بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Serological Detection of Peste des Petits Ruminants Virus (PPRV) Antibodies among Sheep and Goats in River Nile State, Sudan

الكشف المصلي عن الاجسام المضادة لفيروس طاعون المجترات الصغيرة بين الضأن والماعز في ولاية نهر النيل ، السودان

A thesis submitted to the College of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Preventive Veterinary Medicine (M.P.V.M.)

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January, 2020

Declaration of the Status of Thesis

By Student

The work described in this master degree thesis was carried out at the Virology Laboratory, Department of Pathology, Parasitology and Microbiology at the College of Veterinary Medicine, Sudan University of Science & Technology from April 2018 to January 2020 under the supervision of Dr. Nussieba Ahmed Osman Elhag.

The experimental work is original and the thesis has not been submitted partially or fully to any other University.

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Declaration of the Status of Thesis

By Supervisor

The work described in this master degree thesis was carried out at the Virology Laboratory, Department of Pathology, Parasitology and Microbiology at the College of Veterinary Medicine, Sudan University of Science & Technology from April 2018 to January 2020 under my supervision.

The experimental work is original and the thesis has not been submitted partially or fully to any other University.

Dr. Nussieba Ahmed Osman Elhag

Supervisor

January, 2020

Dedication

To my dear mother

To my dear father

To my Sisters and Brothers

To my Friends

Acknowledgements

Firstly, my thanks are due to Almighty ALLAH who enabled me to accomplish this research successfully. My deep gratitudes are then extended to my supervisor Dr. Nussieba Ahmed Osman Elhag for her appreciable kind of help, supervision, guidance, technical and close follow up.

My gratitude and words of appreciation extend to those who helped me during the pursuit of the study. I deem it a proud privilege and feel immense pleasure to acknowledge all those who are directly or indirectly involved. I'm thankful to the staff of the Ministry of the Livestock and Rangelands, River Nile State. My gratitude is also to all the staff of the General Directorate of Animal Health and Epizootics Disease Control, River Nile State with special thanks to the staff of the Department of Epidemics Control.

My sincere thankfulness also extends to all the staff of Atbara Veterinary Research Laboratory, Atbara, River Nile State. I'm also thankful to the General Directorate of the Animal Health and Epizootics Disease Control, Soba, Khartoum for their valuable role in sample preservation. Moreover, my special appreciation is due to their excellent of the animal herd owners in the River Nile State who helped me during the pursuit of study.

Finally, my words cannot help me describe and express my special sensation and deep gratitude to my family and friends and to all who helped me in achievement for the humble research.

This research was funded by a research project for Nussieba Ahmed Osman Elhag from the Republic of the Sudan, Sudanese Ministry of Higher Education and Scientific Research, Commission of Scientific Research and Innovation (Grant No. 2017/972).

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Abstract

Between January 2018 to December 2019, many suspected outbreaks of PPR were reported among flocks of sheep and goats in the seven localities of the River Nile State in the northern part of the Sudan. The present study was designed to investigate the presence of peste des petits ruminants virus (PPRV) antibodies among sheep and goats in the River Nile State, Northern Sudan. Serum samples were collected from 19 suspected PPR outbreaks occurred in seven localities of the River Nile State. A total of 483 serum samples were collected from sheep (337 sera) and goats (146 sera). Animals had no previous history of vaccination against PPR.

Sheep and goats sera were screened using haemagglutination-inhibition (HI) test for detection of PPRV antibodies. Sera with HI titers less than 8 were considered as negative whereas sera with HI titers more than or equal to 8 were considered as positive and protective. Of the total 483 serum samples (337 sheep and 146 goats) tested, 470 sera were positive, with an overall antibodies sero-prevalence of 97.3%, while only 13 (2.7%) samples were negative. Results of the haemagglutination-inhibition test revealed titers ranged from 8 to 256 with an overall mean HI titer of 52.6. On the species basis, within sheep 324/337 (96.1%) sera were found positive whereas only 13/337 (3.9%) sera were negative. Within goat all tested sera (146/146, 100%) were found positive. As a conclusion goat sera yielded a higher overall sero-prevalence (100%) compared to sheep sera (96.1%). Results of the haemagglutination-inhibition test revealed titers ranged from 8 to 256 with mean titer of 48.9 among sheep and 61.3 among goats. Sero-prevalence of PPRV antibodies within localities of the River Nile State demonstrated the highest overall sero-prevalence of PPRV antibodies (100%) in Shendi and Berber localities with relatively lower sero-prevalence demonstrated in Ad-Damar (98.8%), Abu Hamad and Elbohira (97.4%) and Atbara (94.8%) and the least sero-prevalence was demonstrated in Elmatama (90.0%). The higher titers of PPRV antibodies detected in sheep and goats sera in all localities of the River Nile State, where outbreaks occurred, suggested the wide exposure of these animals to PPRV although it was, until recently, considered as a disease free zone.

ملخص البحث

خلال الفترة من 2018م الي 2019م ظهرت العديد من الحالات الوبائية التي يشتبه فيها الاصابة بمرض طاعون المجترات الصغيرة في قطعان الضأن و الماعز في كل من محليات ولاية نهر النيل في شمال السودان. صممت الدراسة الحالية بهدف التقصي عن وجود الاجسام المضادة لفيروس مرض طاعون المجترات الصغيرة بين قطعان الضأن والماعز في ولاية نهر النيل ، شمال السودان. تم جمع عينات سيرم من حوالي 19 حالة وبائية يشتبه الاصبة فيها بالمرض في سبعة محليات بولاية نهر النيل. تم جمع عدد 483 عينة مصل ، من الضأن (337 مصل) ومن الماعز (146 مصل). جميع الحيوانات التي تم اخذ العينات منها لم يسبق لها التطعيم بلقاح طاعون المجترات الصغيرة. تم استخدام إختبار تثبيط تلازن كريات الدم الحمراء للكشف عن وجود الاجسام المضاده لفيروس طاعون المجترات الصغيرة في مصل الضأن والماعز. عينات السيرم والتي لها حساب معيار الاجسام المضادة اقل من 8 تعتبر سالبة اما التي لها حساب معيار الاجسام المضادة اكثر من او مساوياً ل8 فتعتبر موجبة وتشكل حماية. من عدد 483 عينة مصل تم اختبارها ، اوضحت النتائج أن 470 عينة وجدت موجبة ، بنسبة عامة 97.3٪ لوجود الاجسام المضادة للفيروس ، في حين أن 13 عينة فقط كانت سالبة بنسبة (2.7٪). كذلك اوضحت نتيجة إختبار تثبيط تلازن كريات الدم الحمراء أن حساب معيار الاجسام المضادة للفيروس تراوح بين 8 و 256 وحدة لتثبيط لتلازن الدم بمتوسط 52.6 وحدة. عند الأخذ في الإعتبار نوع الحيوان ، بالنسبة للضأن فأن عدد 324 عينة مصل من مجموع 337 عينة مصل وجدت موجبة بنسبة 96.1٪ ، بينما وجدت 13 عينة سالبة بنسبة 3.9٪ وبالنسبة للماعز فإن كل عينات المصل 146 عينة مصل والتي تم اختبارها وجدت جميعها موجبة بنسبة 100%. كخلاصة فأن النسبة المصلية للتقصي كانت عالية في الماعز (100%) مقارنة مع الضأن (96.1٪). نتائج إختبار تثبيط تلازن كريات الدم الحمراء اوضحت أن حساب معيار الاجسام المضادة للفيروس تراوح بين 8 و 256 وبمتوسط 48.9 بين الضأن و61.3 بين الماعز. اوضح الفحص المصلي عن وجود الاجسام المضادة لفيروس طاعون المجترات الصغيرة في محليات ولاية نهر النيل وجود نسبة عالية 100% في محليتي شندي وبربر مع وجود نسبة اقل نسبياً في الدامر (98.8%) ، ابو حمد والبحيرة (97.4%) ، عطبرة (94.8%) ، بينما كانت اقل نسبة في محلية المتمة (90.0%).

النسبة المئوية العالية للاجسام المضادة للفيروس والتي تم رصدها في كل محليات ولاية نهر النيل حيث تم ظهور الحالات الوبائية للمرض ترجح أن هذه الحيوانات قد تعرضت بصورة واسعة لفيروس طاعون المجترات بالرغم من أن الولاية كانت تعتبر –حتي وقت قريب- منطقة خالية من الأمراض.

Introduction

Peste des petits ruminants (PPR) is an infectious, acute and contagious viral and a notifiable transboundary disease that threatens small ruminant. Sheep and goats are the primary hosts for PPRV (OIE, 2019).

The causative agent of PPR is peste des petits ruminants virus, which belongs to the *Small Ruminant Morbillivirus* species in the *Morbillivirus* genus of the *Paramyxoviridae* family (OIE, 2019; Maes *et al.,* 2019). Transmission occurs mainly via direct contact of susceptible animals with infected animals and through aerosols formed by the coughing and sneezing of sick animals (Abegunde and Adu, 1977; OIE, 2019).

The disease is characterized by fever, ocular and nasal discharges, erosive and necrotic stomatitis, conjunctivitis, gastroenteritis, diarrhoea and pneumonia (Ozkul *et al.,* 2002). PPR has an economic importance due to losses caused by its highly mortality and morbidity rates (Banyard *et al.,* 2014).

