (MAb) were added and the mixture was left to react with the antigen coated plate; anti-NP, if present, formed an antibody-antigen complex. After incubation, the plate was washed to remove unbound antibodies. Possible binding of the MAb was detected by adding mouse specific conjugate and the substrate. Absence of chromogenic reaction indicated the presence of circulating antibodies whose specificity was defined by the MAb in completion.

Test Procedure

For coating of microplates, PPR antigen was diluted 1:100 in Phosphate Buffer Saline (BPS) and 50 µl of diluted PPR antigen was added to each well of an ELISA plate. Then the plates were covered and incubated at + 4°C over night or placed on a shaker for one hour. Then the plates were washed three times with washing buffer, 40 µl Blocking Buffer (BB), PBS 0.1% Tween 20 + 0.3% negative serums, were added to all wells and further 10 µl was added to the monoclonal control wells (F1, F2, G1, G2) as showed in Figure 3, and 60 µl to the conjugate control wells (A1, A2). Columns 1 and 2 were used as control, 10 µl of test serum was added to test wells (vertical duplicates), 10 µl of strong positive control serum to controls (B1, B2, C1, C2), 10 µl of weak positive control serum to controls (D1, D2, E1, E2), 10 µl of negative control serum to controls (H1, H2) were added as presented in Figure 4. 50 µl of MAb (1:100

in BB) was added to each well except A1 and A2 (conjugate control wells). The plates were covered and incubated at 37°C for one hour in an orbital shaker, washed three times with washing buffer and blotted to dry. Then 50 µl of anti mouse HRPO conjugate (1:100 in BB) was added to each well and incubated at 37°C for one hour in an orbital shaker. The plates were washed three times with washing buffer and blotted to dry. 50 µl of chromogen/substrate (4 µl of H_2O_2 added to each ml of OPD) were added to all wells. The plates were incubated at room temperature without shaking and avoiding direct light for 10 minutes. The reaction was stopped by the addition of 50 µl of sulphuric acid 1M to each well. OPD/H_2O_2 + H_2SO_4 in one column were used as blank. Optical Density (OD) values were read at 492 nm with an ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems, Finland). The cELISA plate layout for PPR is as presented in Figure 13. The absorbance was converted to Percentage Inhibition (PI) using the formula below with the help of the ELISA Data Interchanges (EDI) software manufactured by FAO/IAEA.

$$PI = \frac{\text{Absorbance of the test wells}}{\text{Absorbance of the MAb control wells}} \times 100$$

Interpretation of cELISA Results

Any sample with average Percentage Inhibition (PI) of:

< 50% considered as negative,

51 - 80% considered as weak positive (WP),

> 81% considered as strong positive (SP).

Controls

	1	2	3	4	5	6	7	8	9	10	11	12
A	CC	CC	S1	S5								S37
B	C++	C++	S1	S5								S37
C	C++	C++	S2									
D	C+	C+	S2									
E	C+	C+	S3									
F	Cm	Cm	S3									
G	Cm	Cm	S4									S40
H	C-	C-	S4									S40

Figure (3): Plate Layout of cELISA for PPR

CC	= Conjugate Control	C++	= Strong Positive
C+	= Weak Positive	Cm	= Monoclonal Antibody Control
C	= Negative Control	S	= Sample

2.4.1.5 Data analysis and presentation:

All collected data like age, sex, breed of individual animals and locations during sampling and the laboratory results were entered, coded, and stored electronically in a Microsoft Excel for Windows 2007 data. The Statistical Package for Social Sciences (SPSS) for Windows version 17.0 was used for all appropriate statistical analyses. First a univariate analysis by using of 2-tailed chi-square test was conducted to test the difference hypothesis between the investigated 14 risk factors and the ELISA positive and negative animals. In the second step, a logistic regression model was used to assess the association between the 9 significant risk factors (P- value$\leq$ 0.05)in the univariate analysis and the PPR Abs (+)ve and PPR Abs (-)ve . Data and results displayed in tables and Choropleth Maps were produced using GIS software ArcMap 9.1.

2.4.2 Methodology of the case- control study:

2.4.2.1 Study design:

One hundred and fourteen localities of the 14 Sudanese states were selected for case- control study (Which represent all the states in Sudan except Western Darfur because of the irregular report` flow from this state during the study period). Localities were divided to 47 cases and 67 controls as shown in annex 2; controls are the localities which have no outbreaks records during the period from 2008 to 2012 while the cases are the localities which have PPR outbreaks even if have one outbreak during the study period as shown in Figure (4).PPR outbreaks in different Sudan states during the study period are explained in figure (5) and (6).

2.4.2.2 Categorical variables

Seven dichotomous categorical variables were investigated in this study as explained in Table (8). Figure (7) explains the numbers of vaccination against PPR comparing to sheep and goat population per state.

The ecological zone divided the localities to ones located in desert and semi-desert and localities in low rainfall woodland savanna as presented in Figure (8). Wildlife density in states was divided into two categories; localities in states with low and medium density of wildlife, and localities within states with high density as shown in Figure (9). The annual rainfall for the period from 2008 to 2012 divided the localities into localities with low rainfall and others with high rainfall as in Figure (10). Localities were also divided into two groups according to their state areas and state population. Vaccination coverage against PPR for every state was calculated by dividing the annual vaccination for the period from 2008 to 2012 over the total state population of sheep and goats.

Table (8): Categorical variables analysed for association with PPR status in Sudan localities in a case- control study for the period from 2008 to 2012.

Variable	Variable categories and description
Ecological zone	There were three major ecological classes in Sudan: desert, semi-desert and low rainfall woodland savanna (. Anonymous, AHEDC, 2005). So accordingly the ecological zone was defined and coded for every state in two categories as 1:desert and semi-desert and 2: low rainfall woodland savanna
Wild life	The wild life were classified according to Sayied (2004) into Low, medium and high density for every country region; **Kassala, Gedarif and Red Sea:** Limited groups of Gazelles, Red fronted gazelles and Barbary sheep. **White Nile:** Limited groups of Dorcas gazelles. **Gezira, Sennar and Blue Nile:** Red- fronted gazelles, buffaloes, Roan antelopes, Bushbucks and Reedbucks. **Khartoum:** Very few groups of Dorcas gazelles in west of Omdurman. **Northern kordofan and Northern Darfur :** Red- fronted gazelles, Dorcas gazelles, Oryx dammah, Oryx beisa and Sommering`s gazelles. **Southern Kordofan and Southern Darfur:** Red- fronted gazelles, Roan antelopes, giraffes and reedbucks In this study two categories were considered for each; 1: low and medium and 2: high density of wild life
Rainfall	The total of the annual rainfall during the period from 2008 to 2012 for each state was calculated according to the Statistical year books (Anonymous, CBS, 2009- 2010) and classes into 1: low rainfall (1 mm to 300 mm) and 2: high rainfall (400 mm to 700 mm).
Bordering foreign country	Localities were divided into two categories; 1: localities belong to bordering states and 2: localities in states not at borders.
Sheep and Goat population	Sheep and goats population were calculated for each state according to 2009 census (Anonymous, MoLFR, 2009). Classified as 1: Medium size population (1,000,000 to 4,000,000 head) and 2: large size population (5,000,000 to 10,000,000 head).

Variable	Variable categories and description
State area	The areas of each studies state were calculated in square kilometer according to(Anonymous, CBS, 2009) and divided into; 1: states with small area (22.000 Km^2 to 160,000 km^2) and 2: states with large area (170,000 to 350,000 km^2).
Vaccination coverage against PPR	The vaccination coverage percentages were calculated for each state by the formula: (The total of PPR vaccinated head in state for the 5 years 2008- 2012 / state population of sheep and goats (Animal census, 2009)) %. Then the percentages were classes as; 1: weak coverage (0.50% to 60%) and 2: wide coverage (70% to 95%)

2.4.2.3 Descriptive analysis

To assess the distribution of the variables, Choropleth maps were produced and frequency tables were generated for all the categorical risk factors.

2.4.2.4 Univariate analysis

All categorical variables were cross tabulated; and the association of the studied factors with the PPR outbreak occurrence was tested by calculating the Odds Ratio (OR) using Mantel Haenszel test. A risk factor with *p*-value ≤ 0.25 was considered to be significantly associated with the occurrence of PPR outbreaks, either positively (OR>1) or negatively (OR<1).

2.4.2.5 Multivariate Analysis

Potential risk factors in the univariate analysis (p- value $\leq .25$) were entered and analysed multivariably using logistic regression to assess the association between PPR outbreaks occurrence and these potential risk factors. Risk factors with P-value $\leq .05$ were considered to have a significant association with the occurrence of PPR outbreaks.

2.4.2.6 Data analysis and presentation:

Data on PPR outbreaks and risk factors were entered into excel sheet for primary summations and coding. Tables, histogram and Choropleth Maps and maps with proportional point symbols were produced using GIS software ArcMap 9.3. All statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) for Windows version 17.0.

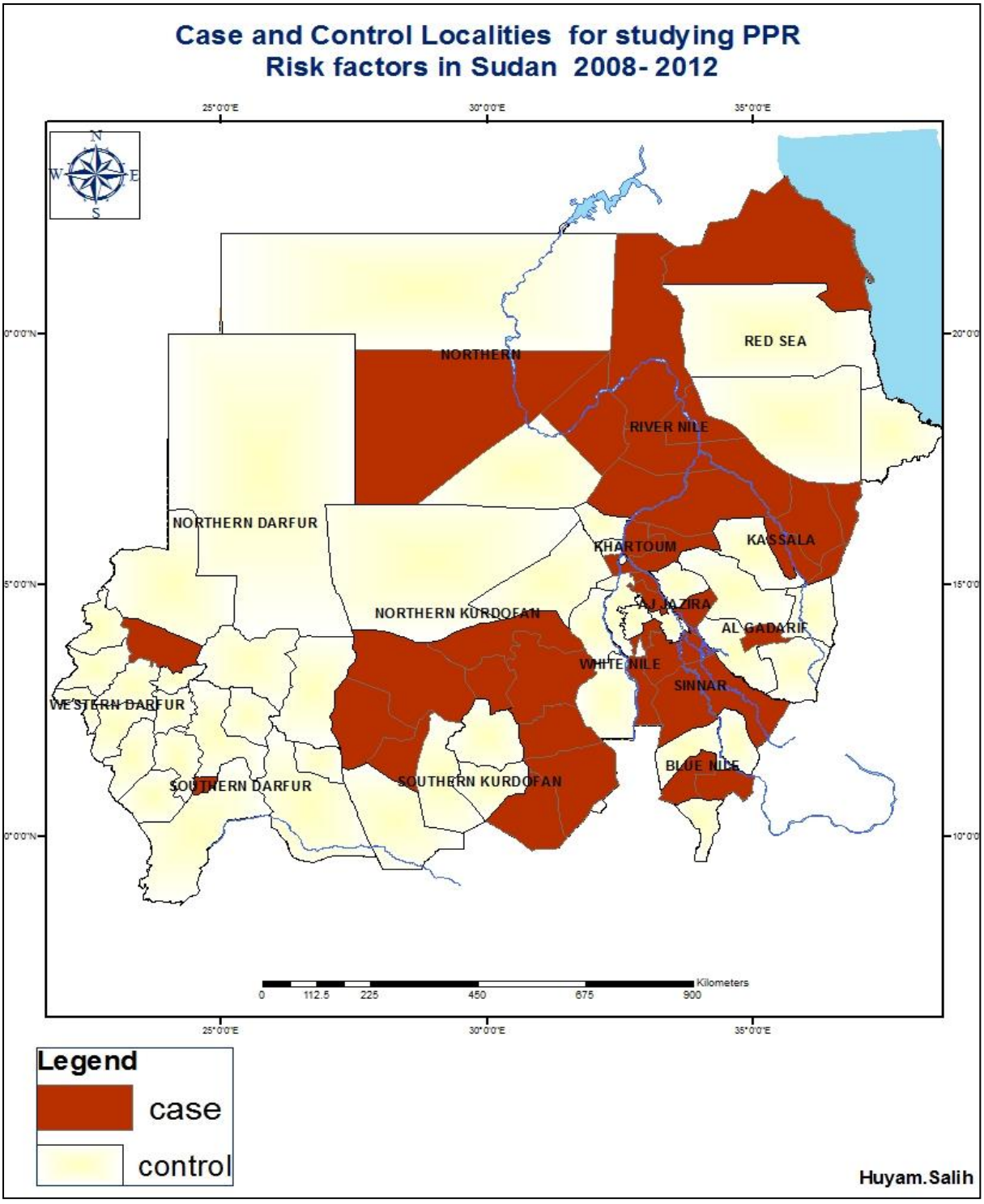
Case and Control Localities for studying PPR
Risk factors in Sudan 2008- 2012
25°0'0"E
30°0'0"E
35°0'0"E
20°0'0
15°0'0
10°0'0
RED SEA
NORTHERN
RIVER NILE
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AL JAZIRA
AL GADARIF
WHITE NILE
SINNAR
WESTERN DARFUR
BLUE NILE
SOUTHERN DARFUR
SOUTHERN KURDOFAN
0 112.5 225 450 675 900
Kilometers
Legend
case
control
Huyam.Salih

Figure (4)

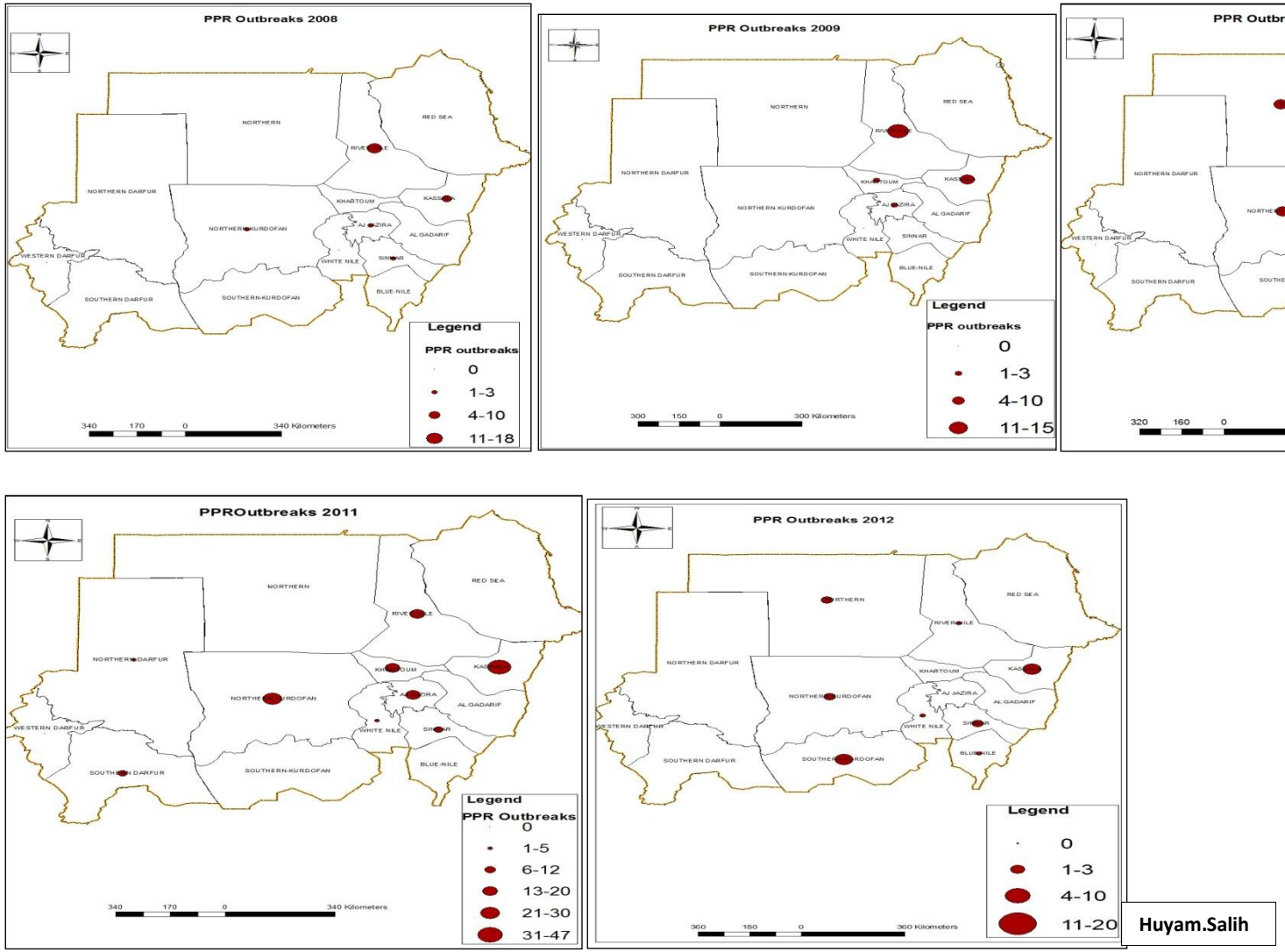

Figure (5): Shows comparison between the numbers of PPR reported outbreaks per state in the years 2008 to 2012.