The first recognition of PPR was made in 1942 in the Ivory Coast in West Africa (Gargadennec and Lalanne, 1942). Further reports in the neighboring countries increased the geographic spread of the disease from West to East Africa, and then to the Middle East and Asia. PPRV is considered to be endemic across Africa, the Middle East and Asia (Dhar *et al.,* 2002; Banyard *et al.,* 2010; Kwiatek *et al.,* 2011; OIE, 2019). Alongside this, within Europe the disease had been described in Turkey and Bulgaria (OIE-WAHIS, 2018).

PRV can be divided into four genetically distinct lineages based on the nucleo-capsid (N) gene (Shaila *et al.,* 1996). These lineages correlate well with the geographic distribution of the virus, with lineages I and II mainly restricted to western and central Africa, lineage III to Eastern Africa and the Arabian Peninsula, and lineage IV to Southeast Asia, the Middle East, and more recently has been recognized in northern Africa (Barnyard *et al.,* 2010).

In the Sudan, outbreaks of the disease were first reported in sheep and goats in south Gedarif (Eastern Sudan) in 1971 (Elhag Ali, 1973). Initially it was wrongly diagnosed as rinderpest but later confirmed as PPR (Elhag Ali and Taylor, 1984). Outbreaks of the disease were reported since then. Recently PPR still occurs and had been reported during the year 2018 in 11 States of the Sudan namely Northern, River Nile, Khartoum, Kassala, Gedarif, Sinnar, White Nile, Southern Kordofan, Northern Darfur, Eastern Darfur and

Southern Darfur (OIE-WAHIS, 2019). In the River Nile State, 52 outbreaks had been reported during 2007 to 2018 (OIE-WAHIS, 2019).

PPR can be diagnosed by clinical observations, characteristic symptoms, epidemiology, and post-mortem lesions. Final diagnosis can be made by laboratory confirmatory tests of PPR and differential diagnosis with other diseases which have the same clinical signs (OIE, 2019).

Control of the disease is by using PPR homologous attenuated tissue-culture vaccine that elicit a protective immunity that has been shown to be effective for at least three years post-vaccination (Diallo *et al.,* 2007; Sen *et al.,* 2010). Current vaccination schedules require the immunization of susceptible animals at least every three years (Diallo *et al.,* 2007; Saravanan *et al.,* 2010).

Objectives:

The present study was designed to accomplish the following objectives:

1- To determine sero-prevalence of peste des petits ruminants virus (PPRV) antibodies among sheep and goats in the River Nile State in order to assist planning the strategy to control and prevent spread of the disease in this zone.

2- To investigate the distribution and the current situation of the disease in the seven localities of the River Nile State.

Chapter I
Literature Review

1.1. Definition:

Peste des petits ruminants (PPR), is known also as goat plague, kata, pseudo-rinderpest, stomatitis-pneumoenteritis complex (Obi *et al.,* 1983). PPR is a notifiable disease to the World Organization for Animal Health (OIE) (OIE, 2019). PPR is a transboundary disease that threatens small ruminant production in many developing countries (Banyard *et al.,* 2010). PPR is an acute, contagious and an endemic viral disease of goats and sheep in Africa and Asia (Taylor, 1984) that is clinically and patho-morphologically closely resembles rinderpest (RP).

1.2. Economic Importance of PPR:

The economic importance of PPR is direct loss and indirect losses principally due to its high contagious nature, with a case fatality rate reaching 100%. The economic losses include the cost for the treatment of sick animals, loss of animal body condition, reduction in market value, increased labour and veterinary services (Banyard *et al.,* 2010). This is of particular concern for smallholder rural farms where small ruminants are reared as the sole source of income (Emikpe and Akpavie, 2011). PPR is currently considered as one of the main transboundary animal diseases (TAD) that hampers livestock production in many developing countries particularly in West Africa and South Asia. In all countries, 62.5% of the global domestic small ruminant population is at risk of PPR (OIE, 2013). The estimated loss due to the disease is 1.45 to 2.1 billion USD every year (OIE, 2015).

1.3. History of the disease:

The first report of PPR was made in 1942 in Cote D'Ivoire "The Ivory Coast" (Gargadennec and Lalanne, 1942). After the first recognition of PPR in the Ivory Coast, increased awareness led to further reports in neighboring countries in the order, Senegal, Chad, Togo, Benin, Ghana, Nigeria, Oman, Sudan, Saudi Arabia, India, Jordan, Israel, Ethiopia, Kenya, Uganda and Pakistan (Sen *et al.,* 2010). This appears to be the geographic spread of the disease from West Africa to East Africa, and then to the Middle East, Asia and Turkey. PPRV is currently considered to be endemic across Africa, the Middle East and Asia (Banyard *et al.,* 2010; Dhar *et al.,* 2002; Kwiatek *et al.,* 2011).

In recent years PPRV has extended its boundaries southwards in Africa as far as southern Tanzania (2008 and 2010) (Banyard *et al.,* 2010) and the Democratic Republic of Congo and Angola (2012). PPR outbreaks have also been reported across North Africa including within Tunisia (2006), Morocco (2008) and Algeria (2011) (Parida *et al.,* 2015). In southwest Asia, the virus spread to Tibet (2007) and has recently been reported all over China (2013-2014) (FAO, 2013). Recently occur in south Asia in Bhutan (2010-2015-2018) (OIE-WAHIS, 2019). Alongside this, within Europe, Turkey PPR outbreaks in sheep and goats were reported during 2011-2012 (Parida *et al.,* 2015) and Bulgaria in 2018 (OIE-WAHIS, 2018).

1.4. Etiology:

Peste des petits ruminants is caused by a virus called peste des petits ruminants virus (PPRV) that belongs to the *Small Ruminant Morbillivirus* species in the *Morbillivirus* genus of the *Paramyxoviridae* family (OIE, 2019; Maes *et al.,* 2019). The Morbillivirus genus also includes other six disease-causing viruses namely: rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV), dolphin morbillivirus (DMV), porpoise distemper virus (PDV) and phocine morbillivirus (PMV). PPRV is antigenically related to rinderpest virus (RPV) from which it can be differentiated by the serum neutralization test (SN) (Taylor, 1979; Gibbs *et al.,* 1979).

1.4.1. Virus classification:

PPRV belongs to the order *Mononegavirales,* family *Paramyxoviridae*, sub family *Paramyxovirinae,* which is consisted of these genera: Aquaparamyxovirus, Avulavirus, Ferlavirus, Henipavirus, Respirovirus, Rubulavirus and Morbillivirus (Maes *et al.,* 2019). Other important viral pathogens of the morbillivirus genus include Rinderpest virus (RPV), Measles virus (MV), Canine distemper virus (CDV), Phocine distemper virus (PDV) and the morbilliviruses of marine mammals or the cetacean morbilliviruses (Maes *et al.,* 2019). The characterization of some novel morbilliviruses have recently been described, including Feline morbillivirus in cats (FMV) (Woo *et al.,* 2012) and numerous morbilli-like viruses in rodents or bats (Drexler *et al.,* 2012). This viral order contains some of the most significant viral pathogens in the medical and veterinary fields.

In general, morbilliviruses are considered restricted in their ability to infect different animal species. Measles virus infections appear to occur exclusively in humans and non-

human primates, rinderpest was restricted to members of the Order Artiodactyla and to date cetacean morbilliviruses have only been reported in aquatic mammals (Buczkowski *et al.,* 2014). In contrast, peste des petits ruminants virus, whilst initially thought to be restricted to the infection of small ruminants, has recently been determined to be the cause of mass mortalities in camelids (Roger *et al.,* 2001; El-Hakim, 2006) and has been described, on a single occasion, in buffaloes (Govindarajan *et al.,* 1997). The seemingly most promiscuous morbillivirus is Canine distemper virus. Initially thought restricted to infection of canids, the virus has been described in numerous species (Buczkowski *et al.,* 2014).

Following genetic analysis, the majority of morbillivirus species were divided into monophyletic lineages or clades (Maes *et al.,* 2019). Morbilliviruses are characterized at the molecular level most extensively through studies with the prototype virus, Measles Virus and to some extent Canine distemper virus and Rinderpest virus. PPRV remains largely uncharacterized with respect to virus replication and transcription. However, the viruses are known to be conserved across the genus with different species sharing similar characteristics (Taubenberger *et al.,* 2000; Stephens *et al.,* 2014).

1.4.2. Viral genome and proteins:

The genome of PPRV is categorized as a non-segmented, linear molecule, single stranded negative-sense RNA of 15,948 nt in length (Mahapatra *et al.,* 2006). The PPRV genome encodes six genes, each is responsible for transcription of a single structural protein in the following order: the nucleoprotein (N), the matrix protein (M), the phosphoprotein (P) in addition to two non-structural proteins (C and V), the haemagglutinin (H), the fusion protein (F), and the large protein or polymerase (L) (Bailey *et al.,* 2005; Chard *et al.,* 2008) (Figure 1).

The F-protein is the first viral glycoprotein, and one of the highly preserved proteins among morbilliviruses. In all paramyxoviruses, the F-protein is embedded in the viral lipid bilayer envelope and projects as spikes on the viral surface (Diallo, 2003).

The H-protein of PPRV is the second viral glycoprotein and is the most diverse among all the members of Morbilliviruses. It serves attaching the virus to the host cell (Diallo, 2003). H-proteins of RP and PPR virus may have significant potential for differentiation of infected from vaccinated animals (DIVA) strategies. Since the H-protein determines the cell tropism, most of the protective host immune response is raised against the H-

protein (Renukaradhya *et al.,* 2002) for this reason, and the preponderance of the neutralizing antibodies against the H-protein, it has remained under continuous immunological pressure. The H-protein is not only involved in cell-tropism but studies indicated that it may have a role as a neuraminidase and by possessing this function PPRV is considered unique among Morbilliviruses (Seth and Shaila, 2001; Dhar *et al.,* 2006).

The Matrix (M) protein is considered one of the smallest proteins between the whole structural proteins of morbilliviruses. It mediates the viral budding process preferentially at specialized regions of the host membrane (Peeples, 1991; Diallo, 2003). The M-protein is linked to the nucleo-capsid and the two surface proteins (F and H) (Mahapatra *et al.,* 2006). The three viral proteins (M, F and H) are associated with the host-derived envelope (Barrett, 1987; Takimoto and Portner, 2004).

The L protein is considered as the largest protein in PPR virion and acts as RNA-dependent RNA-polymerase (Lamb and Parks, 2007).

The N-protein, in all members of the genus Morbillivirus including PPRV, is the most abundant viral protein due to its presence at the extreme 3′-end of the viral genome. The N-protein is considered as the most immunogenic, but the immunity produced against the N protein does not protect the animals from the disease. By virtue of the nature of the H- and N-proteins, these remain the most acceptable targets for the design of PPRV diagnostic tools (Munir, 2011).

P protein is also a component of the RNP and acts as a co-factor for the RNA-dependent RNA polymerase (RdRp). P protein is heavily phosphorylated at serine and threonine residues and interacts with both L and N proteins (Barrett *et al.,* 2006). The association of P-protein to the N- and L-proteins is linked to the viral cycle control, transcription and translation regulation.

The protective immune response is usually elicited against the surface F- and H-proteins of PPRV. However, among the viral proteins most of the neutralizing antibodies are directed against the H-protein during PPRV infection (Diallo *et al.,* 2007).

C and V proteins are the viral non-structural proteins, C-protein is the smallest viral protein that originates in the infected cells and V-protein of PPRV is greatly variable among morbilliviruses (Blom *et al.,* 1999). The V-protein, in contrast to the C-protein, undergoes phosphorylation (Blom *et al.,* 1999).

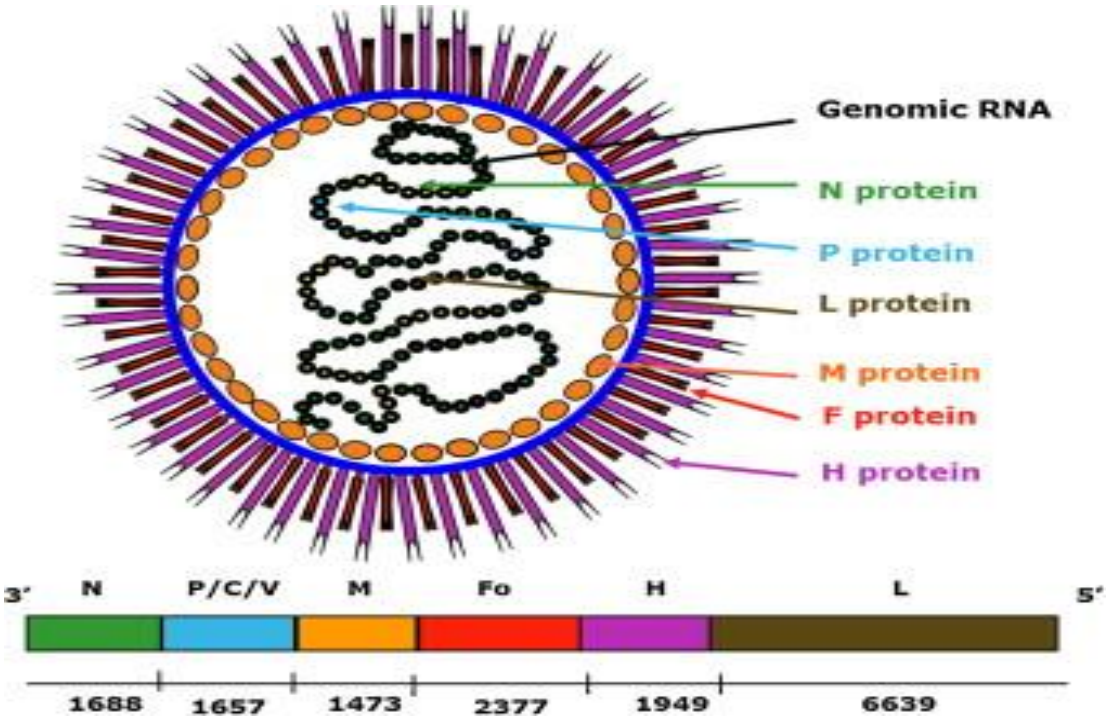

Figure 1. A schematic diagram of peste des petits ruminants virion structure. The RNA viral genome and the six structural proteins are shown (Banyard *et al.*, 2010).

1.4.3. Virus replication cycle:

The replication cycle for different paramyxoviruses is similar. The first step of viral replication is the attachment of the virus on the cell surface then membrane fusion to release the genome into the cell cytoplasm (Moss and Griffin, 2006). The H-protein is responsible for the attachment of the virus to the cell surface through recognition of and binding to the host cell receptor molecules, e.g., salicylic acid, immune cell marker signaling lymphocyte activation molecule (SLAM)/CD150 (Seki *et al.*, 2003; Adombi *et al.*, 2011) or the epithelial cell receptor Nectin-4 (Birch *et al.*, 2013). Attachment of the H-protein to cell receptors activates the fusion activity of the F-protein, enabling a fusion of the viral envelope with the host cell membrane and release of the viral genetic material into the cell cytoplasm. Morbilliviruses replicate solely in the cytoplasm of the host cells (Duprex *et al.*, 1999) (Figure 2).

The genome of PPRV is never found as naked RNA and is fully encapsulated by the N-protein to form the helical ribonucleo-proteins. This ribonucleo-protein complex protects the RNA from host RNAase (Lamb and Parks, 2007). The ribonucleo-proteins may consist of genome sense (–ve) or anti-genome sense (+ve) strands. The ribonucleo-protein

complex, containing the RNA encapsidated by the N-protein, in conjunction with the P- and L-proteins makes up the minimal replicative unit for these viruses (Longhi *et al.*, 2003; Kingston *et al.*, 2004). In an infected cell, the viral genome is released into the cytoplasm and acted upon by viral polymerase complex, which binds to the genome promoter and starts transcribing short leader RNAs. The polymerase works its way across the genome transcribing each gene in turn falling off at each intergenic region. The dissociation of the polymerase at each intergenic region across the genome leads to the build-up of a transcriptional gradient as the polymerase can only commence transcription at the genome promoter (Muniraju *et al.*, 2014). The mRNAs produced are 5′ methylated and 3′ poly-adenylated by the viral polymerase and are translated by host cell machinery. At a certain time point post-infection, the polymerase complex switches its action from the production of mRNA to the production of a full-length positive-sense RNA (Banyard *et al.*, 2005). This switch is thought to be linked to the accumulation of the viral proteins within the host cell, although the precise mechanism for an alteration in polymerase activity remains unclear. Following the production of a full-length anti-genome (+ve) RNA, the polymerase now binds to the anti-genome RNA at the anti-genome promoter (3′ end) and generates nascent full-length negative-sense genomes. The synthesis of viral components within the cell eventually leads to viral egress from the host cell. The M-protein plays an important role in bringing the nascent RNPs and viral glycoproteins to the host cell membrane, which results in the packaging, budding and release of nascent virions (Harrison *et al.*, 2010) (Figure 2).