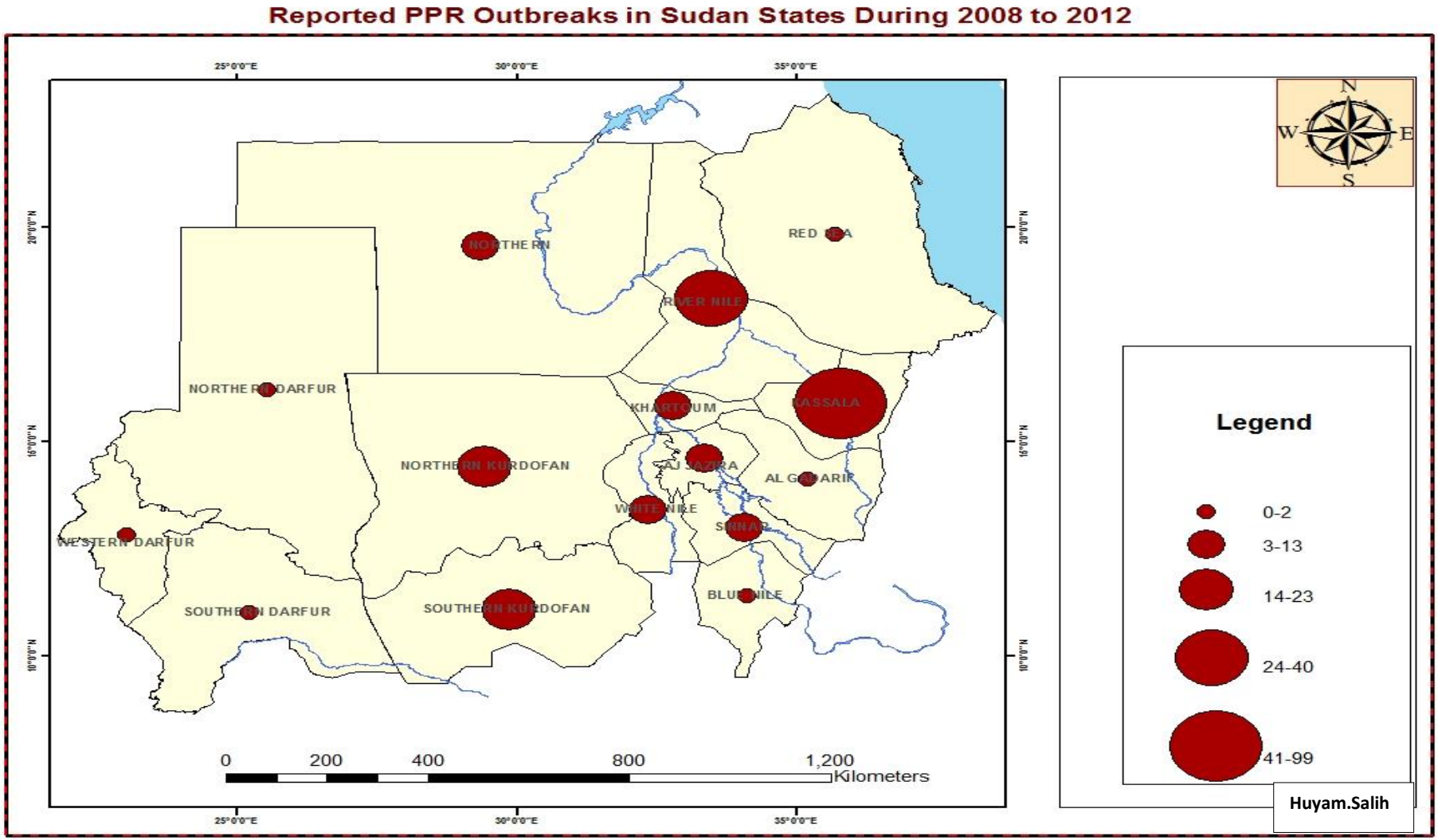
Reported PPR Outbreaks in Sudan States During 2008 to 2012
25°0'0"E
30°0'0"E
35°0'0"E
20°0'0"N
16°0'0"N
10°0'0"N
NORTHERN
RED SEA
RIVER NILE
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AL GADARIF
WHITE NILE
SENNAR
WESTERN DARFUR
SOUTHERN DARFUR
SOUTHERN KURDOFAN
BLUE NILE
0
200
400
800
1,200
Kilometers
N
W
E
S
Legend
0-2
3-13
14-23
24-40
41-99
Huyam.Salih

Figure (6)

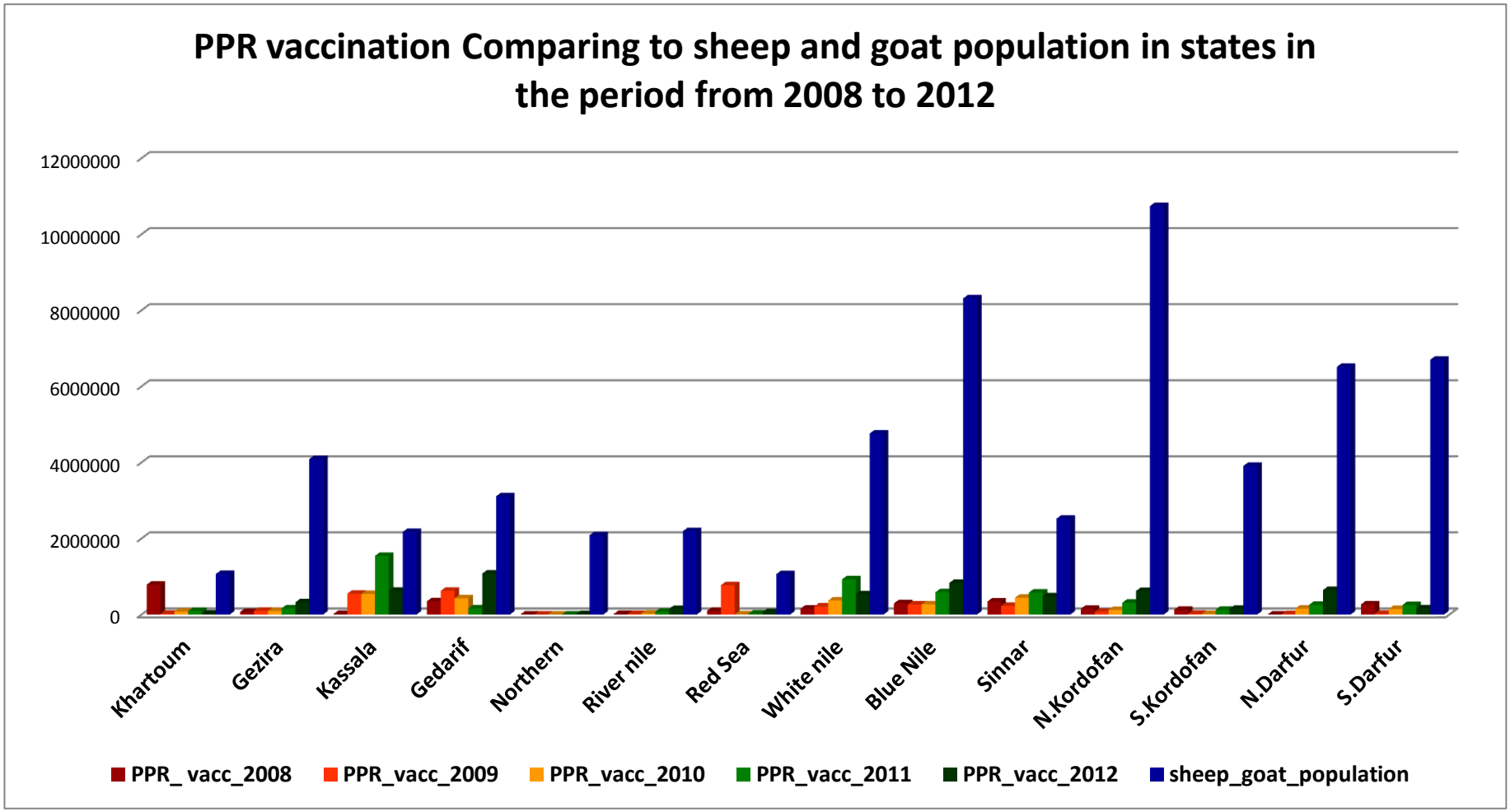

Figure (7): Chart showing vaccination against PPR disease comparing to sheep and goat population per states during the years 2008- 2009- 2010- 2011- 2012.

Source: Source: Annual Reports of The General Directorate of Animal Health and Epizootic diseases control (AHEDC) for the years 08- 09- 10-11 and 2012.

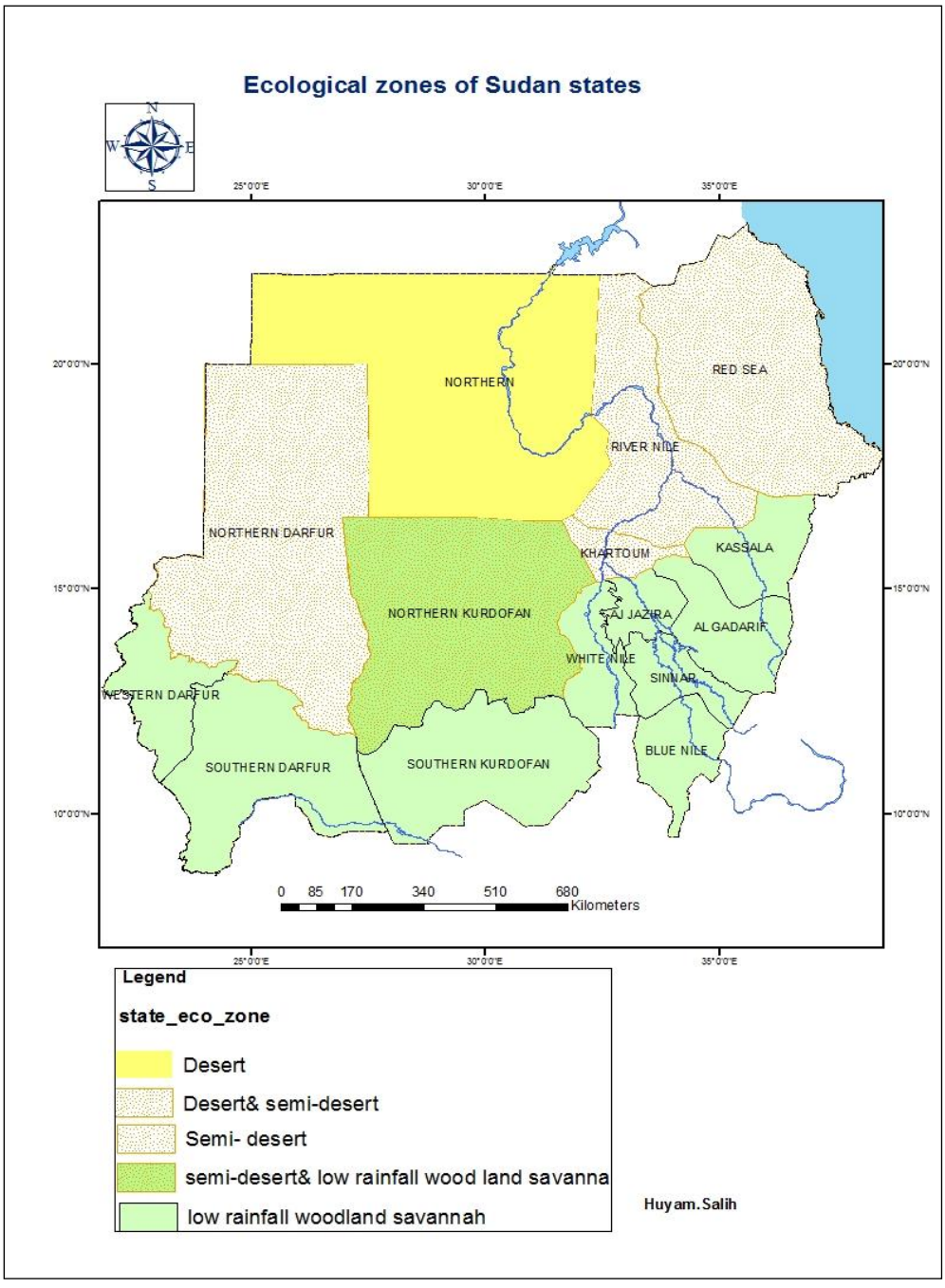
Ecological zones of Sudan states
N
W
E
S
25°0'0"E
30°0'0"E
35°0'0"E
20°0'0"N
15°0'0"N
10°0'0"N
NORTHERN
RED SEA
RIVER NILE
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AJ JAZIRA
AL GADARIF
WHITE NILE
SINNAR
WESTERN DARFUR
SOUTHERN DARFUR
SOUTHERN KURDOFAN
BLUE NILE
0 85 170 340 510 680
Kilometers
Legend
state_eco_zone
Desert
Desert& semi-desert
Semi- desert
semi-desert& low rainfall wood land savanna
low rainfall woodland savannah
Huyam. Salih

Figure (8)

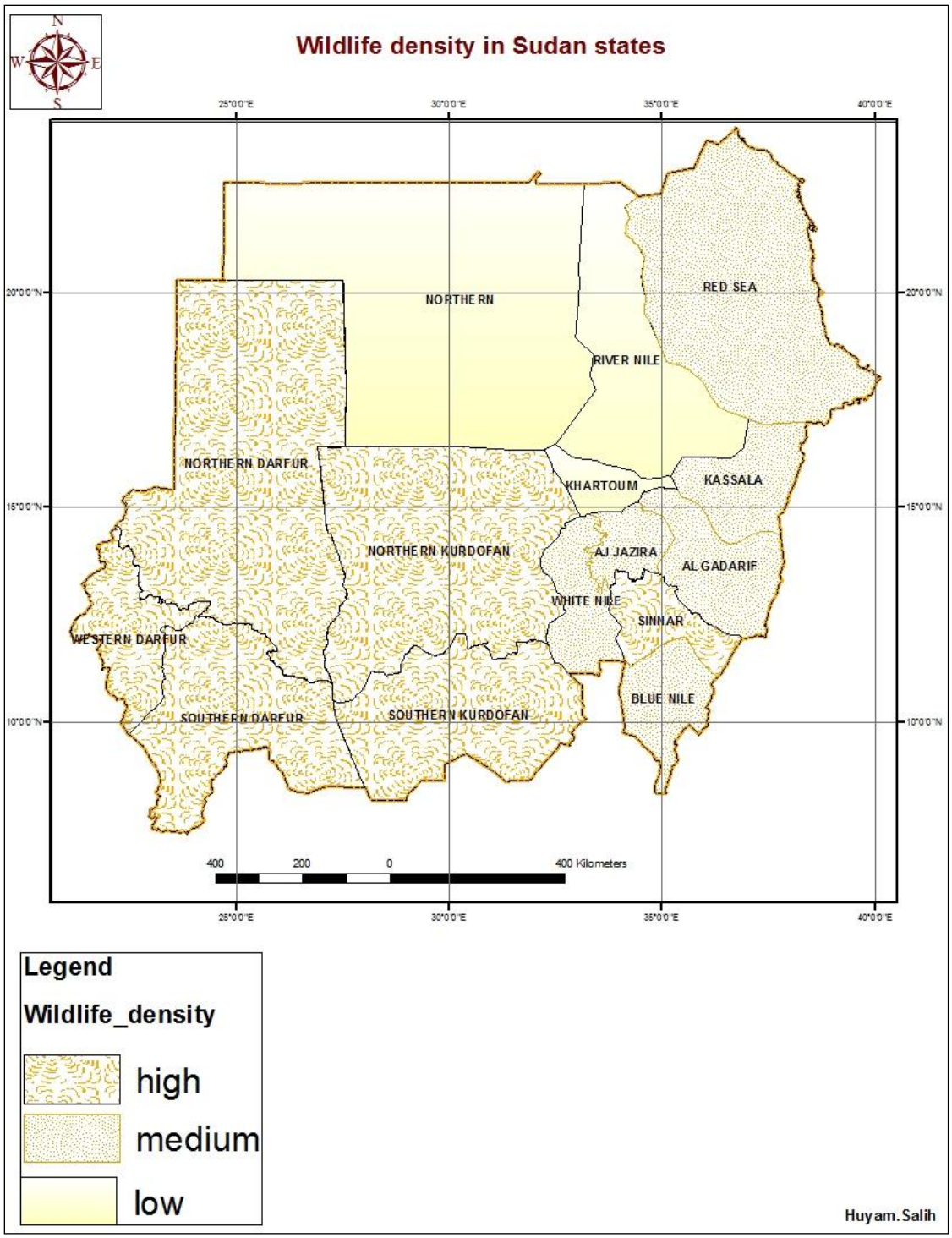
Wildlife density in Sudan states
N
W
E
S
25°0'0"E
30°0'0"E
35°0'0"E
40°0'0"E
20°0'0"N
15°0'0"N
10°0'0"N
NORTHERN
RED SEA
RIVER NILE
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AJ JAZIRA
AL GADARIF
WHITE NILE
SINNAR
WESTERN DARFUR
BLUE NILE
SOUTHERN DARFUR
SOUTHERN KURDOFAN
400
200
0
400 Kilometers
Legend
Wildlife_density
high
medium
low
Huyam.Salih

Figure (9)

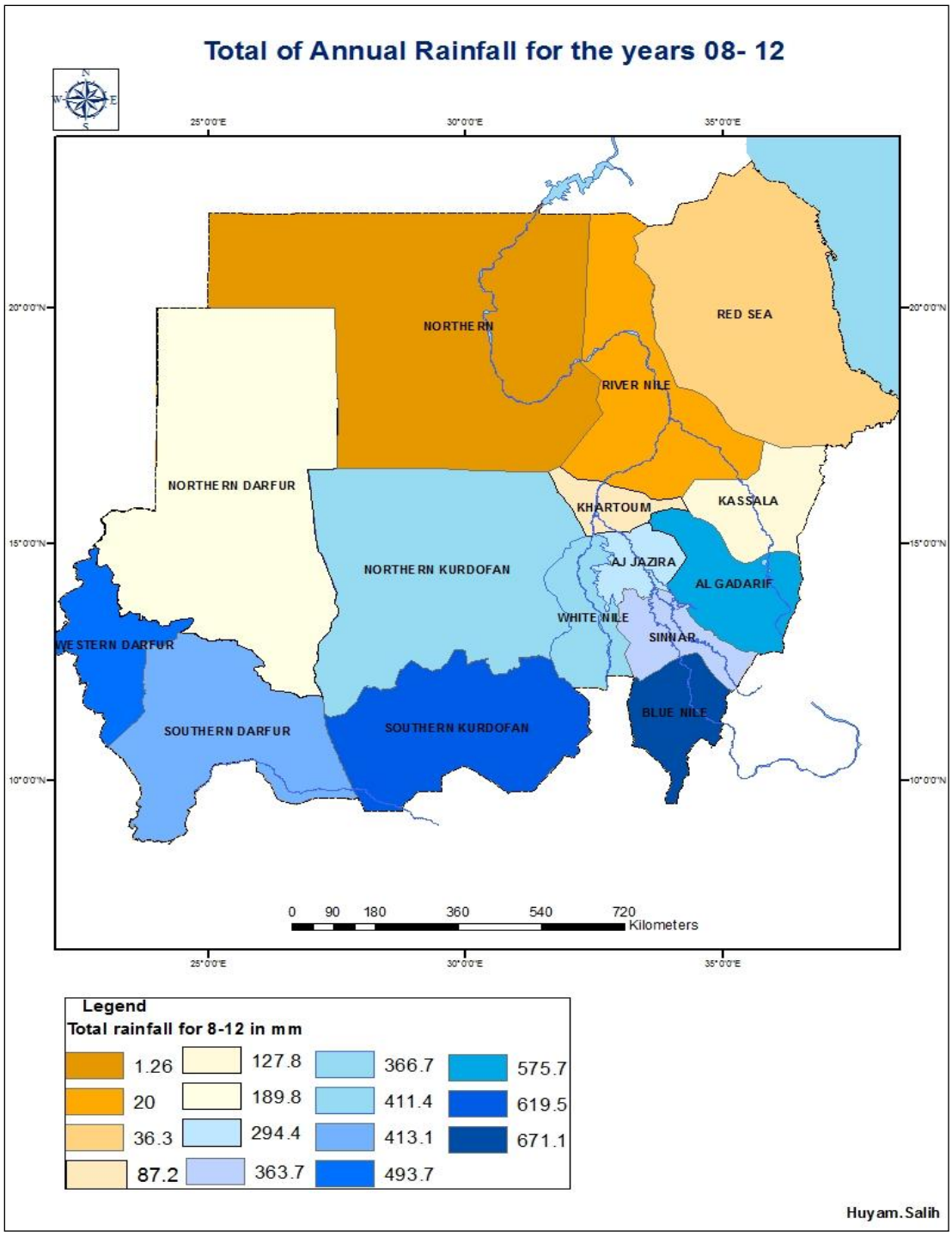
Total of Annual Rainfall for the years 08- 12
25°0'0"E
30°0'0"E
35°0'0"E
20°0'0"N
15°0'0"N
10°0'0"N
NORTHERN
RED SEA
RIVER NILE
NORTHERN DARFUR
KHARTOUM
KASSALA
NORTHERN KURDOFAN
AJ JAZIRA
AL GADARIF
WHITE NILE
SINNAR
WESTERN DARFUR
SOUTHERN DARFUR
SOUTHERN KURDOFAN
BLUE NILE
0 90 180 360 540 720
Kilometers
Legend
Total rainfall for 8-12 in mm
1.26
20
36.3
87.2
127.8
189.8
294.4
363.7
366.7
411.4
413.1
493.7
575.7
619.5
671.1
Huyam.Salih

Figure (10)

2.4.3 Methodology of the Preliminary Qualitative Risk Assessment:

2.4.3.1 Description of the exported sheep from Swakin terminal quarantine;

The study was carried out on the sheep selected and prepared for exportation through Swakin port to the Kingdom of Saudi Arabia during the year 2012. Sheep entered Swakin terminal quarantine and their sources are shown in Table (9).