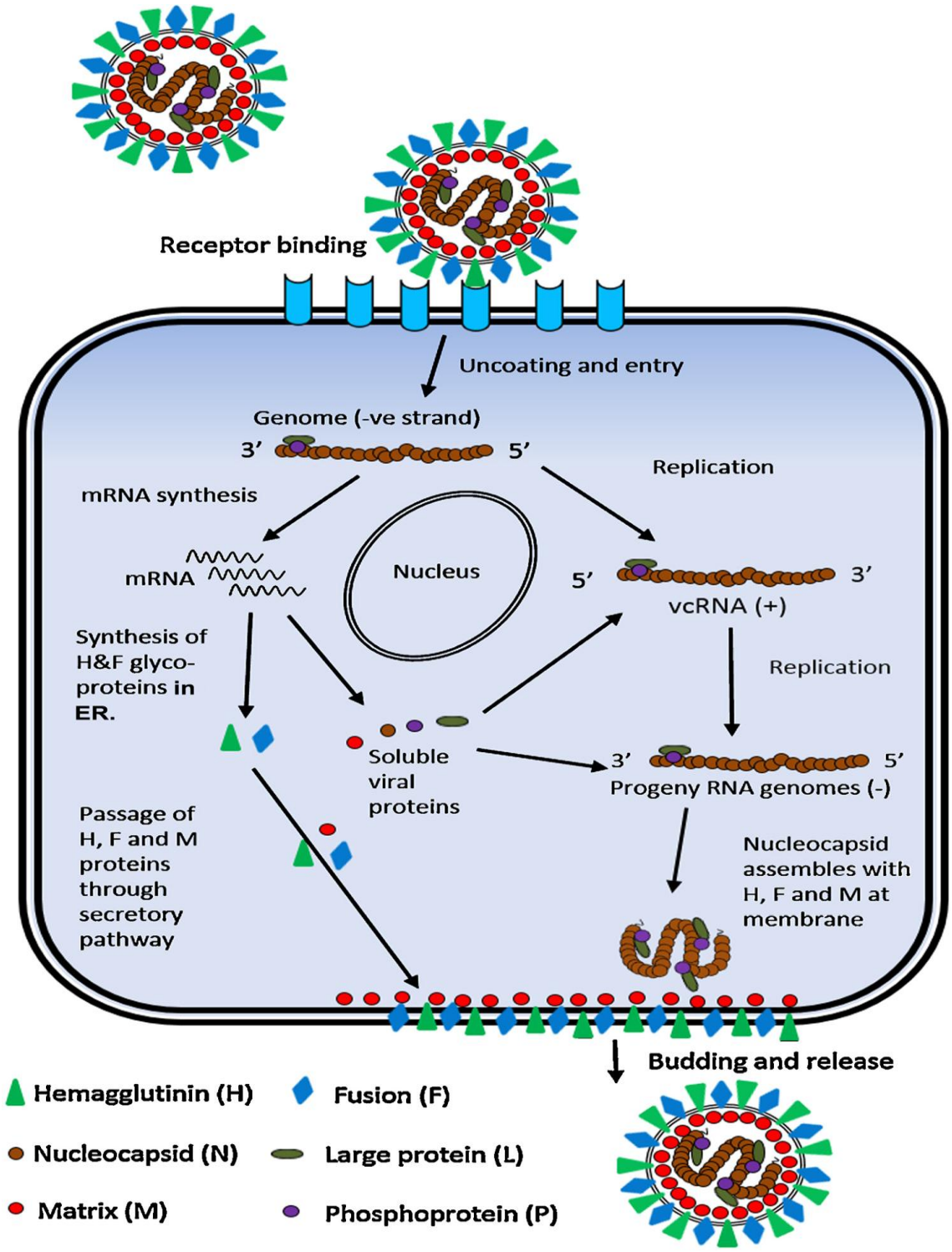

Figure 2. A schematic diagram of morbillivirus replication (adapted from Moss and Griffin, 2006; Parida *et al.*, 2015).

1.5. Epidemiology of PPR:

The first recognition of PPR was made in 1942 in the Ivory Coast (Gargadennec and Lalanne, 1942). Increased further reports in neighboring countries in the order, Senegal, Chad, Togo, Benin, Ghana, Nigeria, Oman, Sudan, Saudi Arabia, India, Jordan, Israel, Ethiopia, Kenya, Uganda and Senegal (Mornet *et al.,* 1956; Provost *et al.,* 1972; Bourdin, 1973; Hamdy *et al.,* 1976; Taylor and Abegunde, 1979). The disease was first recognized in the Sudan during 1971 (Elhag Ali, 1973). The disease was then described in Ethiopia (Roeder *et al.,* 1994; Abraham *et al.,* 2005), Kenya and Uganda (Wamwayi *et al.,* 1995). Outside Africa PPR has been reported in Saudi Arabia (Furley *et al.,* 1987; Abu Elzein *et al.,* 1990), India (Shaila *et al.,* 1989; Nanda *et al.,* 1996), Jordan (Lefèvre *et al.,* 1991), Israel (Perl *et al.,* 1994), Oman (Hedger *et al.,* 1980) and Pakistan (Amjad *et al.,* 1996). There is a marked rise in the regional incidence of PPR outbreaks during recent years (Nanda *et al.,* 1996; Shaila *et al.,* 1996; Ozkul *et al.,* 2002) which indicates the trend of the disease to spread. The presence of a circulating virus was confirmed by serological determination in Syria, Niger, India, Turkey, Jordan and Pakistan whereas the virus presence was detected in Ethiopia and Eritrea (Roeder *et al.,* 1994; Sumption *et al.,* 1998; Abubakar *et al.,* 2008).

In recent years PPRV has extended its boundaries southwards in Africa as far as southern Tanzania (2008-2009) (Swai *et al.,* 2009), the Democratic Republic of Congo (2012-2014) and Angola (2012) (Libeau *et al.,* 2014). PPR outbreaks have also been reported across North Africa including Tunisia (2006), Morocco (2008-2009), Algeria (2011-2012-2013) and Gabon (2011) (Kwiatek *et al.,* 2011; Parida *et al.,* 2015).

Alongside this, within Europe, Turkey reported PPR outbreaks in sheep and goats during 2011-2012 (1996, 2000, 2006, 2007, 2008, 2009, 2010, 2011) (Alcigir *et al.,* 1996; Kwiatek *et al.,* 2007; Banyard *et al.,* 2010) and in Bulgaria during 2018 (OIE-WAHIS, 2018).

In southwest Asia, the virus spread to Tibet, Israel (2011-2015-2017) and central Africa Uganda, Kenya (2007) and has recently been reported all over China (2008-2010-2013-2014-2015) (FAO, 2013; OIE, 2019). In central Asia the disease is prevalent in many countries and the virus detected in Kazakhstan (2014) was found to be genetically similar to the virus circulating in the mainland of China but was distinct to the virus isolated from 2007 outbreaks in Tibetian part of China (Kock *et al.,* 2015). The disease has recently

occurs in south Asia in Bhutan (2010-2013-2014-2016-2018) (OIE-WAHIS, 2019), Libya and Israel during 2019 (OIE-WAHIS, 2019).

This appears to be the geographic spread of the disease from West Africa to East Africa, and then to the Middle East and Asia. PPRV is considered to be endemic across Africa, the Middle East and Asia (Banyard *et al.,* 2010; Dhar *et al.,* 2002; Kwiatek *et al.,* 2011).

1.5.1. Lineages identification of PPRV:

PPRV exists as a single serotype but at the genetic level it's divided into four distinct lineages. This lineage differentiation is based on partial gene sequence of either the N or the F gene (Couacy-Hymann *et al.,* 2002; Forsyth and Barret, 1995; Banyard *et al.,* 2010; Senthil Kumar *et al.,* 2014). Four lineages (I, II, III, IV) of PPR virus were identified. Historically, lineages I–III were found in Africa (Abraham *et al.,* 2005) and were numbered according to the apparent spread of the virus from West Africa (I and II) to East Africa (III). Lineage I virus although historically reported in West Africa has not been detected for several years (since 1997). Lineage II viruses have been mainly reported in north-west, western and central Africa and recently in Tanzania, East Africa (Mahapatra *et al.,* 2015; Parida *et al.,* 2015). Lineage III viruses are currently circulating in East African countries and have been historically reported in Oman and in the UAE in the Middle East (Banyard *et al.,* 2010).

Lineage IV was mainly restricted to the Middle East and Asia with a few exceptions of Lineage III in Yemen and Oman and mixed lineages of III and IV in UAE and Qatar. However, lineage IV has now established its presence all across the PPR endemic areas with frequent outbreaks in Africa (Kwiatek *et al.,* 2011; Parida *et al.,* 2015).

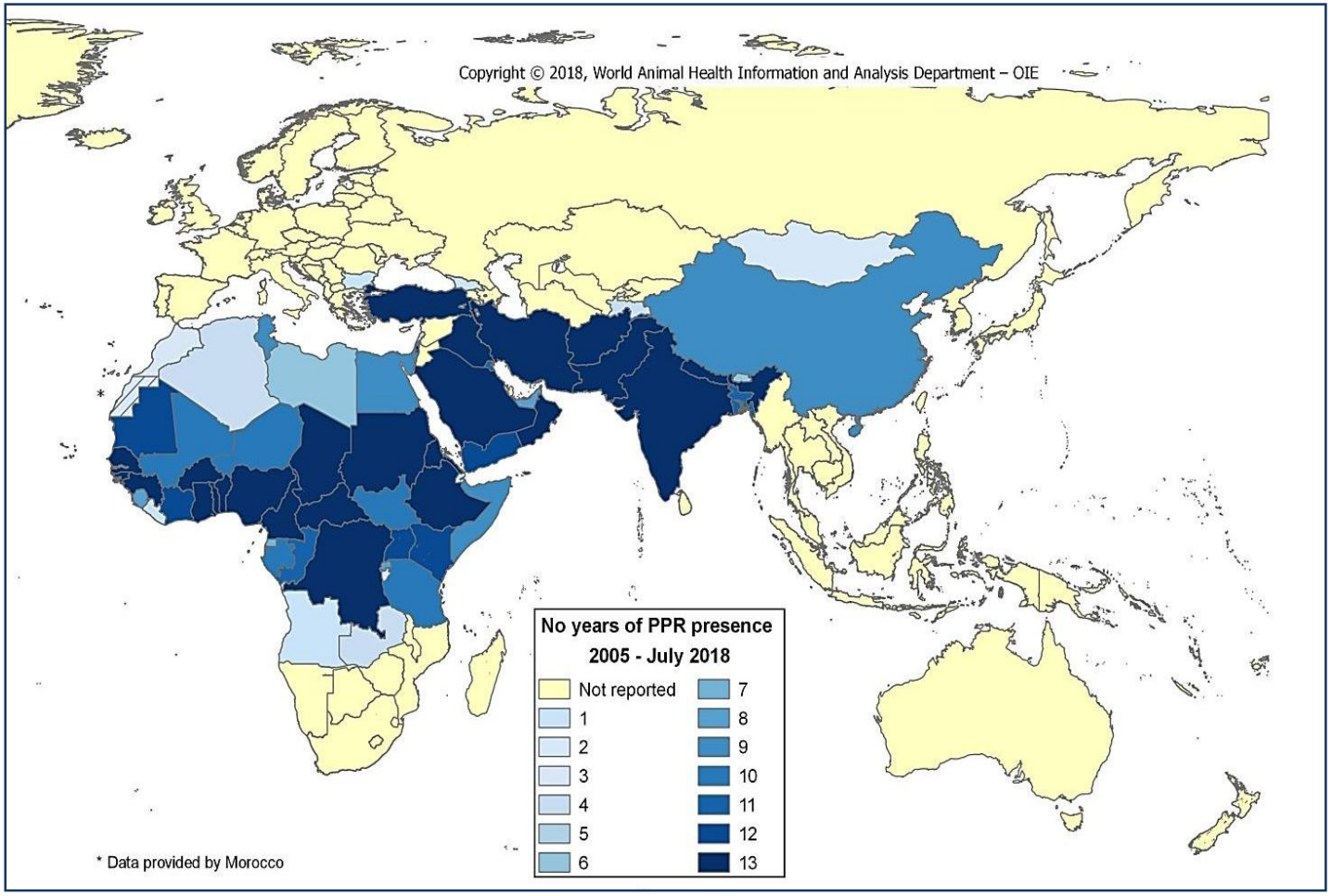

Figure 3. Geographic distribution of PPR. Map based on the information received through six-monthly reports up to July 2018. World Organization for Animal Health (OIE, 2018): http://www.oie.int/en/animal-health-in-the-world/ppr-portal/distribution/

1.6. PPR in the Sudan:

The first outbreak of the disease in sheep and goats in Sudan was in three areas in south Gedarif (Eastern Sudan) in 1971 (Elhag Ali, 1973) which was firstly wrongly diagnosed as rinderpest but later confirmed to be PPR (Elhag Ali and Taylor, 1984). The disease outbreaks were then reported in goats in Sinnar area, Central Sudan during 1971-1972 and in Mieliq in 1972 (Elhag Ali and Taylor, 1984). Later, outbreaks of PPR were reported in sheep in Western Sudan (Rasheed, 1992), in sheep and goats in Central Sudan (El Hassan *et al.,* 1994); in sheep and goats in Khartoum State (Zeidan, 1994; El Amin and Hassan, 1998). PPR was detected and isolated from different parts of the Sudan (Saeed *et al.,* 2004; Osman, 2005; Osman *et al.,* 2008; 2009a; Saeed *et al.,* 2010; 2017). Many serological survey studies of PPR in Sudan were carried out during last years, indicating the high prevalence of PPRV antibodies (El Hassan *et al.,* 1994; Elamin and Hassan, 1998; Haroun *et al.,* 2002; Osman *et al.,* 2009b; Saeed *et al.,* 2010; Ishag *et al.,* 2015; Saeed *et al.,* 2017; Osman *et al.,* 2018). Khalafalla *et al.* (2010) reported a new emerging respiratory disease of camels in Eastern Sudan during 2004, where the disease was associated with high morbidity and mortality rates. PPR antigen was detected using IcELISA and PCR, PPR was supposed to be the main causative agent of that outbreak (Khalafalla *et al.,* 2010).

Continuous outbreaks of the disease are reported annually and almost in all areas (Saeed *et al.,* 2010; Enan *et al.,* 2013; Saeed *et al.,* 2017; Osman *et al.,* 2018). Recently, serological detection of PPRV antibodies in cattle sera in the Sudan was documented (Ali *et al.,* 2019). An outbreak of PPR in Dorcas gazelles was also reported during 2016-2017 (Asil *et al.,* 2019). However, the role of camels, cattles and gazelles in the epidemiology of the disease has never been elucidated and needs more investigation.

1.7. Host range:

Sheep and goats are the primary hosts for PPRV. The disease virus exhibits different levels of virulence in sheep and goats. Goats are severely affected while sheep generally undergo a mild form of the disease (Munir, 2014). An observation detected variations in breed susceptibility within goats in West Africa. For instance, the dwarf breeds of goats have been found to be more susceptible to PPR than the Sahelian breeds (Diop *et al.,* 2005; Couacy-Hymann *et al.,* 2007).

Presence of other diseases and other stress factors precipitate the occurrence of the disease. The infection of wildlife, mainly living under semi-free range conditions, has also been reported small wild ruminant species like antelope can also be severely affected by PPR (Abu Elzein *et al.,* 2004).

Other wild animals which can be affected by PPR include gazelles (Gazella dorcus), ibex (Capra ibex nubiana), gemsbok (Oryx gazelle) and with a few reports of disease outbreaks in camels (Roger *et al.,* 2001; Khalafalla *et al.,* 2010; Kwiatek *et al.,* 2011). Cattle are also considered susceptible to subclinical infection by PPR either by direct contact with infected sheep and goats or via direct inoculation with PPRV (Anderson and Mckay, 1994; Lembo *et al.,* 2013). However, it is not excreting virus and does not pose any potential role in the transmission of PPRV (Parida *et al.,* 2015). Buffalo (Govindarajan *et al.,* 1997) and pigs (Nawathe and Taylor, 1979) develop subclinical infection with PPRV and are not thought to be capable of excreting virus and contributing to the disease epidemiology.

1.8. Transmission of PPR:

PPRV is present in all secretions and excretions from infected animals for approximately ten days after the onset of fever. Animals that have been infected with PPRV either die or acquire firm immunity. There appears to be no chronic carrier state (Hamdy *et al.,* 1976).

PPRV is transmitted by direct contact between susceptible and infected animals through exhaled aerosols, particularly during coughing, or through clinical excretions (lachrymal, nasal, saliva and faeces). Virus may be spread over large distances through the movement of infected animals for trade or during migration, particularly those animals incubating the disease without clinical manifestations. Recently, PPR is classified as a transboundary animal disease (TAD) (Zahur *et al.,* 2011). It is also believed that there is no known carrier state for PPRV, although there are speculations of virus adaptability in other animal species without showing overt clinical signs.

PPRV is temperature labile and is readily inactivated outside its host in a dry environment. Infected and recovered animals develop life-long protective immunity (Hamdy *et al.,* 1976). However, the virus can spread in animals as a mild virulent form that can later lead to severe disease where transmission occurs to naïve susceptible populations

(Couacy-Hymann *et al.,* 2007). There is also a possibility that the virus may be transmitted through fomites.

1.9. Clinical Signs:

Goats and sheep are the primary hosts for PPRV. Goats are more susceptible to disease than sheep with some breeds of goats considered to be more susceptible than others (Couacy-Hymann *et al.,* 2007).

Depending on the severity of the disease, lineage, species, breed, immune status of animals, PPR may manifest in different forms which include per-acute, acute and mild (OIE, 2019). Infected animals present clinical signs similar to those of rinderpest in cattle but with the eradication of this disease worldwide, its differentiation is of little or no importance. The incubation period is typically 4-6 days, but may range between 3-10 days. The clinical disease is acute, with a fever up to 41°C that can last for 3-5 days; followed by depression, anorexic and development of a dry muzzle (OIE, 2019). Animal develops Watery nasal and lachrymal discharge which gradually become mucopurulent with excessive salivation. Erosive lesions formed in the oral cavity that may become necrotic. In severe cases of the disease, these necrotic lesions progress with the appearance of a deposit of brin fibrin (caseous deposit) on the tongue. In the later stages of the disease, animals develop diarrhoea and coughing with labored, abdominal breathing. Finally, the animal may become dyspneic, suffering progressive weight loss and emaciation, ultimately leading to death (OIE, 2019). In some cases, particularly in mild infections, animals may convalesce, returning to a pre-infection health status within 10-15 days of infection. The morbidity rate can reach 100% with a high case fatality rate in the acute form of the disease (Pope *et al.,* 2013).

1.10. Post-mortem lesions:

The carcass is often emaciated and dehydrated with presence of evidence of diarrhoea, serous mucopurulent oculo-nasal discharges, inflammatory and necrotic lesions in the mouth and the gastrointestinal tract (Roeder *et al.,* 1994). The lips often have prominent crusty scabs and necrotic stomatitis is common. Shallow and sharply erosions which are demarcated from the normal epithelium may be present inside the mouths of some animals. In severe cases, the hard palate, pharynx and upper esophagus can also be involved. Similar lesions may be found on the vulva and vaginal mucous membranes

(Saliki and Wohlsein, 2008). The rumen, reticulum and omasum are not significantly involved, although erosions are occasionally found on the pillars of the rumen. Erosions are common also in the abomasum. Hemorrhagic streaks and erosions sometimes occur in the duodenum and the terminal ileum (Diallo, 2003).

Unlike RP, severe interstitial pneumonia commonly occurs with PPR (OIE, 2019). Erosive or hemorrhagic enteritis is usually present and the ileo-caecal junction is commonly involved. Peyer's patches may be necrotic at the caeco-colic junction and in the rectum. In the posterior part of the colon and the rectum, discontinuous streaks of congestion ("zebra stripes") form on the crests of the mucosal folds (Munir *et al.,* 2013). Lymph nodes are enlarged, and the spleen and liver may show necrotic lesions (OIE, 2019).