During the year 2012 about 3,399,421 head of live sheep were exported to the Kingdom of Saudi Arabia via Swakin port as shown in figure (11).

Sheep selected for exportation collected from local livestock markets, inspected and vaccinated in vaccination and inspection centers and quarantined in the collective quarantine of Elkadro then transmitted to Swakin terminal quarantine. During the year 2012 sheep were collected from five internal quarantines as shown in Figure (12).

Sheep is quarantined and monitored in Swakin, any animal shows apparent disease sign is rejected from exports as explained in Table (10).

Table (9): Numbers and sources of sheep entered Swakin quarantine during 2012.

STATE	**Khartoum**	**North kordofan**		**Gedarif**	**Kassala**
MONTH	**Elkadro** central quarantine	**Elkhiwai** Inspection and vaccination centre	**Elrahad** Inspection and vaccination centre	**Elshwak** Inspection and vaccination centre	**Kassala** Inspection and vaccination centre
JAN	13665	97026	12275	151151	35531
FEB	21217	88060	7691	135832	40439
MAR	20233	108284	12745	139648	43598
APR	20560	102708	20189	172894	21224
MAY	20143	109360	13933	132704	40886
JUN	5963	61260	14428	13892	29664
JUL	15384	55456	4761	66367	14152
AUG	2449	39215	2527	52291	39141
SEP	5577	248868	3689	0	45176
OCT	24058	211733	9274	47008	86302
NOV	9073	51022	17913	111252	40757
DEC	13505	114071	17368	175606	56542
TOTAL	171,827	1,287,063	136,793	1,323,145	493,412
PERCENTAGE	5.3%	37.7%	4%	38.8%	14.5%

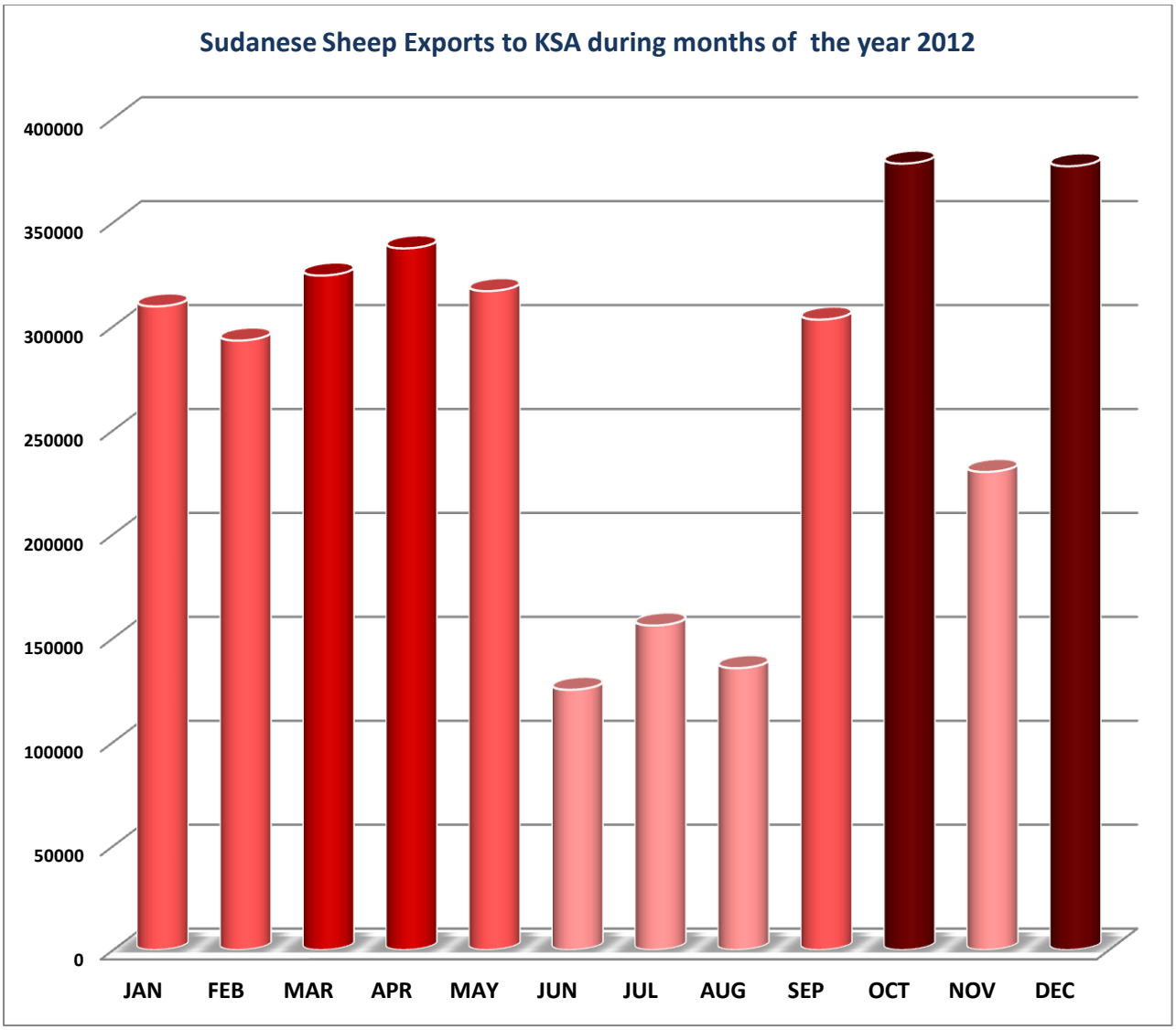
Sudanese Sheep Exports to KSA during months of the year 2012
400000
350000
300000
250000
200000
150000
100000
50000
0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Figure (11)

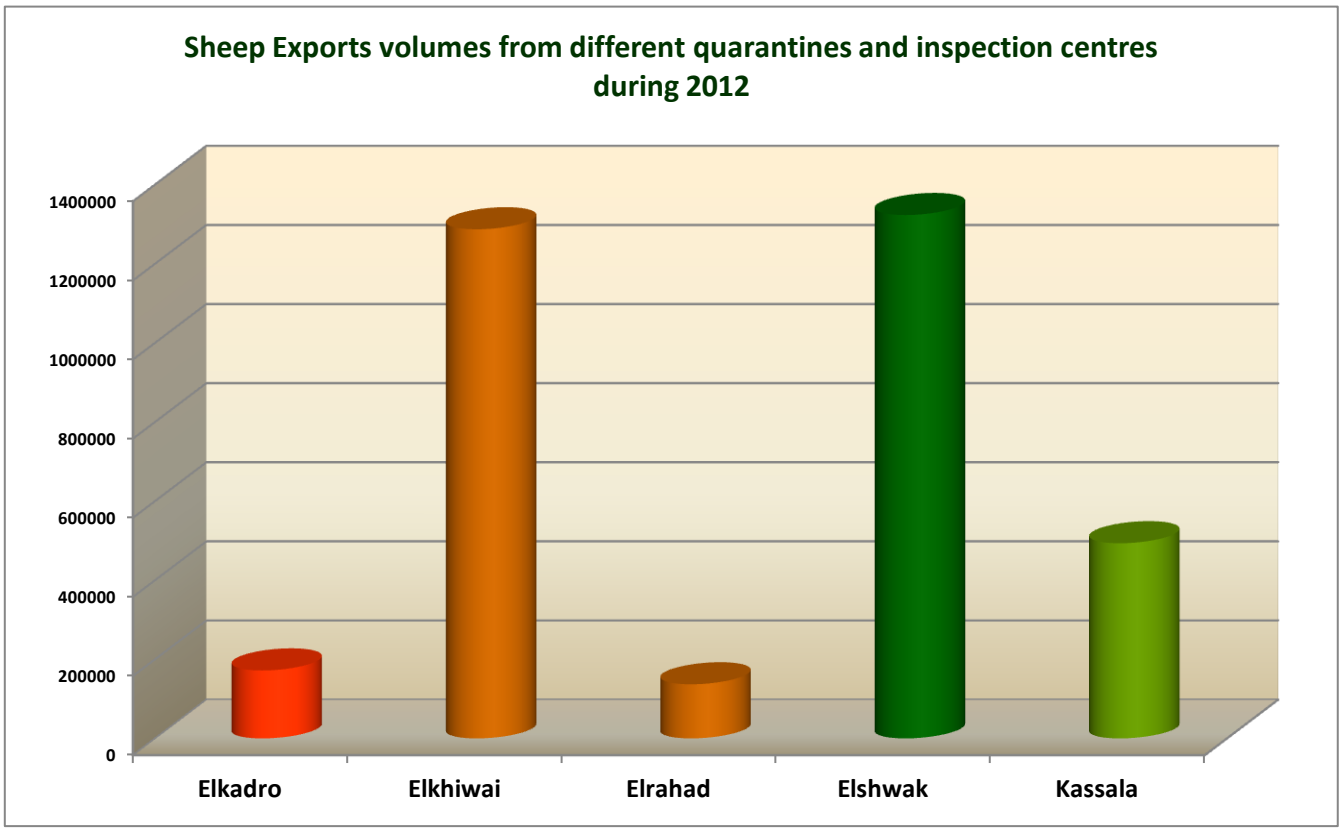
Sheep Exports volumes from different quarantines and inspection centres during 2012
1400000
1200000
1000000
800000
600000
400000
200000
0
Elkadro
Elkhiwai
Elrahad
Elshwak
Kassala

Figure (12)

Table (10): Numbers of sheep and reasons for rejecting from exports in Swakin quarantine during 2012

Month	Reasons for rejecting sheep from exports in Swakin quarantine during 2012						
	Swelling of lymph nodes	**Mange**	**Sheep pox**	**Diarroehea**	**Postulates**	**Emaciation**	**Others**
Jan	1751	720	2110	1268	0	491	18
Feb	1153	778	45	354	83	993	435
Mar	1365	787	112	300	75	412	814
Apr	0	530	85	847	25	634	1215
May	2667	226	114	239	24	365	890
Jun	648	596	34	259	119	175	765
Jul	1114	216	29	255	0	196	45
Aug	600	200	0	400	244	240	0
Sep	2192	596	101	97	0	183	17
Oct	1890	101	65	125	0	486	175
Nov	928	21	39	28	0	266	0
Dec	650	675	195	270	0	95	156
Total	14868	5446	2929	4412	570	4536	4530
Percentage	38.7%	14.2%	7.6%	11.4%	1.5%	11.8%	11.8%

2.4.3.2 Descriptive epidemiology for PPR

Characters of causative agent PPR virus (PPRV)

Peste des petits ruminants is caused by PPRV which belongs to the family paramyxo viridae, genus morbillivirus (OIE, 2010). PPRV is fragile and it cannot survive for long time outside the host, its half life has been estimated to be 2.2 minutes at 56 C and 3.3 hours at 37 C (Chauhan et al., 2009). PPRV is stable between PH 5.8 to 10.0 but inactivated at PH less than4 or more than 11, it is effectively disinfected with alcohol, ether and common detergents while it is susceptible to most disinfectives like phenol, sodium and sodium hydroxide 2% over 24 hours (OIE, 2014).

Four lineages of PPRV were identified through the genotypic classification, which appears to be an efficient tool to survey virus spread worldwide. Viruses of lineage I and II are restricted to western and central Africa; Lineage III is common to eastern Africa and the southern part of the Middle East. In Asia only viruses of lineage IV have been detected (Kwiatek *et al*., 2011).

PPRV transmission:

The virus is highly contagious and exists in all discharges from sick animals, but since it is an enveloped virus, it is extremely sensitive to inactivation by environmental factors such as heat, sunlight and chemicals (Chauhan et al., 2009).

PPR disease situation in Sudan

Circulating PPR virus in Sudan:

In Sudan continuous outbreaks of PPRV have occurred for more than thirty years in sheep and goats, most of the PPRV strains collected during 2000- 2009 were clustered in lineage IV while only a few strains remained in lineage III. Viruses of IV cluster were detected in sheep and goats co circulating with camel viruses during the same period and in the same areas of northern Sudan, Khartoum and Blue Nile. Camels were not regarded as possible hosts for PPRV until 1992, then surveillance in camels resulted in detecting the virus in consecutives outbreaks in Eastern Sudan, Northern Sudan and blue Nile region during 2004, 2005 and 2007 respectively (Kwiatek *et al.*, 2011)

Temporal pattern of PPR occurrence in Sudan

According to the reported outbreaks of PPR in Sudan states during the period from 2008 to 2012(Anonymous, AHDEC, 2008- 2012); PPR outbreaks seems to be generally spreading all over the year, but in 2011 and 2012 the incidence of PPR outbreaks appears to have a clear similar pattern for the two years. That could be due to the regular flow of complete reports from states during 2011- 2012 compared to the previous three years.

During 2011 and 2012 PPR outbreaks reached their peak in months of January and February (winter season), then begin to decline from March until May (summer season) with no or few outbreaks during June, July and August (rainy season). PPR outbreaks then started to increase gradually from October (Post rainy season and beginning of winter) to reach the peak again in January as shown in figure (13).

Similar patterns were described by Gopilo, 2005, when he mentioned the migratory flocks as the important transmitter for PPR in East Africa. The migratory flocks come in contact with local sheep and goat population from where they pick up the infection or spread the disease if the migratory flocks are pre-exposed. With the stat of rains, the movement of animals is restricted due to the availability of local fodder and the nutritional status of animals gets improved during rains. This may reduce PPR transmission during rainy season.

Also Sarker and Islam, 2011 have reported same findings in Bangladesh, they stated that; the seasonal variation is practically responsible for the occurrence of PPR in goats in Bangladesh. The disease was higher in the months of December and January, and lowest in the months of June and July. The dusty and dry winds that characterize winter season of the year have been shown to enhance the spread of PPR.

Spatial distribution of PPR in Sudan

The highest number of reported outbreaks of PPR during the period from 2008 to 2012 was received from Kassala state in the Eastern region of Sudan, followed by River Nile state, North and South kordofan and Northern states. Few outbreaks were reported by sates of Khartoum, Gezira, Sennar and White Nile in central and South Sudan. Relatively, very few outbreaks reported in Red Sea, Gedarif, Blue Nile and Darfur states as shown in figure (14) (Anonymous, AHDEC, 2008- 2012).

There are differences in the epidemiological pattern of PPR in the different ecological zones (Gopilo, 2005). In Sudan the first outbreak of PPR in sheep and goats was discovered in 1971 in south Gedarif which considered as an agricultural state with high rain fall (eastern Sudan, low rainfall woodland savanna ecological zone). Then the disease was reported in Sennar (state with high density of wildlife and sharing borders with Gedarif and both states are in Sudan east border with Ethiopia). In 1972 PPR was found in Mieliq area in Gezira state in central Sudan, also considered in the low rainfall woodland savanna ecological zone. Then PPR was reported in Western Sudan in 1992. Between 2000 and 2002 the disease was detected in different parts of the country, since then continuous outbreaks occurred annually. High seroprevalence was noticed in eastern Sudan (Kassala and Gedarif) followed by central states Gezira and White Nile (Saeed et al., 2010).That is in agreement with findings of Salih et al., 2014, who found PPR seroprevalence in non vaccinated sheep and goats in Gedarif and Sennar states is higher

than those in River nile and North kordofan states (both states are in semi-desert ecological zone). From the previous studies and reported outbreaks; it could be concluded that PPR has a high seroprevalence in the low rainfall woodland savanna ecological zone of east Sudan more than in semi-desert ecological zones.

Current PPR control in Sudan:

A cold chain vaccine is used and it is produced in the Veterinary Research Laboratories in Khartoum-Soba. The governmental veterinary authority in the country facilitate a vaccination program, that controlled by the Head quarter of the federal ministry but since 2003 vaccination is implemented by the state ministries.

PPR surveillance and monitoring is performed by the teams of the head quarter. From the state reports it is obviously that, the numbers of vaccination coverage in sheep and goats in every state comparing to the sheep and goat population as shown in Figure (15).

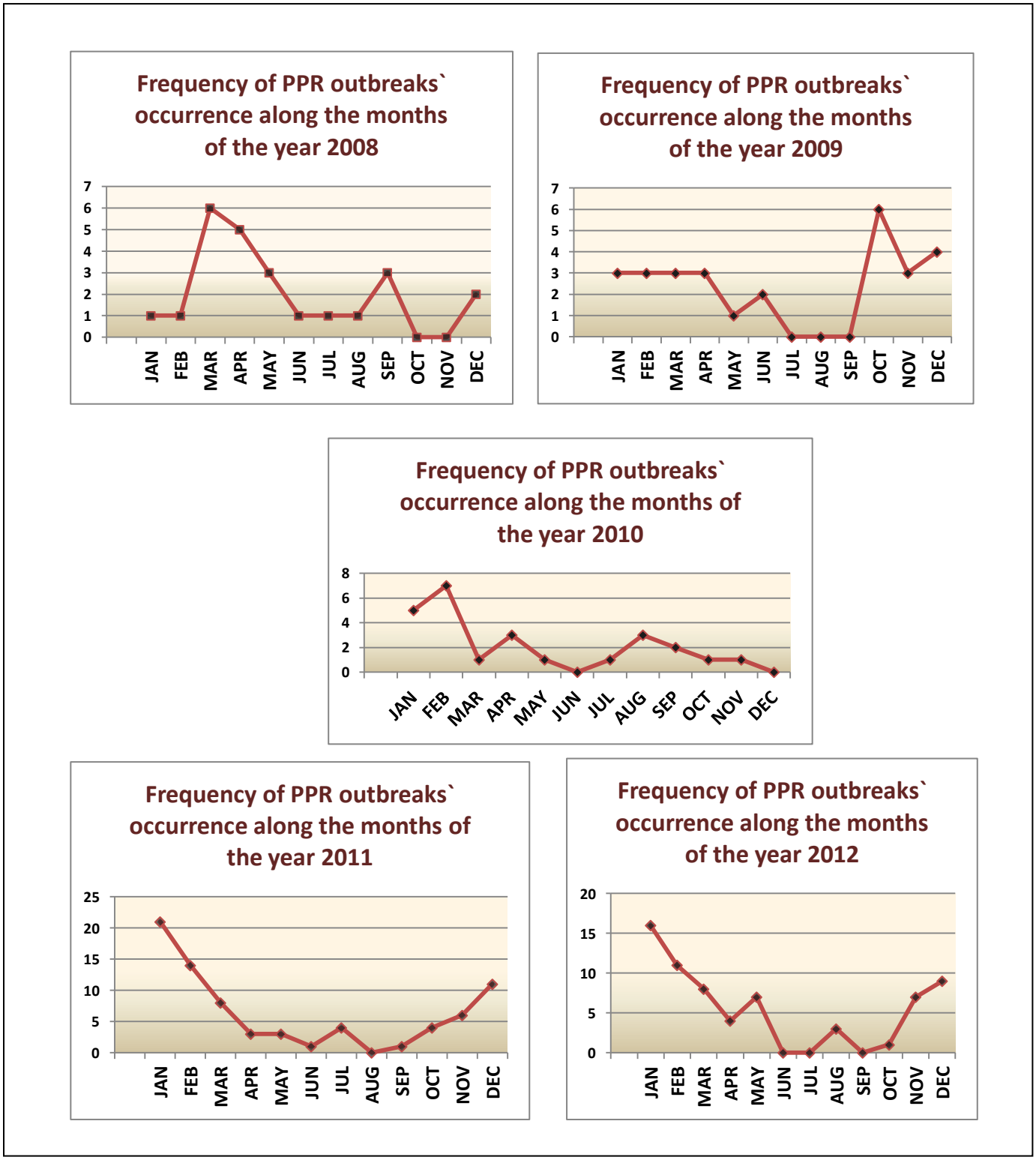

Figure (13): Line charts show the temporal patterns for PPR outbreaks` occurrence in the years 2008, 2009, 2010, 2011 and 2012.

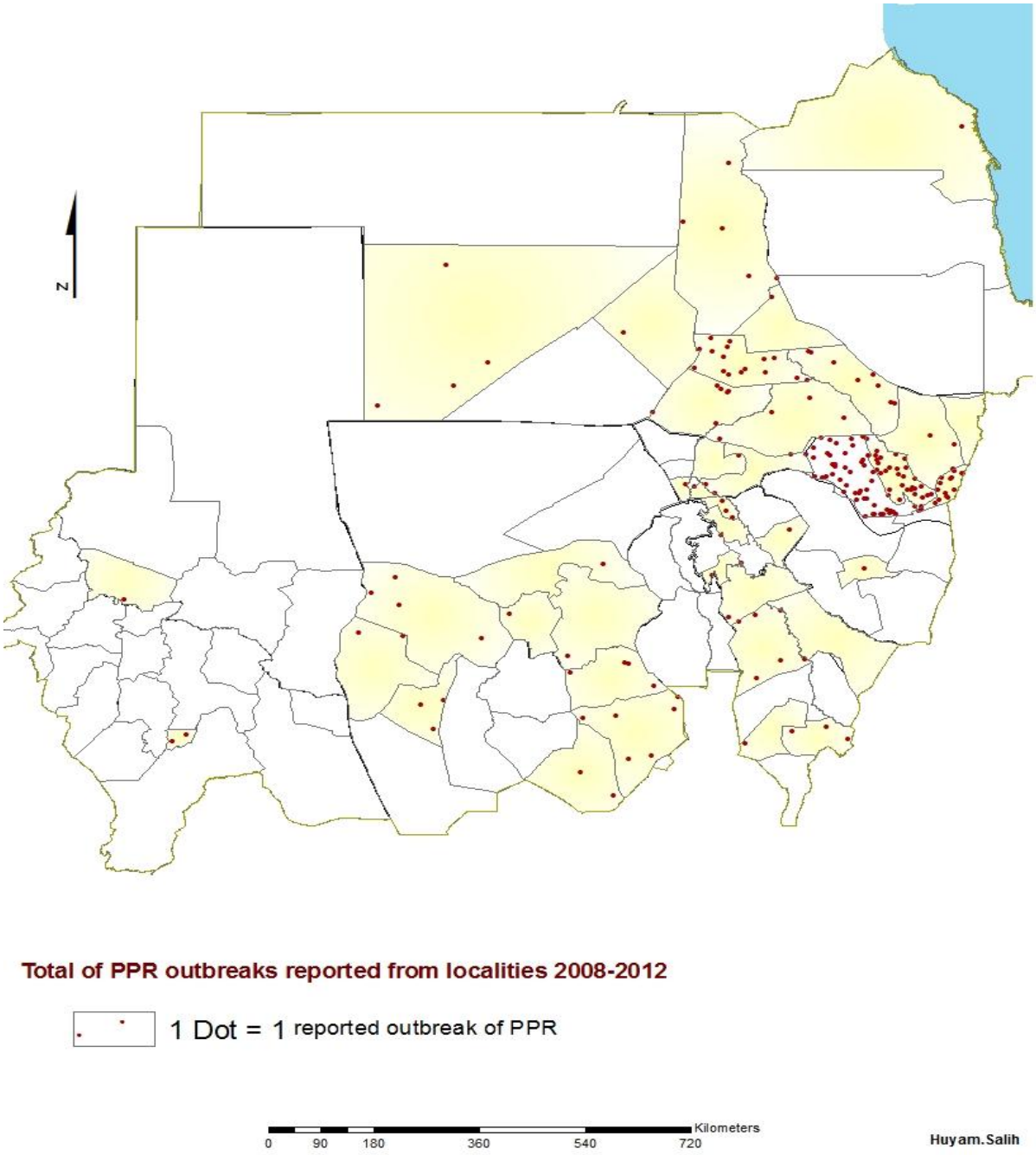

Figure (14): Spatial distribution for the reported outbreaks of PPR in the localities of Sudan during the period from 2008 to 2012.

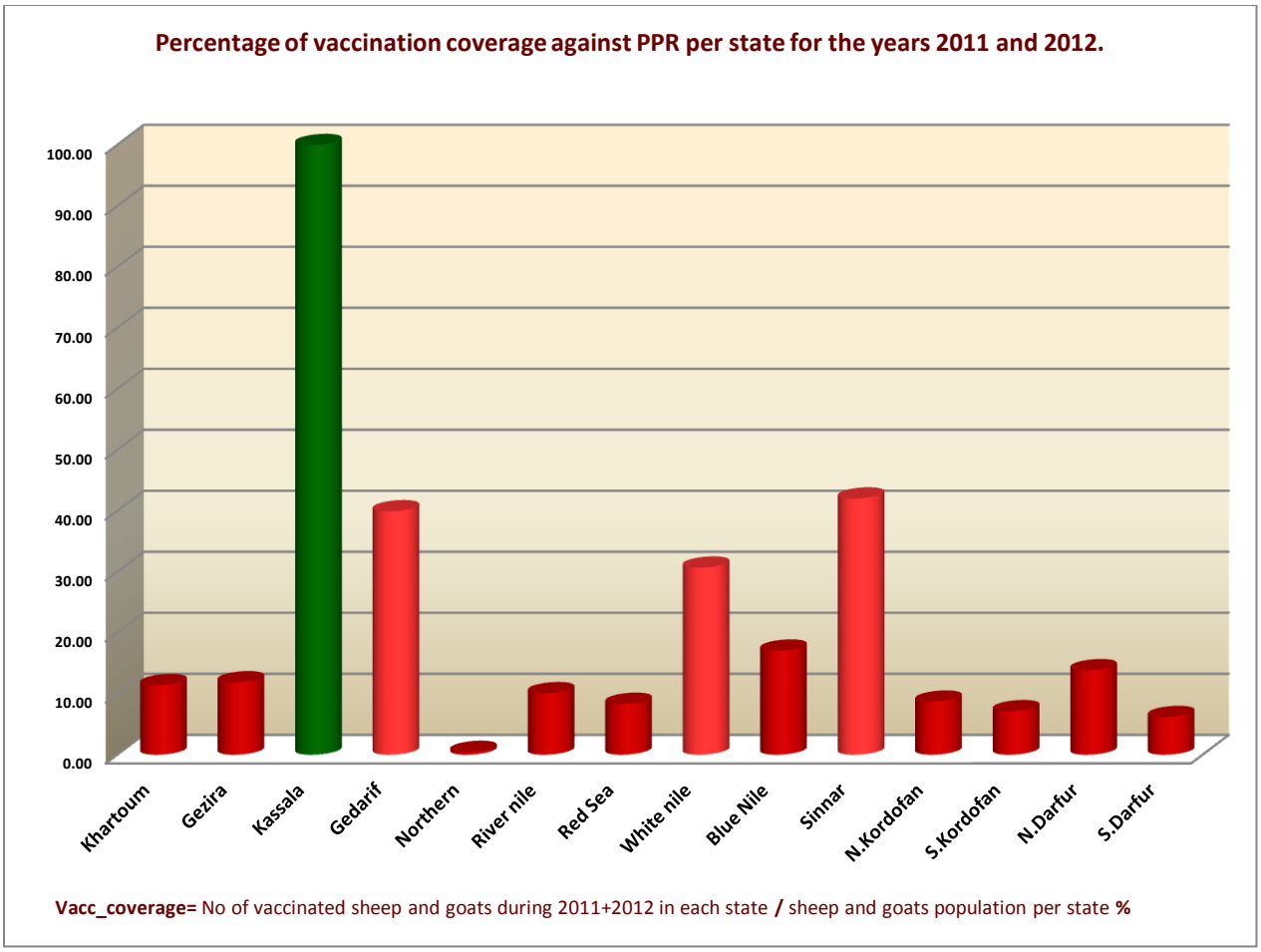
Percentage of vaccination coverage against PPR per state for the years 2011 and 2012.
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Khartoum
Gezira
Kassala
Gedarif
Northern
River nile
Red Sea
White nile
Blue Nile
Sinnar
N.Kordofan
S.Kordofan
N.Darfur
S.Darfur
Vacc_coverage= No of vaccinated sheep and goats during 2011+2012 in each state / sheep and goats population per state %

Figure (15)

2.4.3.3 Description of production and marketing of exported sheep in Sudan

Sheep production and marketing systems in Sudan

Livestock production in Sudan has three systems; traditional low input- low output system, modern or intensive system and feedlot. The pastoral system is predominant and has three components; 1) pastoral nomadism and transhumance system, 2) Sedentary and semi-sedentary agro-pastoralists and 3) Urban and peri-urban scavenging system (ElDirani *et al.*, 2009). According to the 2008 census, 2.7 million people (500,000 households) qualified for the category of 'Nomadic' based on tribal affiliation as well as their livelihood strategy (UNEP, 2013).

In analysis of livestock mobility according to the three components of the pastoral group, high degrees of routine livestock mobility were found in all of them. Nomads are defined as "those who travel in search of pasture and water depending on the environmental conditions, across known tracks, and their animal stocks are the source of life". But livestock mobility is not a marginal issue concerning nomadic groups only- since sedentary condition of livestock keeping households does not result in sedentary animal production. The settled sheep keepers practice at least four types of mobility. First, during the rainy season the flocks is taken to pasture sites about one day from the village, this mobility called "*Almunshag*" and has a goal of keeping sheep flocks as long as possible on the highest quality pastures and sheep leave pasture only for drink water from *rahad* that usually within three hours range.

Second, after the rainy season sheep are moved south (*Moatta*). This involves a three days trek from the village to reach the pasture. Water is brought in tanks from neighboring villages. Third, during the cold season until mid February the sheep are taken to pasture that is particularly rich in water (rich with grasses such as *argassi* and *aldaib*) , and it is about 140 km from the village with no need for watering the animals. Finally, during the hot dry season, the flock kept in the village and taken to graze daily, not further than 5-12 km and watering is carried out in the village.

Mobile strategy is not limited to primary production. Large numbers of animals are taken to the terminal market on the hoof during the wet season, taking advantage of a long journey through

pastoral land for improving the state of animals to the point of not requiring feedlot for export (UNEP, 2013).

Marketing of sheep is systematic and all transactions are money based. Hamari sheep with a red or blonde fleece are preferred on the export market. Male lambs born during the rainy season, then sold in the following season at the age of four or five months before the hot season. Also unproductive females are sold to be replaced with productive ones which accelerate the herd growth. The newborn when not sold, are kept to suckle for 12 months. Part of the money from the sale is reinvested in annual vaccination and veterinary drugs (UNEP, 2013).

Production areas and seasonal migration patterns significantly influence domestic, cross-borders and formal export trade routes for livestock. These two factors have developed a unique internal livestock marketing system composed of four tiers:

- Direct sales from pastoral herd,
- Primary markets,
- Secondary markets and
- Terminal markets

Primary markets: - Usually located within a village or near a livestock producing villages.

- Have no physical infrastructure (such as fences, water and feed for animals) or market information.
- Animals are not kept in the market overnight.
- Market days are variable; once or twice a week. Some primary markets operate only during wet or dry seasons.
- With no veterinary certificates issued for movement of purchased animals.

Secondary markets: - May or may not have facilities and infrastructures.

- Animals are inspected by veterinary officer and veterinary health certificate is issued.
- Animals may be kept overnight in fenced area.

Terminal markets: - Have infrastructures and facilities like fencing, water and feed, veterinary clinic and pharmacy and loading rumps.

- Officials from veterinary authorities and market management are present in the market.
- Live sheep destined for exportation are inspected, vaccinated and health certificates are issued by federal authorities.

Elmuwelih in west Omdurman is the largest terminal market in Khartoum state. Elkhiwai and Elshwak are primary markets but due to the establishment of the inspection and vaccination centers in both towns, veterinary health certificates are issued and they serve as terminal markets from which live sheep are send to Swakin terminal quarantine directly (ElDirani *et al.*, 2009).

Marketing channels for exported sheep

Market channels from production sites to the final export markets and main domestic consumption areas; are dynamic. Elmuwelih livestock market was the major market for export for many years and sheep were sent from it to Elkadaro central quarantine then to the terminal quarantine on the Red sea. The establishment of Elkhiwai inspection and vaccination center in 2005 in the heart of the main sheep production area in Sudan (North Kordofan); encourage the main brokers in the sheep export business to move their activity from Omdurman to Elkhiwai, making it the most important export market for sheep. In 2012 the sheep trade channels appeared to have been changing again. A large part of export trade was taken place in Gedarif, with about 400,000Hamari sheep being channeled there from Elkhiwai in 2011.

Many exporting companies prefer to buy sheep from Elkhiwai to be transported and exported through Gedarif center to Swakin due to many reasons; the state Ministry of Animal Resources in Gedarif has eliminated export fees, when exporting directly from Gedarif the time between purchase on domestic market and export is shortened and more time is saved with the different way of processing the compulsory *Brucella* test: in Elkhiwai the samples are processed in Elobeid veterinary research laboratory, and results come the next day, while in Gedarif the Laboratory is near and results are immediate (UNEP, 2013).

2.4.3.4 PPR risk assessment pathway and analysis of the live sheep exports ` value chain:

1) Risk assessment for PPR spread in sheep exports from Sudan:

i. Hazard identification:

It is a necessary first step, a hazard being something potentially harmful to animals, human, plant and environment (FAO, 2011). This step is defined as the process of identifying any pathogenic agent which could potentially be introduced in the commodity considered for importation, and since risk analysis is equally applicable for other areas such as disease surveillance and control programme; so hazard identification is merely the step of identifying what is that might be go wrong in whatever activity is being considered (MacDiarmid and Pharo, 2003).

In this study the hazard is the PPR virus.

ii. Risk question:

To proceed to risk assessment from hazard identification requires the framing of a "risk question", which should be composed as: What is the risk of [outcome] associated with [hazard] in [location/ population] during [time period]? (FAO, 2011).

Study risk question: What is the risk of exporting live sheep which is infected with PPRV to the Kingdom of Saudi Arabia during the year 2012?

iii. Risk pathway:

Risk pathway is a graphical depiction of the biological pathways to provide a useful frame work "mind map" in a simple and transparent manner to provide the following:

- Identify pathways and variables
- Identify information requirements
- Ensure a logical chain of events in space and time
- Provide a framework for the development of a mathematical model
- Ensure the appropriate estimate is calculated
- Clarify ideas and understanding of problems and
- Assist with communicating the model structure.

Scenario trees are the most appropriate and effective way in depicting biological pathways (MacDiarmid and Pharo, 2003). The risk pathway is a series of conditions that must be met, or events that have to occur in order for the unwanted outcome to occur. And the analysis of pathway is the main tool used in risk assessment (FAO, 2011).

Release assessment pathway:

It is describing the biological pathway for introducing hazard to the animals and estimates the probability of its occurring (OIE, 2014). In this study the release assessment estimated the probability or likelihood of introducing the PPRV to the selected sheep herds within the local markets and collectives quarantines and vaccination centers as shown in Figure (16).

Exposure assessment pathway

It consists of describing the biological pathways necessary for exposure of animals to the hazard (OIE, 2014). In this study the exposure assessment estimated the probability or likelihood of the exposure of sheep herds (destined for exportation), to the PPRV during transportation to the terminal quarantine, within terminal quarantine and during shipping to importing country as shown in Figure (17).

Qualitative estimation for the probability (likelihood):

It involves two steps:

1- Information (derived from collected data) was put together with the risk pathway in a tabular frame work in order to make a systematic process and evidence-based assessment and encourage transparency.

2- Logical conclusions were extracted by comparing the requirements for each step with the actual situation.

Tools used in qualitative approach for estimating the likelihood (Probability):

- Identification and characterization of risk factors within value chains,
- Scoring methods,
- Risk ranking based on scores
- Expert opinion (FAO, 2011).

The risk scoring of the Department of environment, food and rural affaires agency (Defra) in the United Kingdom was used in this study for estimating the likelihood as in Table (11).

Table (11): Explain the meaning of the different levels of the likelihood provided by Defra-UK.

Likelihood	Description
VL Very low	Rare (the risky event may occur in exceptional circumstances)
L Low	Possible (the risky event may occur in the next three years)
M Medium	Likely (the risky event is likely to occur more than once in the next three years)
H High	Almost certain (the risky event is likely to occur this year or in frequent intervals)

Influential Diagram: It is another approach of depicting a model graphically, to show how different variables interact with one another (MacDiarmid and Pharo, 2003). Probability of exporting at least on live sheep infected with PPRV is explained by an influential diagram as shown in figure (18)

Consequences assessment:

It describes the potential consequences of a given exposure and estimates the probability of them occurring. A causal process should exist by which exposure produce adverse health or environment consequence, which may in turn lead to socio- economic consequences (OIE, 2014). The impact or consequences of the risky event can be ranked on a scale similar to that used for likelihood by Defra, from low to high. This assessment would be based on various socio-economic and epidemiological criteria, specific to the hazard and risk in question (FAO, 2011).

Using Tabular framework for risk pathway, value chain description and risk factors:

The tabular framework explains the key information for describing the location of risk in the pathway and geographically referring to the value chain mapping. Also it contains detailed description for the main risk factors was organized using tabular framework. The risk factors represent all the factors which influence the steps of risk pathway, either increasing the risk or decreasing it. The last section of the table illustrates the criteria for scoring the risk and the risk score or estimate.

Overall risk estimation:

Consist of integrating the results from release, exposure and consequence assessments to produce overall measures of risks associated with hazard identified at the outset (OIE, 2014).

The overall level of risk is defined as the product of the likelihood of an unwanted outcome is occurring and the impact resulting should it occur; Risk= likelihood× impact (FAO, 2011).

The overall assessment of risk is made based on:

- The probabilities along the pathway,
- The degree of exposure (number of times pathway active, volume of exports per year),
- The impact of the unwanted outcome.

A qualitative risk assessment scheme used by Defra in UK was used to estimate the overall risk as shown in Figure (19).