1.11. Diagnosis of PPR:

1.11.1. Laboratory Diagnosis:

1.11.1.1. Samples Required for Diagnosis:

In order to perform a laboratory diagnosis of PPR, samples should be collected from live animals that include: swabs from the conjunctival discharges, from the nasal, buccal and rectal mucosa and whole blood in anticoagulant during the early stage of the disease. Also, samples can be collected from dead animals at necropsy include tissues from dead animals such as lymph nodes especially the mesenteric and bronchial nodes, mucosa of lungs and intestine and spleen (OIE, 2019).

1.11.1.2. Methods for virus isolation in cell culture:

PPRV had been isolated, adapted and passaged in cells of sheep, goat and bovine origin. Lamb kidney and lung cell monolayer were reported as the most sensitive for isolation and assay of the virulent strain of PPRV (Nigeria 75/1) (Gibbs *et al.,* 1979) and some cell lines (Vero, B95a) (Sreenivasa *et al.,* 2006). PPRV isolation using such cells may require multiple blind passages. Recently, derivatives of cell lines (Vero, CV1) expressing the morbillivirus receptor, the signaling lymphocyte activation molecule (SLAM or CD150), have been developed for isolation of field viruses from pathological specimens in less than one week, without requirement for blind passages. These include a derivative of the

monkey cell line CV1 expressing goat SLAM (Adombi *et al.,* 2011) and derivatives of Vero cells expressing dog SLAM (OIE, 2019).

The virus manifests specific cytopathic effect (CPE) after 3-5 days of infection, which include initial rounding of the infected cells in grape-bunch-like clusters, followed by vacuolation, granulation of the cell cytoplasm, fusion of the monolayer cells and formation of syncytia, which are characteristics of PPRV (Sreenivasa *et al.,* 2006; Singh *et al.,* 2010).

1.11.1.3. Methods for antigen detection:

Detection of PPRV antigens can be performed using an immunocapture ELISA (IC-ELISA) (Libeau *et al.,* 1994), counter immunoelectrophoresis (CIEP) (Durojaiye *et al.,* 1983; Majiyagbe *et al.,* 1984; Osman *et al.,* 2009b), agar gel immunodiffusion (AGID) (Anderson *et al.,* 1996; Osman *et al.,* 2008), haemagglutination (HA) test (Ezeibe *et al.,* 2004; Osman *et al.,* 2008), immunofluorescence (Sumption *et al.,* 1998), immunoperoxidase staining (IP) and Immunohistochemistry (IHC) (Bundza *et al.,* 1988).

1.11.1.4. Methods for serological diagnosis of PPR:

The demonstration of antibodies in PPRV infected goats and sheep can be used to support a diagnosis based on clinical signs, but such antibodies may also arise from vaccination with any of the current PPRV vaccines. Tests that are routinely used are the virus neutralisation test (VNT) and the competitive ELISA (OIE, 2019).

1.11.1.4.1. Virus neutralization (VN) test:

It is a gold standard test for the diagnosis of PPR and RP, although it is very reliable, sensitive and specific but on the other hand it is expensive and time consuming (Rossiter *et al.,* 1985; OIE, 2019). It is considered to be the ‘gold standard’ test for diagnosis of the disease, although it is a time-consuming method that requires tissue culture facilities. This test usually carried out in 96-well microtitre plates although roller-tube cultures may also be used (OIE, 2019). Vero cells are preferred, but primary lamb kidney cells may also be used and has recently been validated under field conditions for diagnosis as early as 4 days post-infection, before onset of severe clinical signs (Baron *et al.,* 2014).

1.11.1.4.2. Competitive enzyme-linked immuno-sorbent assay (C-ELISA):

Competitive ELISA (C-ELISA) is one of the most extensively used tests for serological screening and diagnosis of PPRV in infected animals. A competitive ELISA based on the use of MAb anti-nucleo-capsid protein and a recombinant nucleo-capsid protein produced in the baculovirus has been described (Libeau *et al.,* 1994; 1995). It is used to determine the presence of PPR specific antibodies both pre and post vaccination.

Another competitive ELISA technique, based on the use of monoclonal anti-haemagglutinin (H), has also been described (Choi *et al.,* 2005).

1.11.1.4.3. Haemagglutination-inhibition (HI) test:

It has been demonstrated that PPRV and MV are unique among morbilliviruses in carrying haemgglutination abilities (Wosu, 1985). Using this haemagglutinating character of PPRV, HA and HAI tests have successfully been employed for the confirmatory diagnosis of PPRV (Wosu and Ezeibe, 1992; Osman *et al.,* 2008). This test is widely used for the quantitative measurement of PPRV antibodies usually in a suspension. In this regard, a two-fold serial dilution of serum is practiced in a micro-well plate. The dilution of antibodies still able to inhibit agglutination is regarded as the titer of the serum sample in the suspension. However, it is also possible to titerate the PPRV antigen using HA and HAI tests (Osman *et al.,* 2008).

1.11.1.5. Methods for RNA genome/Nucleic acid detection:

Molecular technique based on the amplification of parts of the N and F protein genes have been developed for the specific diagnosis of PPR. There are sensitive and specific detection methods, such as the standard reverse-transcription polymerase chain reaction (RT-PCR) (Forsyth and Barrett, 1995; Couacy-Hymann *et al.,* 2002) and currently real-time PCR assays specific for PPRV (Bao *et al.,* 2008; Kwiatek *et al.,* 2010) The generation of a standard RT-PCR product is, however, necessary in order to perform sequence analysis and subsequent phylogenetic characterization of novel virus isolates.

1.12. Control:

The State or federal authorities should be notified immediately after appearance of disease outbreaks (OIE, 2019). Control and eradication strategies are based on three

epidemiological principles, to prevent contact between PPRV and susceptible animals, to stop the production of PPRV in infected or exposed animals and to increase the disease resistance of susceptible animals to PPRV or reduce the shedding of PPRV in infected or exposed animals (Diallo, 2003).

Preventive measures employed against the disease in free area were achieved by using many different measures such as strict restrictions on the importation of animals from disease infected regions, quarantine, slaughtering infected animals, decontamination of affected premises, sanitary measures, raise awareness among all value chain actors on sanitary measures and surveillance (FAO, 2015). If an outbreak occurs, isolation, quarantine measures should be imposed and supported by vaccination of the population at risk (OIE, 2019).

Maternal antibodies against the virus can be detected in young animals, and remain able to neutralize the virus for three to four months, enabling a level of protection in newborn animals (Libeau *et al.*, 1992).

Immunization can be performed with commercially available PPR homologous attenuated tissue culture vaccine that elicit a protective immunity for at least three years post-vaccination (Diallo *et al.*, 2007; Sen *et al.*, 2010). Current vaccination schedules require the immunization of susceptible animals at least every three years (Diallo *et al.*, 2007; Saravanan *et al.*, 2010). Vaccination in animals aged 4–6 months is recommended for control of PPR in endemic areas (Balamurugan *et al.*, 2014).

Chapter II

Materials and Methods

2.1. Materials:

2.1.1. Chemicals and reagents:

Name	Company
PBS tablets	Caisson Laboratories, USA
NaCl	Sigma, USA
KCl	Sigma, USA
Na_2HPO_4	Sigma, USA
KH_2PO_4	Sigma, USA
Na citrate	Sigma, USA
Citric acid	Sigma, USA
D-glucose	Sigma, USA
Ethanol Absolute	Romil, UK

2.1.2. Antibiotics and antifungal:

Name	Company
Benzylpenicillin Sodium	NCPC, China
Streptomycin Sulphate	NCPC, China
Gentamycin	NCPC, China
Mycostatin	NCPC, China

2.1.3. Apparatuses, Equipment and Instruments:

Name	Company
Laminar Flow Safety Cabinet – Class II	BDSL (Biological & Diagnostic Supplies Ltd.)
Autoclave	Autoclave SANO clav, Germany
Sanyo OMT Oven	Gallenkamp, UK
Refrigerated Centrifuge	Centurion Scientific Ltd., UK
Bench Centrifuge EBA 20	Hettich, Germany
Jenway pH Meter	Bibby Scientific Ltd., UK

Orbital Shaker SO3	Stuart Scientific, UK
Refrigerator (+4°C)	Ocean, Italy
Deep Freezer (-20°C)	Ocean, Italy
Single channel micropipette	Biohit and Labtech, Germany
Multichannel micropipette	Biohit and Labtech, Germany
Glassware	Pyrex
Plasticware	-
Mortar and pestle	-
Scissors	-
Forceps	-
Scalpel and blades	-
96-well V-bottom micro-well haemagglutination plate	-

2.1.4. Disposables:

Name	**Company**
Plain Vacutainer and Multi Sample Needle	Mrlab, China
Syringes	Ava-med Medical Industries, Sudan
Sealed plastic bags	-
Ice bags or Ice	-
Container for preservation of samples	-
Latex Examination Gloves	Okjo Medical Consumables, Malaysia
Face mask	-
Yellow Tips (Sterile)	Marina Pharma, China
Blue Tips (Sterile)	Marina Pharma, China
Trough	-
Eppendorf tubes (Sterile)	Zhejinag Medicines & Health Products, China
Boxes for Eppendorf tubes	-
Tube Rack	-
50 ml White cap tubes, Conical Centrifuge Tube (Sterile)	Nalge Nunc International, USA
15 ml Blue cap tubes (Sterile)	Zhejinag Medicines & Health Products, China
Stainless steel tray	-
Absorbent Cotton Wool	Anji Speng Industrial Co. Ltd.
Aluminum Foil	-
Detergent Micro-90	International Products Corporation, USA
Disinfectant/Dettol	-

2.1.5. Study area:

River Nile State is located in the northern part of the Sudan and lies between altitudes 16-22 north and longitudes 32-35 east. It is bordered in the north by Egypt, in the west by the Northern State, in the east by Kassala and Red Sea States, and in the south by Khartoum State. River Nile State is composed of seven localities namely Ad-Damar, Atbara, Berber, Abu Hamad and Elbohira "part of Abu Hamad", Shendi and Elmatama (Figure 4).