↑ **Likelihood**	**H**	M	M	H	H*
	M	L	M	M	H
	L	VL	L	M	M
	VL	VL	VL	L	M
		VL	**L**	**M**	**H**
	Impact →				

Figure (19): Risk estimation scheme by Defra.

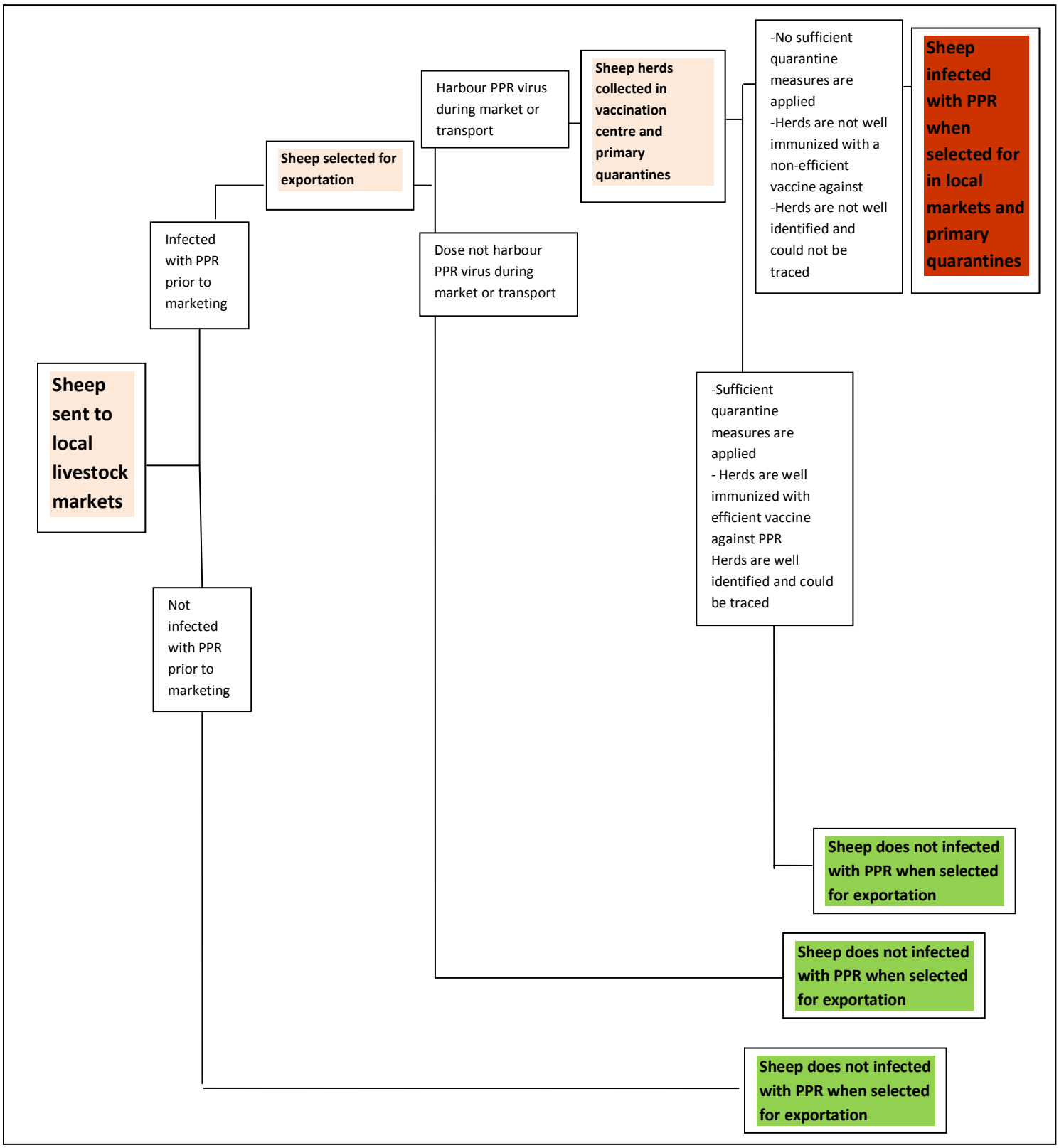

Figure (16): Scenario tree for a release risk assessment

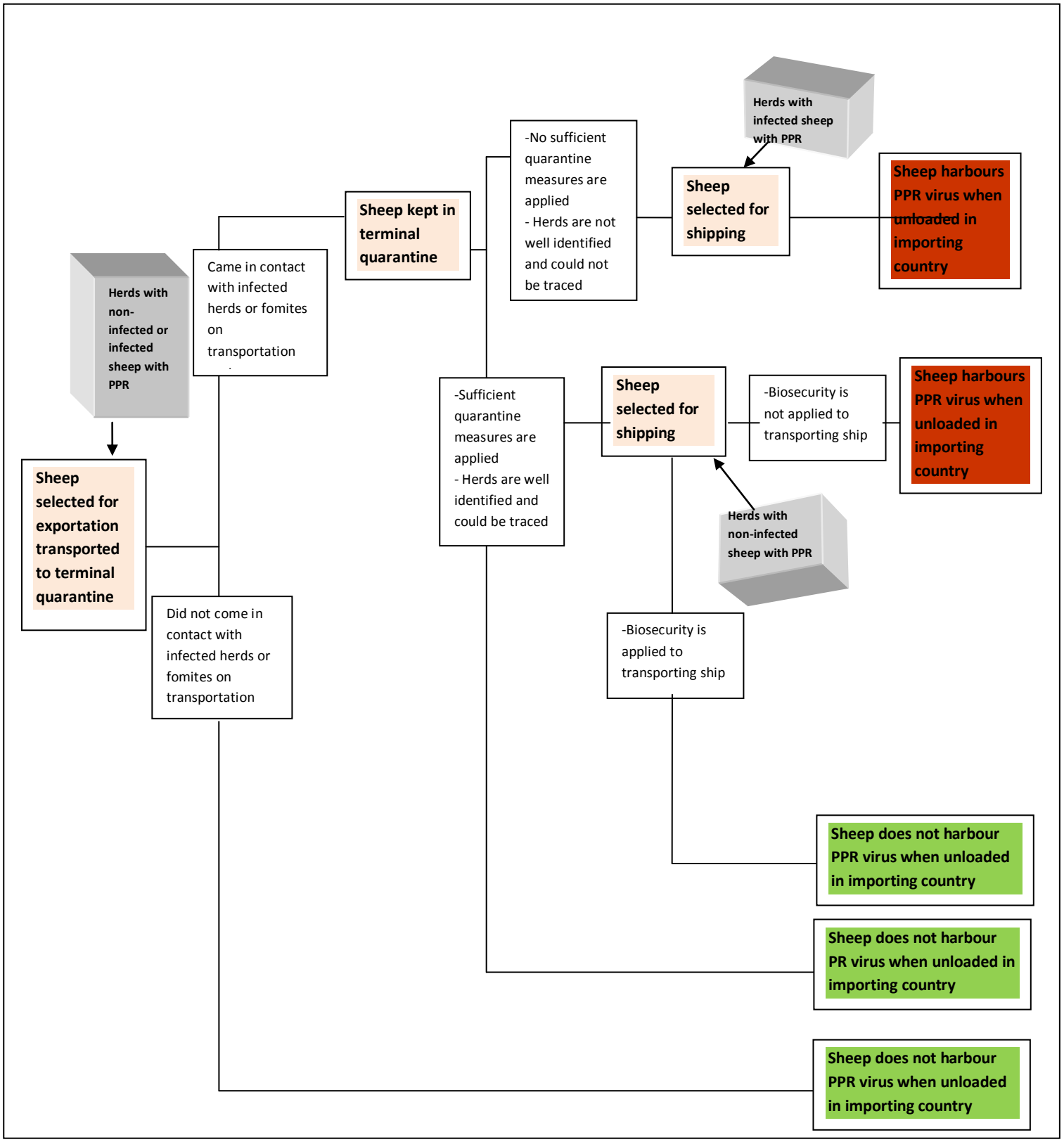

Figure (17): Scenario tree for an exposure risk assessment

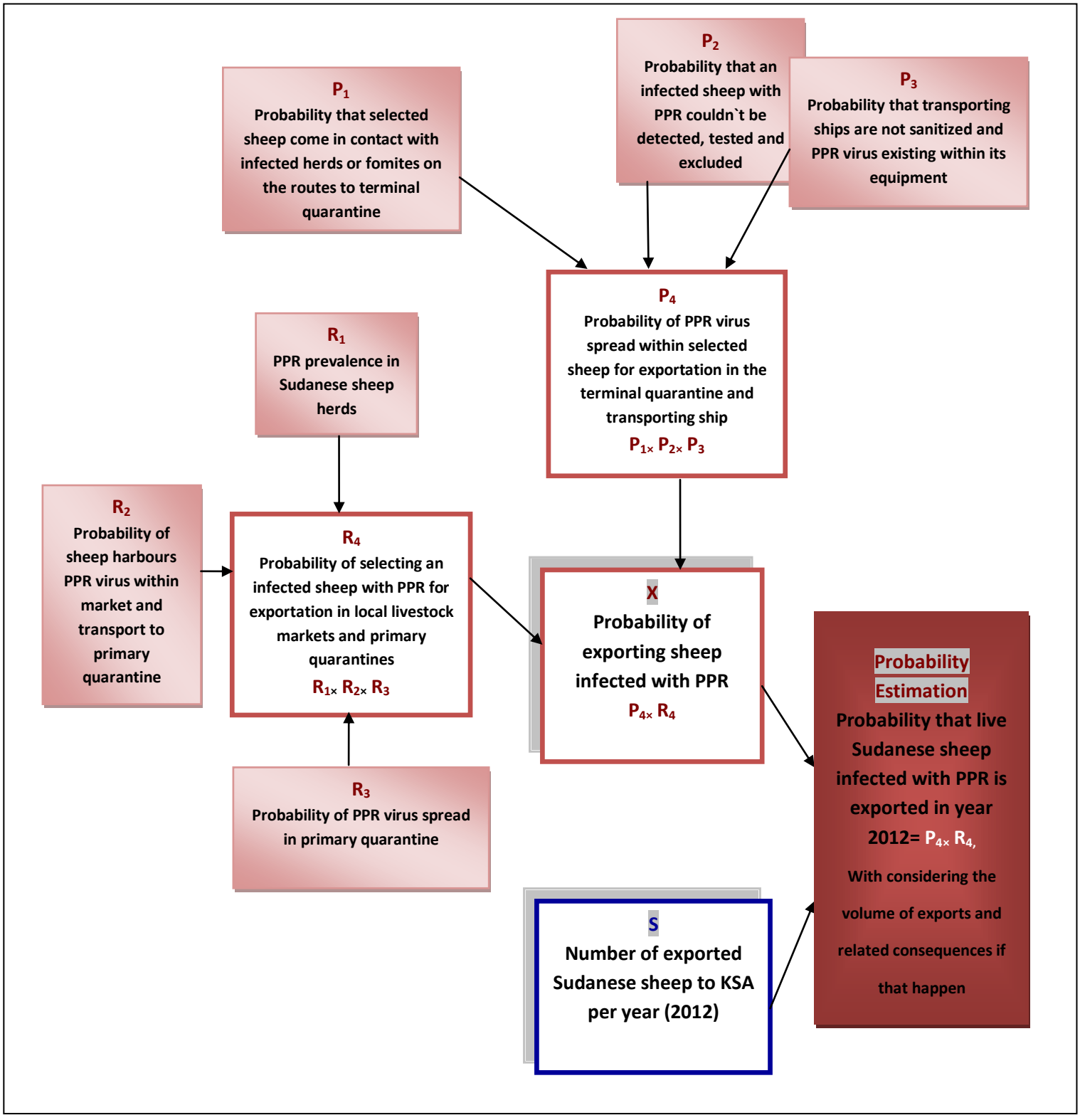

Figure (18): Influencial diagram modeling risk probabilities of exporting Sudanese live sheep infected with PPR virus.

2) Value chain analysis:

Value chains are groups of people linked by an activity to provide, process, produce, transport and supply a specific commodity. Value chain analysis is the study of the chains that link production system, markets and consumers to determine risk hotspots and to suggest an effective risk reduction intervention (FAO, 2011). Sheep exports value chains were studied in purpose of assessing the risk hotspots, which considered being any behaviour or process contribute to the transmission or spread of PPRV among sheep in the exports chain.

(Based on the FAO practical framework- A value chain approach to animal diseases risk management, 2011); a value chain analysis is split into three basic steps:

1-Value chain mapping for live sheep exportation; it is a graphical representation of the exported sheep value chain. This diagram was prepared through primary data collection from scientific literature and governmental reports and studies.

2- Identification of important routes, people, groups and organizations involved in sheep exportation chain;

- Primary identification was done by reviewing scientific studies and governmental reports to determine the following: - systems of sheep producers using different routes within exports chain,
- Volume estimates of sheep that moves through the export chain,
- Monetary value that moves through the different routes of the chain.

All gained information from the last two steps in value chain analysis are relevant to risk assessment and influencing the magnitude of PPR risk in different locations and processes which considered risk hotspo

3- Assessing the profitability, power and institutional environment of the key people, groups and organizations involved in the chain

CHAPTER THREE

RESULTS

3.1 Results of seroprevalence and risk factors study:

480 serum samples were tested by ELISA for PPRV Abs prevalence in Sennar, Gedarif, River Nile and North Kordofan states.

3.1.1 Frequencies and distribution of tested serum samples by states and localities:

Table (12 A): frequencies and distribution of tested serum samples by states and localities:

Risk factor		Frequency	Relative frequency	Cumulative Frequency
State	**Sinnar**	138	28.8	28.8
	Gedarif	143	29.8	58.5
	River Nile	106	22.1	80.6
	North Kordofan	93	19.4	100.0
	Total	480	100.0	
Localities	**Abuhugar**	68	14.2	14.2
	East sinnar	44	9.2	23.3
	Dindir	26	5.4	28.8
	Elfashga	29	6.0	34.8
	Basonda	23	4.8	39.6
	Elgorisha	61	12.7	52.3
	Western Glabat	30	6.3	58.5
	Atbra	50	10.4	69.0
	Barbar	10	2.1	71.0
	Eldamar	46	9.6	80.6
	Elkhiwai	15	3.1	83.8
	Abozabad	12	2.5	86.3
	Umrwaba	10	2.1	88.3
	Elrahad	56	11.7	100.0
	Total	480	100.0	

Table (12 B): Frequencies and distribution of tested serum samples within husbandry systems, herd species composition and herd size:

Risk factor		Frequency	Relative frequency	Cumulative frequency
Husbandry system: Open grazing		322	67.1	67.1
Pastoralist		72	15.0	82.1
Intensive		86	17.9	100.0
Total		480	100.0	
Herd composition: Sheep and goats		327	68.1	68.1
Sheep		82	17.1	85.2
Goats		71	14.8	100.0
Total		480	100.0	
Herd size:	**Less than 61**	213	44.4	44.4
	61-120	208	43.3	87.7
	121-200	27	5.6	93.3
	More than 200	32	6.7	100.0
	Total	480	100.0	

Table (12 C): Frequencies and distribution of tested serum samples among species, breeds, sex and age groups:

Risk factor		Frequency	Relative frequency	Cumulative frequency
Species:	**Ovine**	261	54.4	54.4
	Caprine	219	45.6	100.0
	Total	480	100.0	
Breed:	**Rufaa**	43	9.0	9.0
	Ashgar	14	2.9	11.9
	Gwasma	5	1.0	12.9
	Kenana	1	0.2	13.1
	Kwahla	6	1.3	14.4
	Baladi	241	50.2	64.6
	Ethio- baladi	20	4.2	68.8
	Garag- baladi	15	3.1	71.9
	Hamary	43	9.0	80.8
	Saanen	11	2.3	83.1
	Shami	4	0.9	84.0
	Nubian- shami	1	0.2	84.2
	Nubi	48	10.0	94.2
	Baladi-saanen	28	5.8	100.0
	Total	480	100.0	
Sex:	**Male**	146	30.4	30.4
	Female	334	69.6	100.0
	Total	480	100.0	
Age:	**1-3 months**	51	10.6	10.6
	4-12 months	186	38.8	49.4
	More than 12 months	243	50.6	100.0
	Total	480	100.0	

Table (12 D): Frequencies and distribution of tested serum samples regarding the climatic parameters in surveyed states at the time of sampling process:

Risk factor		Frequency	Relative frequency	Cumulative frequency
Annual rainfall	**Low rainfall**	199	41.5	41.5
	High rainfall	281	58.5	100.0
	Total	480	100.0	
Wind speed	**Slow wind speed**	249	51.9	51.9
	High wind speed	231	48.1	100.0
	Total	480	100.0	
Max Day Temperature	**Moderate**	236	49.2	49.2
	High	244	50.8	100.0
	Total	480	100.0	
Relative humidity	**Low**	337	70.2	70.2
	High	143	29.8	100.0
	Total	480	100.0	

3.1.2 The overall seroprevalence rate of PPR:

Generally, Antibodies against PPR were detected in all sampled localities in the four studied states. The overall sero-prevalence rate was found to be 45.6% (219/480).

3.1.3 Seroprevalence rate of PPR in Sinnar, Gedarif, River Nile and North Kordofan states:

A total of **(79)57.2%** in Sennar, **(66) 46.2%** in Algadarif, **(37)34.9%** in River Nile and **(37)39.8%** in North Kordofan were found to be Positive for PPR Abs as shown in table (13) and presented in Figure (20).

Table (13) Cross-tabulation for PPR sero-prevalence rate with the potential risk factors:

Risk factor		No of tested animals	No of positive samples	Sero-prevalence rate %
State:	**Sinnar**	138	79	57.2
	Gedarif	143	66	46.2
	River nile	106	37	34.9
	North kordofan	93	37	39.8
Locality :	**Abuhugar**	68	43	63.2
	East sinnar	44	25	56.8
	Dindir	26	11	42.3
	Elfashga	29	11	37.9
	Basonda	23	15	65.2
	Elgorisha	61	29	47.5
	Western glabat	30	11	36.7
	Atbra	50	14	28
	Barbar	10	7	70
	Eldamar	46	16	34.8
	Elkhiwai	15	4	26.7
	Abozabad	12	11	91.7
	Umrwaba	10	1	10
	Elrahad	56	21	37
Husbandarysystem:	**Open-grazing**	322	137	42.5
	Pastoralists	72	49	68.1
	Intensive	86	33	38.4
Housing:	**No house**	175	73	50.3
	Building of bricks	73	35	47.9
	Metal	11	0	0
	Mud	58	18	31
	Shrub fence	186	86	46.2
	Wood& scrap	7	7	100

Risk factor		No of tested animals	No of positive samples	Sero-prevalence rate %
Herd Composition : sheep &goats		327	157	48
	Sheep	82	30	36.6
	Goats	71	32	45.1
Species:	**Ovine**	261	114	43.7
	Caprine	219	105	47.9
Breed:	**Rufaa**	43	27	62.8
	Ashgar	14	5	35.7
	Gwasma	5	4	80
	Kenana	1	0	0
	Kwahla	6	5	83.3
	Baladi	241	108	44.8
	Ethio-baladi	20	6	30
	Garag-baladi	15	5	33.3
	Hamary	43	20	46.5
	Saaneen	11	2	18.2
	Shami	4	0	0
	Nubian-shami	1	0	0
	:Nubi	48	24	50
	Baladi- saaneen	28	13	46.4
Sex:	**female**	334	182	54.5
	Male	146	37	25.5
Age :	**1-3 months**	51	10	19.6
	4-12 months	186	70	37.6
	>12 months	243	139	57.2
Herd size:	**less than& = 60**	213	87	40.8
	61-120	208	100	48.1
	121-200	27	18	66.7
	More than 200	32	14	43.8
Annual Rainfall :	**Low rainfall**	199	74	37.2

Risk factor		No of tested animals	No of positive samples	Sero-prevalence rate %
High rainfall		281	145	51.6
Wind speed :	**Slow wind speed**	249	103	41.4
High wind speed		231	116	50.2
Day Max temperature:Moderate		236	103	43.6
High		244	116	47.5
Relative Humidity :Low humidity		337	153	45.4
High humidity		143	66	46.2

3.1.4 Risk factors associated with PPR sero-positivity in univariate analysis using Chi-Square test:

About **14** risk factors were investigated using structured questionnaire for every samplled herd and other collected data, out of them **9 risk factors** were found to be associated with PPR ser-prevalence (P-value ≤ 0.05) in univariate analysis when analysed by Chi- square.

Sero- prevalence rate of PPR in states and Localities:

Sinnar state was found to have the highest prevalence (57.2%). and within localities Abozabad in Northern Kordofan has the highest prevalence (91.7%) followed by Barbar in River Nile state (70%).

Sero- prevalence rate of PPR within the different husbandry systems and hosing types:

PPR is highly prevalent among pastoralists (68.1%) than the other husbandry systems. Among animal housing types animals kept in scrap fence houses were found to have the highest PPR ser-positivity (100%).

Sero- prevalence rate of PPR in surveyed breeds:

Kwahla breed in sheep is mostly affected by PPR than other breeds (83.3%).

Sero- prevalence rate of PPR among males and females:

Females were found to be more affected with PPR rate of (83.1%).

Sero- prevalence rate of PPR within the age groups:

Regarding age groups; animal over 12 months old have the highest prevalence (57.2%).

Sero- prevalence rate of PPR regarding climate factors under investigation:

Two climatic factors from four investigated, were found associated with PPR prevalence; states with high rain fall and states with high wind speed were found to have the highest PPR prevalence (51.6%) and (50.2%) respectively. As shown in Table (14).

Table (14): Univariate analysis for risk factors association with the PPR sero- positivity using Chi- square test:

Risk factor	No of tested animals	No of positive samples	Sero-prevalence rate %	X^2	Df	p-value
State:						
Sinnar	138	79	57.2	**13.717**	**3**	**.003**
Gedarif	143	66	46.2			
River nile	106	37	34.9			
North kordofan	93	37	39.8			
Locality :						
Sinaar states: 1-Abuhugar	68	43	63.2	**46.017**	**13**	**.000**
2- East sinnar	44	25	56.8			
3- Dindir	26	11	42.3			
Gedarif Localities:1- Elfashga	29	11	37.9			
2- Basonda	23	15	65.2			
3- Elgorisha	61	29	47.5			
4- Western glabat	30	11	36.7			
River Nile localities:1- Atbra	50	14	28			
2- Barbar	10	7	70			
3- Eldamar	46	16	34.8			
N. Kordofan localities: 1- Elkhiwai	15	4	26.7			
2- Abozabad	12	11	91.7			
3- Umrwaba	10	1	10			
4- Elrahad	56	21	37			
Husbandarysystem:						
Open-grazing	322	137	42.5	**17.656**	**2**	**.000**
Pastoralists(Nomadic)	72	49	68.1			
Intensive	86	33	38.4			

Risk factor	No of tested animals	No of positive samples	Sero-prevalence rate %	X^2	Df	p-value
Housing:						
No house	175	73	50.3	**24.038**	**5**	**.000**
Building of bricks	73	35	47.9			
Metal	11	0	0			
Mud	58	18	31			
Shrub fence	186	86	46.2			
Wood& scrap	7	7	100			
Herd Composition:						
sheep &goats	327	157	48	**3.461**	**2**	**.177**
Sheep	82	30	36.6			
Goats	71	32	45.1			
Species:						
Ovine	261	114	43.7	**.874**	**1**	**.200**
Caprine	219	105	47.9			
Breed:						
Rufaa	43	27	62.8	**23.193**	**13**	**.039**
Ashgar	14	5	35.7			
Gwasma	5	4	80			
Kenana	1	0	0			
Kwahla	6	5	83.3			
Baladi	241	108	44.8			
Ethio-baladi	20	6	30			
Garag-baladi	15	5	33.3			
Hamary	43	20	46.5			
Saaneen	11	2	18.2			
Shami	4	0	0			
Nubian-shami	1	0	0			
Nubi	48	24	50			
Baladi- saaneen	28	13	46.4			

Risk factor	No of tested animals	No of positive samples	Sero-prevalence rate %	X^2	Df	p-value
Sex:						
Female	334	182	54.5	**34.793**	**1**	**.000**
Male	146	37	25.5			
Age :						
1-3 months	51	10	19.6	**31.829**	**2**	**.000**
4-12 months	186	43	37.6			
>12 months	243	139	57.2			
Herd size :						
less than& = 60	213	87	40.8	**7.330**	**3**	**.062**
61-120	208	100	48.1			
121-200	27	18	66.7			
More than 200	32	14	43.8			
Annual Rainfall :						
Low rainfall	199	74	37.2	**9.758**	**1**	**.001**
High rainfall	281	145	51.6			
Wind speed :						
Slow wind speed	249	103	41.4	**3.784**	**1**	**.032**
High wind speed	231	116	50.2			
Monthly Day Max temperature:						
Moderate	236	103	43.6	**.734**	**1**	**.222**
High	244	116	47.5			
Monthly Relative Humidity :						
Low humidity	337	153	45.4	**.023**	**1**	**.479**
High humidity	143	66	46.2			

3.1.5 Multivariate analysis using Logistic regression for the risk factors that associated with PPR sero-prevalence:

The significant 9 factors that found significant in the univariate analysis were subjected to multivariate analysis using Logistic Regression model. Out of them **5** risk factors were found to have an association with PPR sero- prevalence (P-value ≤ 0.05); States, Localities, Husbandry system, sex and age as shown in table (15).

Table (15): Multivariate analysis for the association between sero-positivity status and the potential riska factors resulting from the univariate analysis using Logistic regression:

Risk Factor	Sero-prevalence (%)	Exp (B)	95% C.I for Exp(B)		P-value
			Upper	Lower	
States					**.038**
Sinnar	57.2	.205	.025	1.658	.137
Gedarif	46.2	1.104	.186	6.560	.913
North Kordofan	39.8	.018	.001	.427	.013
River Nile (Ref)	34.9				
Localities					**.001**
Abuhugar	63.2	12.949	2.025	82.794	.007
East Sinnar	56.8	11.217	1.562	80.536	.016
Elfashga	37.9	1.528	.300	7.786	.610
Basonda	65.2	5.731	1.221	26.906	.027
Elgorisha	47.5	3.662	.761	17.631	.106
Atbara	28	1.376	.274	6.899	.698

Risk Factor	Sero-prevalence (%)	Exp (B)	95% C.I for Exp(B)		P-value
			Upper	Lower	
Elkhiwai	26.7	.448	.014	4.920	.511
Abuzabad	91.7	9.567	.530	172.768	.126
Elrahad	37	.080	.005	1.355	.080
Umrwaba (Ref)	**10**				
Husbandary system					**.007**
Open grazing	42.5	.061	.011	.348	.002
Pastoralists(Nomadic)	68.1	.073	.008	.633	.018
Intensive (Ref)	**38.4**				
Sex					**.000**
Female	54.5	.414	.219	.782	.007
Male (Ref)	**25.5**				
Age					**.000**
More than 12 months	57.2	.295	.178	.492	.000
4 to 12 months	37.2	.161	.064	.408	.000
1 to 3 months (Ref)	**19.6**				

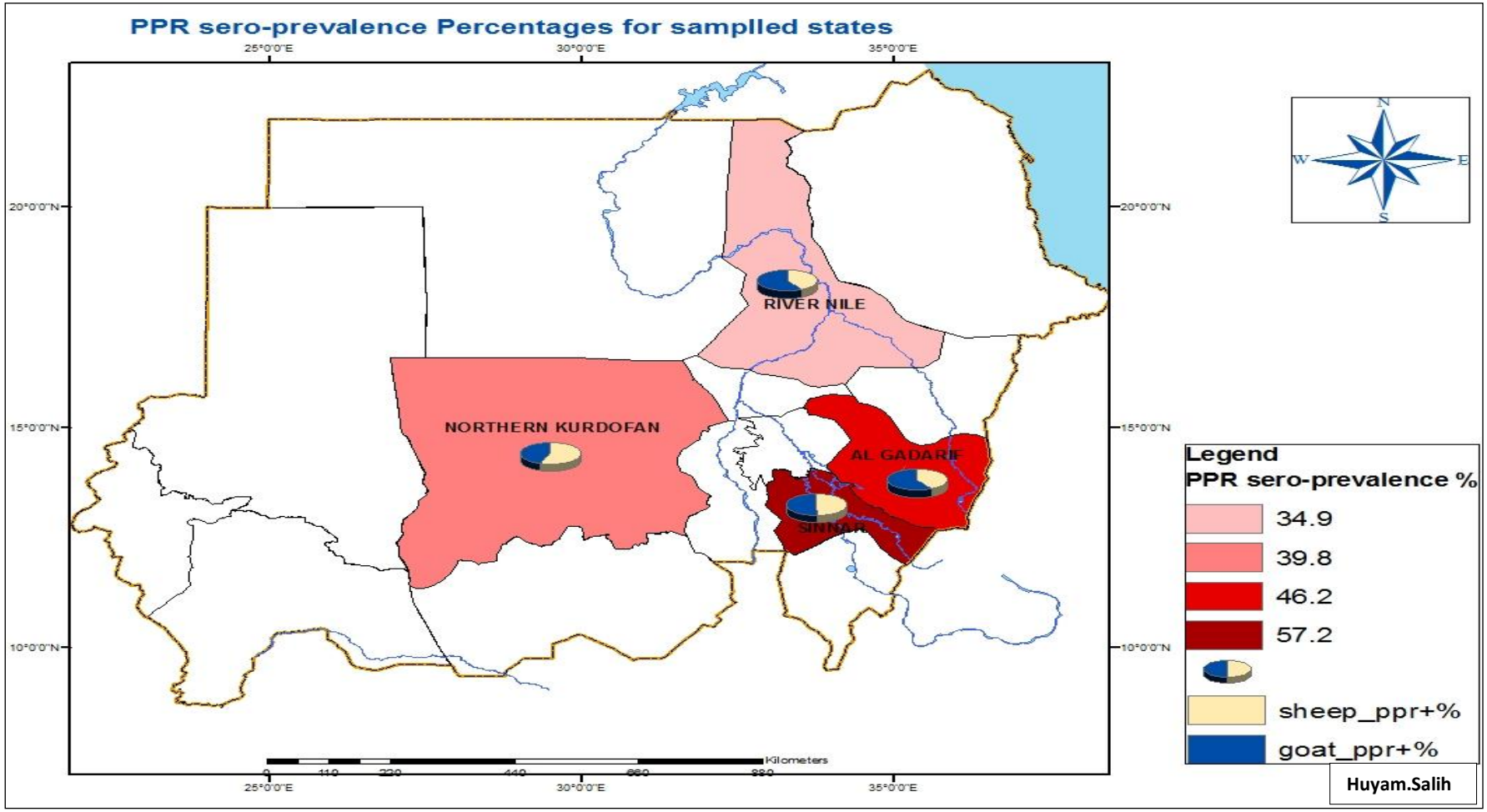
PPR sero-prevalence Percentages for samplled states
25°0'0"E
30°0'0"E
35°0'0"E
20°0'0"N
15°0'0"N
10°0'0"N
RIVER NILE
NORTHERN KURDOFAN
AL GADARIF
SINNAR
N
W
E
S
Legend
PPR sero-prevalence %
34.9
39.8
46.2
57.2
sheep_ppr+%
goat_ppr+%
Kilometers
0
110
220
440
660
880
Huyam.Salih

Figure (20)

3.2 Results of the case- control study:

3.2.1 Descriptive analysis

Frequencies of the seven categorical variables were investigated in the 114 localities according to their status as cases and controls as presented in Table (16).

Table (16): frequencies of variables in cases and control localities:

Risk factor	Risk factor categories	Case(n=47)		Control(n=67)		Total
		No	(%)	No	(%)	
Ecological zone	Low rainfall woodland Savanna	23	(33.8)	45	(66.2)	46
	Desert and semi-desert	24	(52.2)	22	(47.8)	68
Wild life	High rainfall	27)	(38	44	(62)	64
	Low rainfall	20	(46.5)	23	(53.5)	50
Annual Rainfall	High density	18	(36)	32	(64)	43
	Low/ medium density	29	(45.3)	35	(54.7)	71
Bordering foreign countries	At border	31	(35.2)	57	(64.8)	26
	Not at border	16	(61.5)	10	(38.5)	88
Sheep and goat population/ state	Large population size	14	(34.1)	27	(65.9)	73
	Small population size	33	(45.2)	40	(54.8)	41
PPR Vaccination coverage	Weak coverage	36	(43.9)	46	(56.1)	82
	Wide coverage	11	(34.4)	21	(65.6)	32
State Area	Large/ medium area	25	(37.9)	41	(62.1)	48
	Small area	22	(45.8)	26	(54.2)	66

3.2.2 Univariate analysis

The Odds ratios were calculated using Mantel- Haenszel test. Six risk factors were positively associated with PPR occurrence (Odds ratio >1). Three factors were found to have significant effect on PPR outbreaks occurrence (*p*- value ≤ .25); the ecological zone, being at country borders and the large sheep and goats population per states. Only vaccination was negatively associated with PPR occurrence, (Odds ratio<1), as shown in Table (17).

3.2.3 Multivariate analysis The risk factors showing significant association (*p*- value ≤ .25) in the Univariate analysis were entered into logistic regression model for multivariate analysis, only the risk factor of being at country borders with foreign country was found to be significantly associated with PPR outbreaks occurrence (*p*- value ≤ .05), as shown in Table (18).

Table (17): Univariate analysis for risk factors associated with PPR outbreaks occurrence by calculating the Odds ratios in cases and controls using Mantel Haenszel test

Variable	Variable categories	Case(n=47) No (%)	Control(n=67) No (%)	Odds ratio	95% CI Lower	95% CI Upper	*P*-value
Ecological zone	Low rainfall woodland Savanna	23 (33.8)	45 (66.2)	2.134	.992	4.592	.052*
	Desert and semi-desert	24 (52.2)	22 (47.8)				
Annual Rainfall	High rainfall	27 (38.0)	44 (62.0)	1.417	.658	3.052	.373
	Low rainfall	20 (46.5)	23 (53.5)				
Wild life	High density	18 (36.0)	32 (64.0)	1.473	.690	3.146	.317
	Low/ medium density	29 (45.3)	35 (54.7)				
Bordering foreign countries	At border	31 (35.2)	57 (64.8)	2.942	1.192	7.258	.019*
	Not at border	16 (61.5)	10 (38.5)				
Sheep and goat population/ state	Large population size	14 (34.1)	27 (65.9)	1.591	.720	3.517	.251*
	Small population size	33 (45.2)	40 (54.8)				
PPR Vaccination coverage	Weak coverage	36 (43.9)	46 (56.1)	.669	.286	1.560	.355
	Wide coverage	11 (34.4)	21 (65.6)				
State Area	Large/ medium area	25 (37.9)	41 (62.1)	1.388	.652	2.952	.392
	Small area	22 (45.8)	26 (54.2)				

Table (18): Multivariate analysis for the association between PPR outbreaks occurrence and Potential risk factors in the univariate analysis using Logistic regression

Variable	Variable categories	B	Exp(B)	95% CI for Exp(B)		*P*-value
				Lower	Upper	
Ecological zone	Low rainfall woodland Savanna	-.689	.502	.224	1.122	.093
	Desert and semi-desert (Ref)					
Bordering Foreign country	At country border	-1.067	.344	.134	.886	.027*
	Not at border (Ref)					
Sheep and Goats population/ states	Large size population	-.694	500	.213	1.173	.111
	Small size population (Ref)					

3.3 Results of the preliminary qualitative risk assessment

3.3.1 Live Sheep exports value chain analysis in Sudan

Sheep selected for exportation collected from local livestock markets, inspected and vaccinated in vaccination and inspection centers and quarantined in the collective quarantine of Elkadro then transmitted to Swakin terminal quarantine as shown in Figure (21).

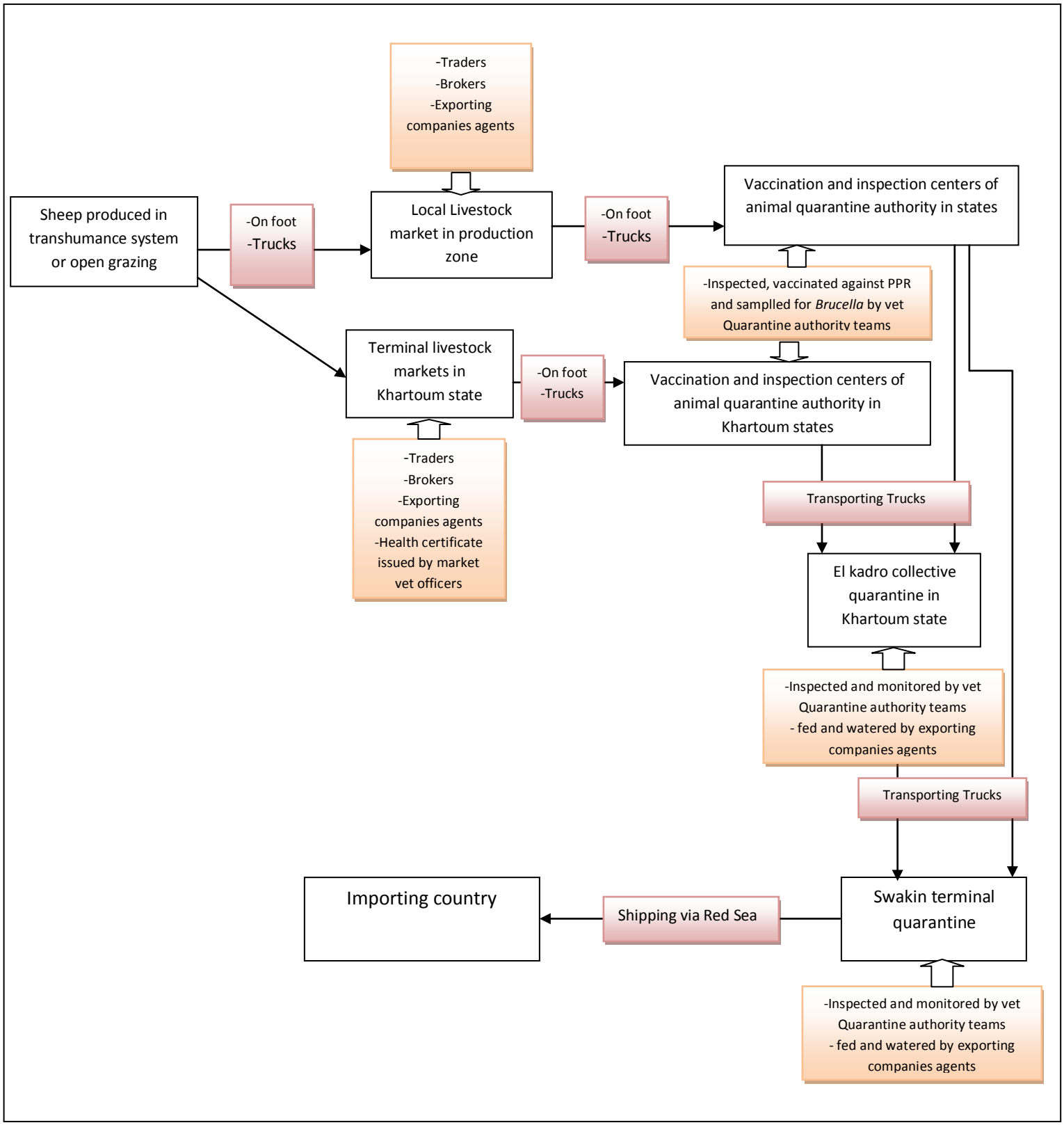

Figure (21): Shows the preliminary mapping for sheep exports value chain in Sudan.