The present study was carried out in order to determine the sero-prevalence of peste des petits ruminant virus (PPRV) antibodies among sheep and goats in the localities of the River Nile State.

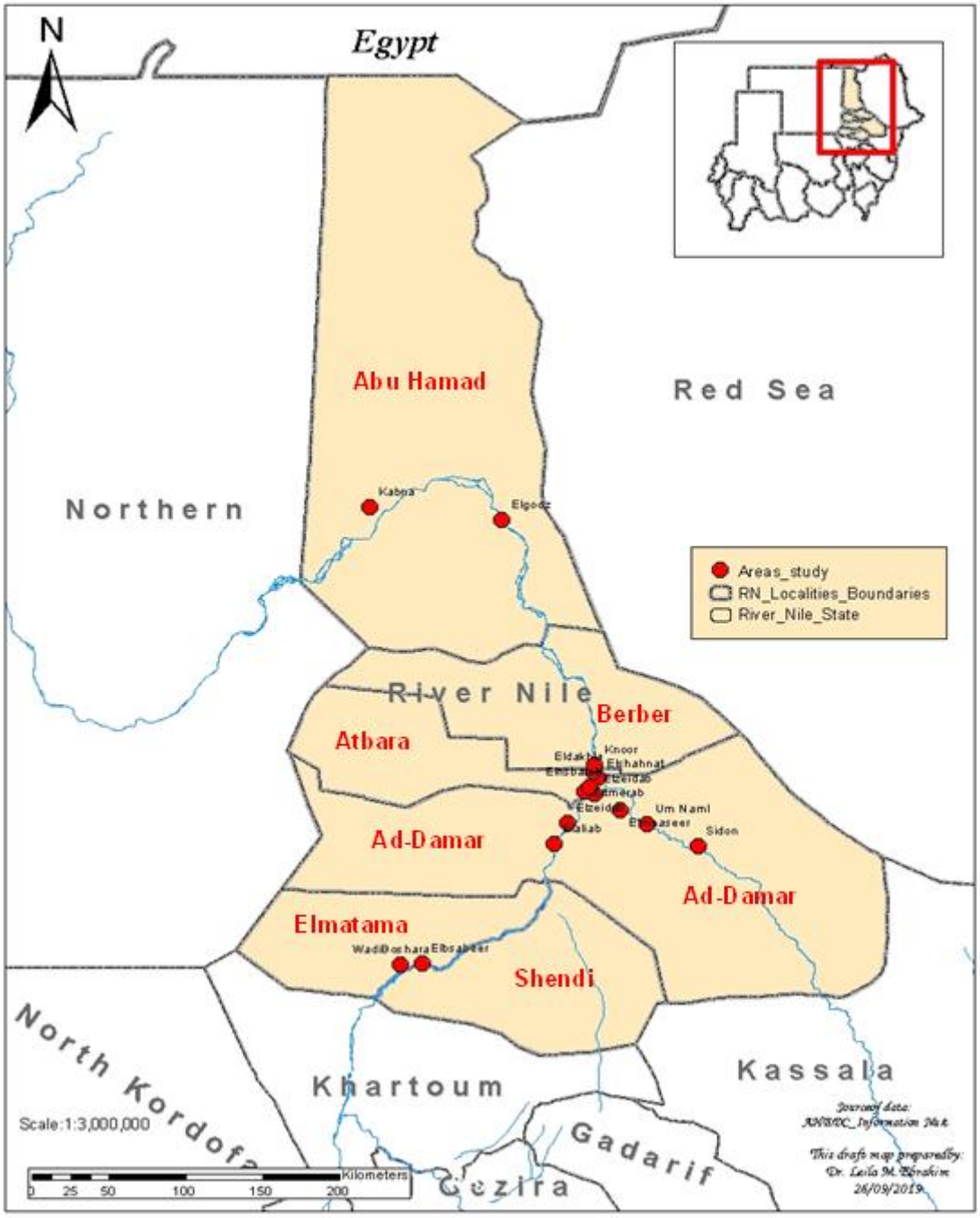

Figure 4. Map of the Sudan showing the study area in River Nile State. The seven localities of the River Nile State (Ad-Damar, Atbara, Berber, Abu Hamad and Elbohira, Shendi and Elmatama) were shown in red colour font.

2.1.6. Livestock in the River Nile State:

There is considerable number of animal wealth in the River Nile State which is adapted to the nature and climate of the State. These constitute local animal breeds like Butana cows and Nubian goats but recently some foreign animal breeds (Friesian cows, Anglo Nubian, Shami and Saanen goats), which produce high milk yield, had been introduced into the State. There are also distinguished camel breeds which include Albushari, Anafi, Dielya and the Arabic camel breeds. Estimation of animal population and their distribution in the River Nile State was presented in Table 1.

Table 1. Estimation of animal population and distribution of livestock in the River Nile State, report of the year 2018.

Locality	Cattle	Goats	Sheep	Camel
Atbara	6512	78404	28221	2524
Ad-Damar	21707	340892	255393	44592
Shendi	39074	538608	482567	22716
Berber	8683	182377	200365	5889
Elmatama	30391	347709	299135	6731
Abu Hamad and Elbohira	2171	216466	145334	1683
Total	108538	1704456	1411015	84135

2.2. Methods:

2.2.1. Sample collection:

A total of 483 serum samples were collected from, infected and non-infected, sheep (337) and goats (146 sera) from 19 suspected PPR outbreaks reported during January 2018 to December 2019 in the River Nile State. These sera were collected from 7 localities of the State including 96 sera (51 sheep and 45 goats) from Atbara locality, 254 sera (195 sheep and 59 goats) from Ad-Damar locality, 15 sera (15 sheep) from Shendi locality, 39 sera (26 sheep and 13 goats) from Berber locality, 40 sera (32 sheep and 8 goats) from Elmatama locality, 39 sera (18 sheep and 21 goats) from Abu Hamad and Elbohira localities (Table 2).

Before or after these outbreaks occurred, animals in these herds have never been vaccinated against PPR.

2.2.2. Collection and preparation of serum samples:

Blood samples were collected aseptically from the jugular vein of sheep and goats using a sterile Vacuum blood collection system/Vacutainers and needles. The method of preparing serum sample from the blood is by placing the Vacutainer collection tube in a diagonal position for 1-2 hour at room temperature then overnight at 4°C. On the next day, serum was separated from blood by centrifugation at 3000 rpm for 5 minutes, the clear serum was aspirated and transferred to serum collection tubes and stored at -20°C.

Table 2. Sheep and goats sera collected from PPR outbreaks in the River Nile State during 2018-2019.

Date of Collection	Locality	No. of Outbreaks	Animal Species		Total No. of Sera
			Sheep	Goats	
January 2018 to July 2019	Atbara	5	51	45	96
January 2018 to December 2019	Ad-Damar	9	195	59	254
January 2018	Shendi	1	15	0	15
April 2018	Berber	1	26	13	39
March 2019	Elmatama	1	32	8	40
March to July 2019	Abu Hamad and Elbohira	2	18	21	39
Total		19	337	146	483

2.2.3. Preparation of antigen samples:

The virus antigen was obtained by preparation of a 50% tissue suspension from the pneumonic sheep lung samples in Phosphate Buffer Saline (PBS, pH 6.8). The procedure was performed under cold aseptic conditions inside the laminar flow safety cabinet. A large piece of lung tissue was placed into sterile mortar, using a sterile scissors and forceps tissues were cut into small pieces, then small tissues were ground and minced using mortar and pestle, around 50 ml of cold PBS solution (pH 6.8) supplemented with antibiotics and antifungal was added. The minced preparation were then transferred into 50 ml sterile white cap tubes and centrifuged at 3000 rpm for 10 minutes at 4°C. Then supernatants were aspirated and distributed into 50 ml sterile white cap tubes, stored at -20°C till used. The homogenate was tested against PPRV positive serum then used as an antigen source for PPRV in the HA and HI tests.

2.2.4. Detection of PPRV antibodies in sheep and goats sera:

In order to establish the haemagglutination-inhibition (HI) test for detection of PPRV antibodies in sheep and goats sera, haemagglutination (HA) test for detection and quantification of PPRV antigen was performed.

2.2.4.1. Preparation of red blood cells (RBCs) suspension:

For preparation of red blood cells (RBCs) suspension, the collected chicken blood was centrifuged at 1500 rpm for 10 minutes at room temperature/25°C, then the plasma and buffy coat was aspirated and discarded, the remaining RBCs were washed after addition of 10 ml of PBS (pH 6.8) by centrifugation at 1500 rpm for 10 minutes, supernatant PBS was discarded and washing was repeated 3 times.

A 0.8% chicken RBCs suspension was prepared by addition of 0.8 ml of washed RBCs to 100 ml of PBS (pH 6.8), the suspension was used as indicator in the HA and HI tests.