3.3.2 Risk assessment for PPR spreading in Sudanese sheep selected for exportation

3.3.2.1 PPR virus release assessment in sheep selected for exportation in local livestock markets and primary quarantines and vaccination centers

Release risk of PPRV was assessed using the tabular frame for the release risk pathway as shown in Table (19), and the Probability of releasing PPRV in sheep selected for exportation in local livestock markets and primary quarantines and vaccination centers, is found to be:

High × High × Low= Medium

Table (19): Tabular framework of the release assessment for PPRV among sheep exports value chain and the probability of establishing PPR infection prior sending to the terminal quarantine during 2012.

Risk Location		Risk Factors		Partial qualitative risk (Likelihood) estimation	
In the risk pathway	**Geographically**	**Increasing the risk**	**Decreasing the risk**	**Criteria for risk scoring**	**Risk scoring**
Probability of sheep were infected prior sending to local livestock	States of: - Gedarif -North Kordofan state -Kassala state -Khartoum state	1-PPR prevalence: Gedarif state 80.6% for Kassala and Gedarif (Saeed et al, 2010), 46.2% in unvaccinated sheep and goats (Salih et al, 2014). North Kordofan state: 61.2% (Saeed et al, 2010), 74.5% (Shuaib, 2011), 39.8 % in unvaccinated sheep and goats (Salih et al, 2014). Kassal state:80.6% for (Saeed et al, 2010), 66.2% (Shuaib, 2011). Khartoum state: 55.9% (Osman et al, 2009), 59.7% (Saeed et al, 2010).	Vaccination coverage against PPR comparing to sheep and goats population / state in 2011 and 2012 was: Gedarif state 39.9%. North Kordofan state 8.7%. Kassal state 99.9%. Khartoum state 11.4%.	-PPR disease is prevalent in all states from which sheep are selected for export. Despite the efforts devoted for vaccination, the vaccination coverage comparing to the numbers of sheep populations in the states, it is considered weak coverage except in Kassala state.	**High**

market		2-Practised husbandry systems in Gedarif, Kassala and North Kordofan states are trans-human pastoralists and open grazing, which have been proven to have the highest PPR seroprevalence rates in Sudan (Salih et al, 2014)			
		3-Kassal state had reported the highest number of PPR outbreaks in Sudan during the period of 2008 to 2012(AHEDC, 2008-12).			
		4- Gedarif and Kassala states are located in the eastern borders with foreign country which increase PPR outbreaks; OR=2.942/p-value= .019 (Salih et al, 2015).			
		5-Gedarif and Kassala states are located in the low rainfall woodland savanna, which associated significantly with high outbreaks numbers of PPR (Salih et al, 2015).			
Probability of sheep harbour PPRV Within local markets and during transportation to the primary	**Primary and secondary markets:** Elkhiwai (N.Kordofan) Elsemaih (N.Kordofan) Elshwak (Gedarif) Kassala (Kassala) **Terminal**	-Majority of primary and secondary markets are without separate pens for herds,no market records and no veterinary health certificates are issued except in Elkhiwai and Elshwak due to the establishment of the vaccinations centers (ElDirani *et al.*, 2009).	Terminal markets are well established with fences and pens, veterinary inspection, market records and veterinary health certificates are issued (ElDirani *et al.*, 2009).	- Preventive measures are not in place in primary markets – Sheep trade is controlled by a series of brokers and the sheep source, health and vaccination history could not be	**High**
		-There is no method for animal identification for sheep herds selected for exports like ear tag or electronic microchip(Noticed from personal direct			

quarantine	**markets:** Elobeid (N.Kordofan) Elmuaileh (Khartoum)	visits)		identified.	
		Majority of sheep herds selected from primary and secondary markets are transported on foot to the nearest vaccination centre in the production sites, and during transport it may come across local sheep herds which may be infected(UNEP, 2013). That can accelerate PPR transmission between selected herds and local grazing herds; -highest numbers of sheep are exported in the months from October to December, after the rainy season and at the beginning of winter; which are the season for high numbers of PPR outbreaks (Salih et al, 2014) and (Sarker and Islam, 2011).			
Probability of PPR virus spread in primary quarantine/ Vaccination and Inspection Centers		-The method for animal identification for sheep herds that and tested vaccinated in inspection and vaccination centers is ear tag which could be lost or even cheated, and beside no tracing could be done for source of sheep in case of disease occurrence due infection and improper vaccination (Noticed from personal direct visits)	-All sheep are inspected visually, tested for *Brucella* and vaccinated against PPR by the team of the Inspection and vaccination centers before reach the collective quarantine of Elkadro and the Swakin terminal quarantine.	- Sheep are inspected during the quarantine period before sheep transported to terminal quarantine.	**Low**
		Some brokers during 2011 and 2012 had brought large numbers of hamari sheep from Elkhiwai livestock market to be inspected and vaccinated in Gedarif (UNEP,	-Sheep is quarantined for 7 to 10 days in separated pens		

		2013) this mobility may play a role in diseases spread.	and inspected visually during this period then transported to Swakin terminal quarantine		
		-Vaccination against PPR in vaccination and inspection center is practiced using cold chain attenuated vaccine. The maintenance of cold chain for vaccine efficacy has proven difficult in subtropical countries (Sen et al., 2010), taking into consideration the large numbers of sheep to be vaccinated specially during the season of large numbers of exports.			

3.3.2.2 PPR virus exposure assessment in sheep selected for exportation in terminal quarantine and transportation vehicles:

Exposure risk to PPRV was assessed using the tabular frame for the exposure risk pathway as shown in Table (20), and the Probability of exposing to PPRV in sheep selected for exportation during transportation to the terminal quarantine, within it and during transportation from Swakin port to importing country, is found to be:

V Low × V Low × V Low= V Low

Table (20): Tabular framework of exposure assessment for PPRV and the probability of establishing PPR infection during transportation to and within the terminal quarantine and final shipping to the importing country during 2012.

Risk Location		Risk Factors		Partial qualitative risk (Likelihood) estimation	
In the risk pathway	Geographically	Increasing the risk	Decreasing the risk	Criteria for risk scoring	Risk scoring
Probability of selected sheep come in contact with infected herds with PPR or Fomites during transportation to Terminal quarantine	-Road from Elkhiwai and Elarahad in North Kordofan through White Nile state, Khartoum state and River Nile state to Swakin terminal quarantine in the Red Sea state. 2-Road from Elkadro collective quarantine in Khartoum state through River Nile state to Swakin terminal quarantine in the Red Sea state. 3-Road from Elshwak in Gedarif state through Kassala	-Sheep are transported through states with high numbers of PP outbreaks and with high PPR seroprevalence rates(Saeed et al., 2010) and (Salih et al., 2014)	- Sheep selected for exportation are vaccinated against PPR.	-PPR can be transmitted by aerosole but the virus is fragile and the sheep selected for exportation are vaccinated against PPR, quarantined and inspected by quarantines vet officers.	**V. Low**
		-PPR is transmitted by aerosole and high wind speed during winter season may play an important role in PPR transmission between infected herds and sheep selected for exportation(Salih et al, 2014) and (Sarker and Islam, 2011), especially in the case of in proper vaccination.	-Sheep transported in big trucks examined by quarantines vet officers who issuing the Pass permit with date and sheep numbers, according to the suitable number of sheep in every truck with the attached vaccination certificate and Brucella test results.		
		-There are no strict Biosecurity measures are applied to the cleaning and disinfecting of transporting trucks (Noticed from personal direct visits).	-PPRV is a fragile virus which cannot survive for long time outside the host, its half life has been estimated to be 2.2 minutes at 56 C and 3.3 hours at 37 C (Chauhan et al., 2009).		

	state to Swakin terminal quarantine in the Red Sea state. 4-Road from Kassala center to Swakin terminal quarantine in the Red Sea state.				
Probability that sheep with PPR infection couldn`t be detected, diagnosed and rejected from Swakin terminal quarantine	Swakin terminal quarantine in the Red Sea state	-sheep develop PPR in terminal quarantine after getting in contact with infected herds during transportation due to proper vaccination or incomplete quarantine period after vaccination in the collective quarantine or in case of Sheep come directly from the inspection and vaccination centers to Swakin, especially during the season of large number of exports during Pilgrim.	Quarantine procedures are applied in Swakin terminal quarantine as the following: -Animals are kept for 21 days under monitoring in the quarantine without therapy or vaccination as recommended in the OIE code. - The animals when brought to Swakin quarantine; vet officers verify the vaccination certificate, *Brucella* test certificate, certificate of origin and road document. the animals are counted, and first visual examination is made before the animals enter the quarantine. - A day before shipping, a second inspection is made by visual		**V. Low**

			examination for all animals, and accordingly, apparently diseased animals are rejected from exports. -Thereafter the animals are counted, weighed and examined visually before shipping.		
Probability that **Sheep get** **infected** **By PPRV** **From** **infected fomites** **In the transporting** **Ships**	Transporting ships from Swakin to Jeddah	-PPRV is highly contagious and exists in all discharges from sick animals (Chauhan et al., 2009). -Ship containers for animal transportation are in high humidity environment. -No laboratory specimens are taken from the ship fomites to ensure the hygiene and the efficacy of the disinfectant that used.	-The team of quarantine examine the ship to ensure that ventilation and sanitation, are provided as confirmed by a certificate given to them by the Captain of the ship, finally the responsible veterinary inspector from the quarantine signs the certificates which consist of: -*Brucella* free certificate, certificate of origin , FMD free certificate and the veterinarian health certificate. These documents are given to the exporting company to be handed to the importers.		**V. Low**

3.3.2.3 Risk estimation for PPR disease spreading in exported herds

Probability of exporting live sheep infected with PPRV is found to be; release risk ×exposure risk= Medium× V Low= Low, as explained by an influential diagram as shown in figure (22).

3.3.2.4 Magnitude of the consequences:

- During the year 2012 about (3,399,421) head of live sheep were exported to the Kingdom of Saudi Arabia via Swakin port.
- This number of exports was earning about (318,276,437.45) USD.
- There was no rejection of sheep from the veterinary authority in KSA during the year 2012, although a percentage from annual sheep exports used to be rejected due to sanitary reasons in the past. During the year 1999 (74,868) sheep heads (4.6% of the annual sheep exports) were rejected with suspicion of T.B, vesicular stomatitis and mange, also in 2003 (29,114) head of sheep (2.2% of the annual sheep exports) were rejected with suspicion of vesicular stomatitis and *Brucella* (Salih, 2007).
- In case of disease detection of PPR or any disease, the exported sheep will be rejected from the importing country, which mean loss of hard currency and extra costs and burden on the exporting companies, quarantine staff and affect a lot of people who are working in sheep export value chain.

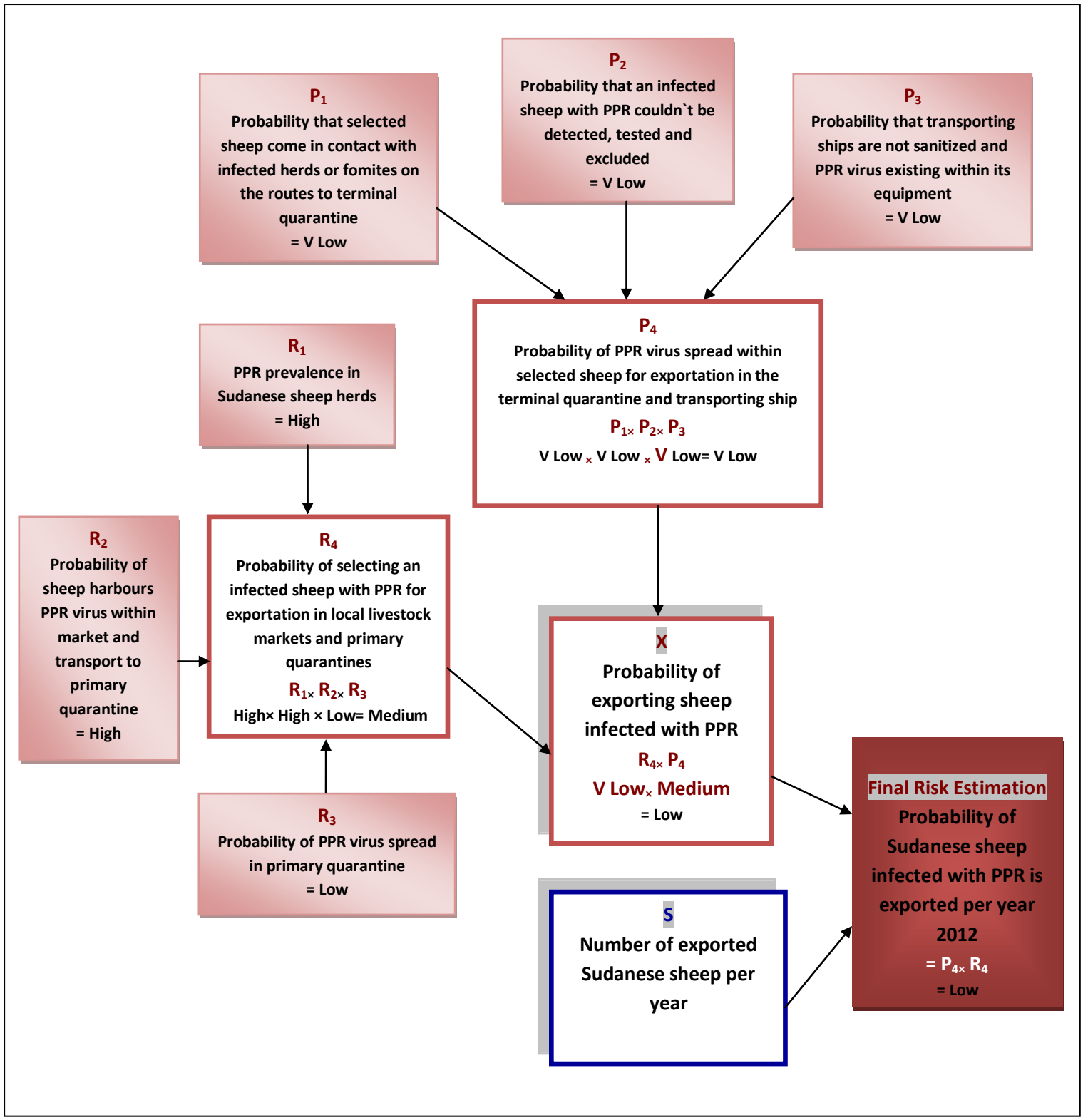

Figure (22): Influential diagram modeling the risk of exporting a Sudanese live sheep infected with PPR virus.

3.3.3 Risk management strategy Suggestion

The management should consider the potential epidemiological impact on the disease risk and the economical impact on the livestock value chain. The most important issue is the likelihood and compliance of the stakeholders with the recommended intervention.

Disease surveillance

PPR surveillance should include an early warning system (which could be used for other diseases), to track the risk factors in particular the climatic factors since the PPR is more spreading after the rainy season and during the dry cold winters. And also surveillance should monitor the efficacy of vaccination.

Surveillance should be risk- based and involve all people, groups and organization in the livestock sector. Therefore it is very important to strengthening surveillance in the parts where risk cannot be reduced by control measures.

Vaccination against PPR

PPR vaccination should be planned, well arranged and implemented by all state before the rainy season according to the livestock movement in nomadic and open grazing systems.

A thermo stable PPR vaccine is recommending to be used in the country to ensure vaccine efficacy in controlling PPR due to the tropical climate of the country which may affect the cold chain vaccine.

Biosecurity measures applied in livestock value chains

- Integration of production from input supply or production till marketing through linked and traceable channels; compartmentalization.
- Health checking in all livestock markets; inspection.
- Strengthening traceability by using reliable identification method for sheep in vaccination campaigns and local markets which will be of great value for inspection in quarantine and export value chain.
- Animal movement control by setting and activating regulation of animals and animal products movement with certification and enforcement of checks on the routes by veterinary check point; to discover diseases earlier and break the transmission cycle.

- The role of other ruminant (wild life and other domestic) in the maintenance of PPRV.

Coordination of control efforts:

All stakeholders in livestock value chain should participate and be aware of the PPR control measures. And an extra and continuous coordination should be established between the head quarter epidemiology unit, vaccine production, quarantine and state epidemiology unit.

CHAPTER FOUR

DISCUSSION

Investigation of risk factor associated with PPR is important for PPR control and eradication. The climatic factors are of most importance, because knowing the seasons of infection, geographical areas with high incidences and the climate conditions in these areas will enable the veterinary authorities to implement the proper control and risk reduction measures that could eventually prevent or mitigate PPR outbreaks and its consequences.

The overall prevalence of PPR was lower in this study (45.6%) compared to its prevalence in previous studies; 54% (Haroun et al., 2002), 50.6% (Osman et al, 2009), 61.8% (Abdalla et al., 2011), 62.8% (Saeed et al., 2010) and 70.2% (Shuaib, 2011). That might be due to the wide coverage of vaccination against PPR which reach 5,200,190 Dose (animal) in 2011 comparing to 2,799,299 Dose (Animal) in 2010 (. Anonymous,AHEDC, 2010, 2011).

The PPR prevalence was found to be significantly higher in Sennar (57.2%) (previously in the central state, now in the southern after Sudan division) followed by Gedarif in eastern Sudan (46.2%) then North kordofan in western Sudan (39.8%), lowest prevalence 34.9% was found in River Nile in Northern Sudan. This agreed with Saeed et al (2010) while it differs from the results reported by Abdalla et al (2012) and Shuaib (2011). Abozabad in Northern Kordofan has the highest prevalence (91.7%) followed by Barbar in River Nile state (70%). It can be concluded that the geographical factor of the state or the province and of localities or counties within the same state; has a significant effect on PPR prevalence, that agree with results addressed by Shuaib (2011) and Muse et al (2012) regarding the geographical factor effects and disagree with that of Ozkul et al (2002).

Apparently goats showed higher seroprevalence (47.9 %) than sheep (43.7%), although statistical significance did not exist, but this observation agrees with the finding of AbdElRahim et al (2010) in Egypt, who reported a higher seroprevalence in goats (88%) than in sheep (53%). Our results also corroborate with the results of Abubakar et al (2009) who found PPR prevalence of 67% in goats and 54% in sheep. However, it disagree with the findings reported in Sudan by

Saeed et al (2010) who found the PPR sero- prevalence to be (67.2%) in sheep and (55.6%) in goats, and also in other countries like in Ethiopia where Gopillo (2005) found a sero-prevalence of (13%) in sheep and (9%) in goats.

Sheep and goats over 12 months old have a significantly higher seroprevalence followed by animals with age from 4 to 12 months while the low prevalence was found in kids from 1 to 3 months. This picture is the same of Abubakar et al (2009) who found the highest PPR seroprevalence in animals aged more than 2 years and also similar apparently to the findings of Shuaib (2011) and that might be referred to the longer time that adult animals have to be vulnerable to PPR infection through and not like younger animals and also younger kids at this age didn't develop a natural immunity after the breakdown of the maternity immunity. But it differs from the results of Sarker and Islam (2011) who found the highest PPR prevalence in the young animals and he referred that to poor immunity and poor nutrition which predisposed them to PPR.

Females were significantly, more affected than males and that agree with Shuaib (2011) and Abdalla et al (2012), the breeding system in Sudan could have a role in this finding because female animals were kept longer time for reproduction than males. But it disagree with Sarker and Islam (2011) who stated according to his results, that males are more affected may be due to genetic factors.

Animals at the pastoralist system (Transhumance) was significantly highly infected (68.1%) followed by open grazing system and the low prevalence was found in the intensive system. This is similar to the findings of Shuaib (2011). Also the interaction between sheep and goats in pastoralist system with wild small ruminants in pasture especially in states with high density of wild life like Sennar and North kordofan could affect the PPR prevalence; as the infectivity and role of PPR transmission through wild ruminants is mentioned by Housawi et al (2004), Zahur et al (2008) and Gopillo (2005).

PPR sero-prevalence was found to differ significantly between housing categories; animals in scrap fences were more affected followed by animals with no houses and the low prevalence in

animals kept in modern houses with metal fences. This is logical with the findings of the husbandry systems since animals in pastoralist and open grazing system are kept in scrap fences houses or with no houses.

Kwahla breed in sheep was the most affected breed significantly followed by Gwasma in sheep. These two breeds belong to transhumans tribes and trans humans has shown the highest sero-prevalence among husbandry systems.

Animals found in States with high rainfall rate and high wind speed were more affected significantly than those in states with low rain fall and slow wind speed in the season of sampling. High rainfall rates lead to cold weather and that is contributing to PPR spread and this agree with Elnoman et al (2011), Elhassan et al (1994) and with Saeed et al (2010).

The association between the positive status for PPRV and the 9 potential risk factors in univariate analysis, was assessed in a multivariate analysis using logistic regression; with confidence interval 95% and a *p*- value ≤ 0.05.

The multivariate analysis showed an association between the PPR seropositivity and the geographical location in State and locality levels, and that is in line with Shuaib (2011). Sheep and goats from three localities were at a high risk for PPR infections; Basonda in Gedarif state (Exp (B) =5.731) Abuhugar (Exp (B)= 12.949) and East Sennar (Exp(B)= 11.217) and in Sennar state.

Regarding the husbandry system, logistic regression found a significant association between the systems and PPR seropositivity. The animals owned by nomadic pastoralists were at high risk for PPR with Exp (B) = 0.073 and ***p*** -value 0.018 comparing to the other systems. This could be due to vulnerability of small ruminant herds in pastoralists and open grazing systems to infected herds in pastures and water points, these herds could be from other Sudan states or from a neighboring country, in particular in state at borders like Sennar and Gedarif which showed the high PPR prevalence in this study, the same observation was mentioned by Kihu et al (2010).

The association between PPR sero-prevalence and females was found by the logistic regression this could be refer to the longer years of keeping females in the herds more than males which are send for slaughtering and exporting in younger ages, so the probability of getting infected is increased for females.

It could be concluded that; the spread of PPR attributed to many reasons some of them are; the free animal movement of pastoralists herds through different states and also to and from neighboring countries, and also the climatic factors especially high rainfall and high wind speed which could accelerate PPR outbreaks spread as result in univariate analysis, since the highest PPR sero-prevalence were found in Sennar followed be Gedarif and the two states have the highest annual rainfall rates among the samples states according to Sudanese meteorology Authority.

The case- control study was based on the data of reported PPR outbreaks by states veterinary authorities to the Head quarter of the veterinary authorities, so the findings of this study is definitely affected by the accuracy of the reporting system and diagnosis method for PPR outbreaks.

In this study, an important potential risk factor for the occurrence rate of PPR outbreaks was found to be the geographical position at the country borders, with odds ratio of 2.942 and *p*-value of .019.

The highest numbers of PPR outbreaks during the study period, were found in the localities of Kassala state, its neighboring state River Nile and Sennar state. Kassala and Sennar states have shared borders with Ethiopia which has the same PPRV lineage III (Kwiatek *et al*., 2011). Hence pastoralists may play an important role in PPR spread (Salih *et al*., 2014). The shared borders with other countries might increase the probability of spreading PPR to and from that country.

Also, PPR outbreaks were found to be associated with the ecological zone of the low rainfall woodland savanna which has rainfall rate higher than in the desert and semi- desert ecological zones, with odds ratio of 2.134 and *p*- value of .052. This finding agrees with the finding of Salih *et al*. (2014) who found the highest seroprevalence of PPR in states with higher rainfall, and also it is in agreement with Grenfell and Dobson (1998) who stated that widely spread epidemics of PPR occur in the beginning and end of the rainy season among the settled farmers. But many

other studies mentioned the positive effect of dry areas and seasons on the PPR outbreaks as stated by Sarker and Islam (2011) and Okoli (2003).

The third potential risk factor which showed significant association in this study is large population of sheep and goats, which having a probability of 1.591times more for developing PPR outbreaks more than the small size populations. Pastoralists in states with large population of sheep and goats in Sudan such as North and South Kordofan, North and South Darfur, Blue Nile, White Nile and Gezira states are practicing either transhumance or open grazing. These two husbandry systems increase the probability of spreading PPR through the common pastures and water sources as found by Shuaib (2011) and Salih *et al*. (2014). Also, the association is in an agreement with Singh (2011) who stated that; the higher population density of animals' results in increased levels of contact between them and this helps to maintain the PPR virus within the environment.

Even though wildlife density was found to have no significant association with PPR outbreaks occurrence in the current study, but the odds ratio; 1.473 was putative. Regarding the husbandry system in Sudan, the contact between pastoralists` herds and wild ruminants is more probable, and some wild small ruminants have been reported to contract severe PPR infections such as (Oryx gazelle, (Gazella dorcas) and (Capra ibex nubiana) as stated by Housawi et al. (2004).

Annual rainfall level was found to be putative factor (OR=1.417) but without a statistical significant *p*-value, although the states with a high rainfall were found to have the highest PPR seropositivity (Salih *et al*., 2014) and also with high numbers of PPR outbreaks.

Only the factor of vaccination coverage against PPR was found to be associated negatively with PPR spread (protective factor, OR=.669), despite that it has no statistical significance (*p*-value=.35). That could be an indicator for the important role of vaccination in PPR control specially when considering that PPR vaccination coverage was very low comparing to the state population all over the country.

The association between the occurrence of PPR outbreaks and the 3 potential risk factors found through univariate analysis was assessed with a multivariate analysis using logistic regression; only the risk factor of bordering foreign country was found to have a significant effect (*p*- value= .027). The majority of at border localities with high rate of PPR outbreaks in Sudan were located in the eastern borders; where the tribes move freely between countries with their small ruminant

herds in search for pastures and trade. Trade of live animals is one of the important risk factors in spreading PPR in Africa as mentioned by Kaukarbayevich (2009) and Singh (2011).
The preliminary qualitative risk assessment provides a framework for PPR risk estimation in sheep exports value chain, which could be extrapolated for PPR risk in sheep value chain in whole country. The qualitative approach adopted in this study has limitations, especially in missed details and risk scoring, but it has the advantage of being simple and may be useful for animal health and quarantine authorities to test their control and inspection measures.

The overall estimated risk for PPR spread in sheep exports value chain was found to be Low. Which means it is possible and may occur in the next years according to Defra risk scoring. Possibility of PPR spreading in export value chain requires a stricter animal health and quarantine measures applied in all steps of value chain to minimize the risk.

The PPR release in sheep value chain was found to be Medium, which means that the risky event is likely to occur more than once in the next three years.
In release assessment, the probability of selected sheep that infected with PPR was found to be high because PPR is considered as an endemic disease in Sudan with estimated prevalence at 54% by Haroun et al. (2002), 50.6% by Osman et al. (2009), 61.8% by Abdalla et al. (2012), 62.8% by Saeed et al. (2010), 70.2% by Shuaib (2011) and 45.6% by Salih etal. (2014).

The second event affect the release of PPRV is its probability in spread within local livestock markets and in the routes to the primary quarantine or vaccination and inspection centers was also found to be high. Majority of primary and secondary livestock markets are lacking for separated pens (ElDirani et al., 2009) and there is no regular application for bio-security measures which considered a risky hotspots that increase the disease transmission. Also some of selected sheep are transported in trucks and some are taken on foot to the nearest vaccination and inspection centers and come into contact with local herds that may be infected with PPR as noticed from direct observations.

PPRV release could be minimized through a risk- based control strategy for PPR. Vaccination is considered the most effective way of controlling PPR (Kumar et al., 2014). The approach to

control the disease can be divided into three inter-dependent stages, based on prioritizing available resources. These stages are; (i) reducing disease intensity through vaccinating targeted populations, (ii) controlling PPR by intensive vaccination and (iii) implementing mass vaccination campaigns that provide high levels of vaccination coverage (Singh, 2011).

Vaccination coverage against PPR in the country during 2011 and 2012 was estimated between 0.46% to 42% in all states except Kassala state which cover 99.9% of sheep and goat population (AHEDC, 2008-12). The coverage considered very weak comparing to the proportion of susceptible population needed to be immune for PPR become stable which estimated by 85.4% (Zahur et al., 2009). Timing is a very important factor in PPR vaccination which is better achieved before the rainy and cold seasons which are characterized with high numbers of outbreaks (Sarker and Islam, 2011) and (Salih et al., 2014).

The third event affect the release risk of PPRV is the probability of PPRV spread into the primary quarantines which is found to be Low. Primary quarantine and vaccination and inspection centers are responsible for sheep inspection during the primary quarantine period for 7 or 10 days, identification, vaccination against PPR and other diseases, and sampling for *Brucell* test. It was discovered that some exporters transport selected sheep from North Kordofan livestock markets to be quarantined, samplled and vaccinated in Gedarif vaccination and inspection center due to its proximity to Swakin from Gedarif (UNEP, 2013). During inspection and sampling many sheep excluded from exportation and may have its way to Gedarif market or farms, this step could be a hotspot for PPR and other diseases spread and should be taken into consideration by animal health authorities of Gedarif.

Control of sheep movement is very important for disease control and couldn`t be achieved without sheep identification which is very crucial for inspection, transportation and disease control in quarantine channels. Animal identification and traceability are tools for addressing animal health (including Zoonoses) and food safety issues and these tools may significantly improve the effectiveness of disease management, control of animal movement, surveillance, early response, vaccination and application of zoning and compartmentalization (OIE, 2014).

Plastic ear tags with serial numbers are used for identification of exported sheep, but more accurate methods could be used like electronic ear tags or microchips to provide more information about the animal origin, vaccination history and movement.

The exposure assessment which represent the probability of PPRV to spread among the sheep herds selected for exportation and it is depending on the contact with an infected sheep or fomites within the transporting trucks to the terminal quarantine or / and in the terminal quarantine or / and in the fomites of the transporting ship to the importing country. In this study the exposure risk was found to be very low (V Low), that means the risk of PPRV spread is rare (the risky event may occur in exceptional circumstances). The justification for this result is built upon the characteristics of the PPRV which is fragile with half-life of 2 hours at 37 and susceptible to the most common disinfectants (Kumar et al., 2014). But since the virus could be found in infected animal discharges which can contaminate materials such as water, feed and bedding to make them another source of infection (Gopilo, 2005), so it is very important for the transporting vehicles to be cleaned and disinfected after every shipment.

The most important factor in reducing the risk of PPRV spread and other disease agents in the terminal quarantine is the application of Biosecurity measures and reliable inspection and disease detection methods. During the study period in 2012 about 37,291sheep were rejected from Swakin quarantine due to emaciation and disease signs including swelling of lymph nodes, mange, Diarroehea, sheep pox and postulates depending on visual inspection.

38.7% of this rejected sheep were excluded from the export herd due to swelling of lymph nodes,11.4% for diarroehea and 1.5% due postulates. The excluded sheep taken out of the quarantine without knowing the exact reason for disease sign. Diseases diagnosis and knowledge about the reason and tracing the origin of infected animals can contribute to the control of many diseases and also may give a clue about the efficacy of vaccination, Biosecurity measures and inspection along sheep export value chain.

The probability of ship fomites get contaminated and may contribute to disease transmission was found to be very low (V LOW), depending on the fragile nature of PPRV and the inspection

made by Swakin quarantine officers in ship after receiving the disinfection certificate form ship captain. Laboratory testing for ship hygiene may be needed to assess ship Biosecurity and assure the health status of exported sheep.

CONCLUSION AND RECOMMENDATIONS

Referring to the findings of the conducted studies in this research of PPR disease in Sudan, it is concluded that:

- PPR disease was found to be endemic spreading all over the country with high rate of occurrence in states near country borders.
- PPR prevalence was mostly associated with high rainfall rated and high wind speed.
- Pastoralists and open grazing among the different husbandry systems were most important for PPR occurrence.
- PPR was found to be more prevalent in female and small ruminant of more than 12 months of age.
- The risk of PPR spread in sheep exports chain was assessed to be Low; which is possible and may occur in the next years.

Small ruminants` production sector in Sudan is very important to national economy by its exports from live and slaughtered animals. Beside the importance of this sector for the rural habitats who depend on sheep and goats in their nutrition and livelihood. Depending on research conclusions the following is recommended:

- ✓ Conducting studies to evaluate the efficacy of the local PPR vaccine when administered in field conditions.
- ✓ Studying the feasibility of producing and using thermo stable vaccine against PPR.
- ✓ Increasing the coverage of PPR vaccination to reach at least 70% of sheep and goats population in every state.
- ✓ Arranging the date of vaccination against PPR before the rainy season.
- ✓ Improving the control and the monitoring of animal movement at country borders and intrastate.

- ✓ Applying stricter Biosecurity measures in livestock markets, quarantines and transporting vehicles.
- ✓ Improving animal traceability in sheep and goats export chains and applying an identification method that gives data about the origin of animal and its vaccination history.
- ✓ Conducting thorough routine laboratory diagnosis for animals excluded from exports due to disease signs in the terminal quarantine.
- ✓ Adopting risk assessment by national veterinary authorities to establish an early warning system for animal diseases and to assess and evaluate animal health and quarantine procedures and regulations.
- ✓ Encouraging scientific researches in the fields of; PPR risk factors and the sanitary status in exports chain.

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Annexes

Annex 1

Questionnaire of seroprevalence and risk factors

Assessing PPR risk factors and prevalence in sheep and goats in some of Sudan states

Questionnaire No…… **Date: ……**

State……..

Locality…… **Area……**

Longitude……. Latitude……

Herd data:

1-Owner Name……

2- Herd size…….

3-Type of Husbandry:

Open grazing ( ) **Close/ intensive ( )** **Pastoralists ( )**

4-Housing type:

Modern metal ( ) **building of bricks ( )** **Mud building ( )**

Wood and scrap ( ) **shrubs fence ( )**

5-Pen area ……..

6-Number of animals per pen……

7-Species: Ovine ( ) **Caprine ( )** **Mixed ( )**

8-Breeds…….....

9-New animals into herd: Yes ( ) No ( )

10-If yes, Date of entry into herd……

11- Source and species of the new animals…….....

12-Date of Last outbreak of PPR ……

13-Last Vaccination against PPR ……

Sample NO	**Species** [Ovine or Caprine]	**Breed**	**Sex** [Male or Female]	**Age** [> year, 4-12 month, 1-3 months**]**

Annex 2

Cases and control localities of the case- control study

State	Cases localities	Control localities
1 Gezira	1 Hasahisa	1 Wad madani
	2 south Gezira	2 Almanagil
	3 UmElgora	3 Sharg Gezira
	4 Kamleen	
State	**Cases localities**	**Control localities**
2 River Nile	5 Shendi	
	6 Aldamar	
	7 Atbara	
	8 Abohamad	
	9 Elmatma	
	10 barbar	
3 Sinnar	11 Abu hugar	4 Elsuki
	12 East Sinnar	
	13 Eldali	
	14 Singa	
	15 sinnar	
4 Blue Nile	16 Eltadamon	5 Eldmazeen

	17 Baw	6 Elrosairis
	18 Elsalam	7 Elkurmuk
5 Gedarif	19 wasat gedarif	8 Algedarif
		9 Elfashaga
		10 Elrahad
		11 western glabat
		12 eastern glabat
		13 Basonda
		14 Elgorisha
		15 Alfaw
		16 Almafaza
		17 Gala elnahal
		18 Butana
6 Kassala	20 Kassala	19 Newhalfa
	21 Sitate	
	22 atbara river	
	23 Elgash	
7 N.Darfur	24 Kabkabia	20 Alfashir
		21 Kutum
		22 maleet

		23 UmKadada
		24 Alkoma
		25 Sarafomera
		26 Kalemendo
		27 Malha
		28 Aliate
		29 Darlsalam
8 Khartoum	25 Bahri	30 Omdurman
	26 Ombeda	31 Khartoum
	27 Shergelneel	32 Karari
	28 Jabal Olia	
9 N.Kordofan	29 Elnihood	33 Sodari
	30 Elkhiwai	34 Jabrtelsheikh
	31 Sheikan	35 Elrahad
	32 Gheibaish	36 Abuzabad
	33 UmRwaba	
	34 Bara	
	35 Wad Banda	
	36 West Bara	
10 Northern	37 Borgaig	37 Wadi halfa

	38 Marawy	38 Dalgo
	39 Algolid	39 Aldaba
	40 Dongola	
11 RedSea	41 Haliab	40 Portsudan
		41 Algonoub&Aloleeb
		42 Swakin
		43 Sinkat
		44 Haya
		45 Dordaib
12 S.Darfur	42 Tulus	46 Nyala
		47 Rehaidelberdi
		48 Adeela
		49 Aldeain
		50 Buram
		51 Baharelarab
		52 kass
		53 Shearia
		54 Eddelfursan
		55 Alradoam
13 S.Korrdofan	43 Abugebaiha	56 kadogli

	44 Rashad	57 Aldalanj
	45 Talodi	58 Abassiya
	46 Elsalam	59 Babanosa
		60 Lagawa
		61 Kailik
		62 Sunnut
14 White Nile	47 Rabak	63 Algeetaina
		64 Alaewaim
		65 Kosti
		66 Algablain
		67 Tandalti