2.2.4.2. Haemagglutination (HA) test:

To quantifying the titer of the virus to be used in the haemagglutiantion-inhibition (HI) test, haemagglutination (HA) test was performed as described recently (Abdalla, 2019). Firstly, 50 µl Phosphate Buffer Saline (PBS, pH 6.8) as diluent was distributed into all wells of the V-bottom 96-well microtiter plate. 50 µl of the 50% lung tissue suspension

as antigen was dispensed into well A1 then 2-fold serial dilution was prepared. Secondly, 50 µl of 0.8% RBCs suspension was added to column 1 and to column 2 which served as RBCs control, then shaking was performed for mixing the contents in the wells of the plate. The HA plate was incubated at room temperature (15°C) for 17-18 minutes, results of the HA test was recorded by the naked eye, positive result were indicated by formation of the haemagglutination sheet in the virus dilution wells while in the RBCs control wells negative results appeared as a sharp red button.

The haemagglutination titer of the 50% tissue suspension PPRV was calculated then 4 or 8 haemagglutination units (HAU) was used as antigen in the HI test.

2.2.4.3. Haemagglutination-inhibition (HI) test:

To detect and quantify PPRV antibodies in sheep and goat sera, haemagglutination-inhibition (HI) test was performed according to the protocol described by Wosu and Ezeibe (1992). Firstly, 0.25 µl of the Phosphate Buffer Saline (PBS) of pH 6.8 diluent was distributed into all wells of the V-bottom 96-well microtiter plate, then 0.25 µl of PPRV positive serum was added to each well A1, 0.25 µl of the antibody samples were added to each well of row A from A2 to A11. Column 12 served as RBCs control or negative control and received only PBS and RBCs.

A 2-fold serial dilution of the sera was prepared by mixing the content of the wells in Row A then transferring 0.25 µl to row B, for the 2-fold serial dilution we continued by mixing and transferring 0.25 µl to the next row till row H then 25 µl volume was discarded away. Secondly, 0.25 µl of PPRV antigen containing 8 HAU was added to all wells of the plate except column 12 (RBCs control), then shaking of the plate for mixing the contents in the wells was performed using Orbital Shaker. The HI plate was incubated in a refrigerator at 4°C overnight. On the next day, 50 µl of 0.8% RBCs suspension was added to all wells of the plate, the HI plate was incubated at room temperature (15°C) for 18 minutes, results of the HI test were recorded by the naked eye. The haemagglutination-inhibtion titers of the control and tested sera were calculated from their respective end-point dilutions and expressed as haemagglutination-inhibtion titer (HAI).

Chapter III
Results

3.1. PPR outbreaks history:

During 2018-2019, many suspected outbreaks of PPR were reported among flocks of sheep and goats in the seven localities of the River Nile State in the northern part of the Sudan. The main clinical signs observed in the affected animals were nasal and ocular discharges, fever (40-42°C), coughing, anorexic, erosive lesions in the oral cavity and lips, diarrhoea, while some goats aborted. The course of the disease ranged from acute to sub-clinical. Sheep and goats of all ages and sex groups were seriously affected, however, infection of PPR in goats of various ages was more severe than sheep and losses were generally encountered in young goats and sheep while mortality in sheep mostly occurred at an age ranging from 1-2 months. Generally, in these suspected PPR outbreaks reported in different localities of the River Nile State, higher morbidity rate was reported among goats (62.0%, ranged between 80.0% and 33.3%) compared to sheep (21.7%, ranged between 58.3% and 10.5%).. Similarly, the mortality rate was higher among goats (38.0%, ranged between 43.3% and 13.3%) compared to sheep (12.8%, ranged between 36.7% and 8.0%) (Table 3).

Table 3. Morbidity and mortality rates estimated among sheep and goats in PPR suspected outbreaks occurred in the River Nile State during 2018-2019.

	Total No. of animals in herds		No. of animals infected (Morbidity%)		No. of animals deaths (Mortality%)	
Locality	sheep	Goats	sheep	Goats	sheep	Goats
Atbara	375	146	75 (20.0%)	84 (57.5%)	30 (8.0%)	55 (37.7%)
Ad-Damar	1470	270	358 (24.4%)	187 (69.3%)	201 (13.7%)	117 (43.3%)
Shendi	1050	0	110 (10.5%)	0 (0.0%)	96 (9.1%)	0 (0.0%)
Berber	60	15	35 (58.3%)	12 (80.0%)	22 (36.7%)	5 (33.3%)
Elmatama	334	45	135 (40.4%)	15 (33.3%)	72 (21.6%)	6 (13.3%)
Abu Hamad and Elbohira	0	45	0 (0.0%)	25 (55.6%)	0 (0.0%)	15 (33.3%)
Total	3289	521	713 (21.7%)	323 (62.0%)	421 (12.8%)	198 (38.0%)

3.2. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in sheep and goats serum samples:

To investigate the presence of PPR antibodies, sheep and goats sera form 19 suspected outbreaks occurred in seven localities in the River Nile State were tested using the haemagglutination-inhibition (HI) test. Of note, HI results were recorded by the naked eye at 17-18 min post-incubation (p.i) with the RBCs suspension and the elution phenomenon (sediment RBCs) occurred at 19 min p.i. Positive HI results appeared as red buttons of sediment RBCs which is larger than that of red blood cells (RBCs) control while negative HI results appeared as diffuse sheet (Figure 5).

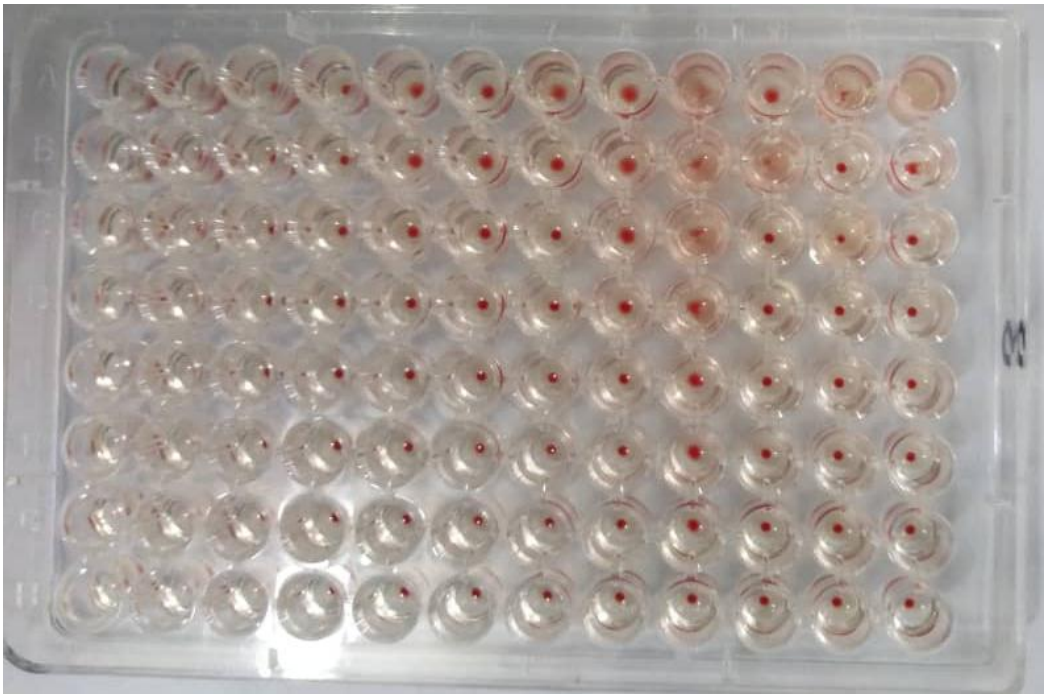

Figure 5. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies. HI test plate showing positive HI result as a red button, red blood cells (RBCs) control (column 12) appeared as a sharp red button.

3.3. Overall sero-prevalence of PPRV antibodies in sheep and goats sera by haemagglutination-inhibition (HI) test:

A total of 483 serum samples (337 sheep and 146 goats) from River Nile State were screened using haemagglutination-inhibition (HI) test for the presence of PPRV antibodies. Sera with HI titers less than 8 were considered as negative whereas sera with HI titers more than or equal to 8 were considered as positive and protective. HI results revealed that 470/483 of the tested sera were positive, with an overall antibodies sero-prevalence of 97.3%, while only 13/483 (2.7%) sera were negative (Table 4; Figure 6). Results of the haemagglutination-inhibition test revealed titers ranged from 8 to 256 with an overall HI mean titer of 52.6, apparently most of the samples (136/483, 28.2%) achieved HI titer of 32 and 26/483 (5.4%) samples achieved the highest HI titer of 256 (Table 4; Figure 7).

Table 4. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in sheep and goats sera from River Nile State. Number of positive and negative sheep and goats serum samples for PPRV antibodies, end point dilutions and number of samples at different HI titers were presented.

		Sheep and Goats	
End-point dilution	HI Titer (HAI)	+ve%	-ve%
0	0	0 (0.0%)	2 (0.4%)
1/2	2	0 (0.0%)	2 (0.4%)
1/4	4	0 (0.0%)	9 (1.9%)
1/8	8	45 (9.3%)	0 (0.0%)
1/16	16	120 (24.8%)	0 (0.0%)
1/32	32	136 (28.2%)	0 (0.0%)
1/64	64	97 (20.1%)	0 (0.0%)
1/128	128	46 (9.5%)	0 (0.0%)
1/256	256	26 (5.4%)	0 (0.0%)
Total		470 (97.3%)	13 (2.7%)

*Sera with HI titers ≥8 were considered as positive and protective.

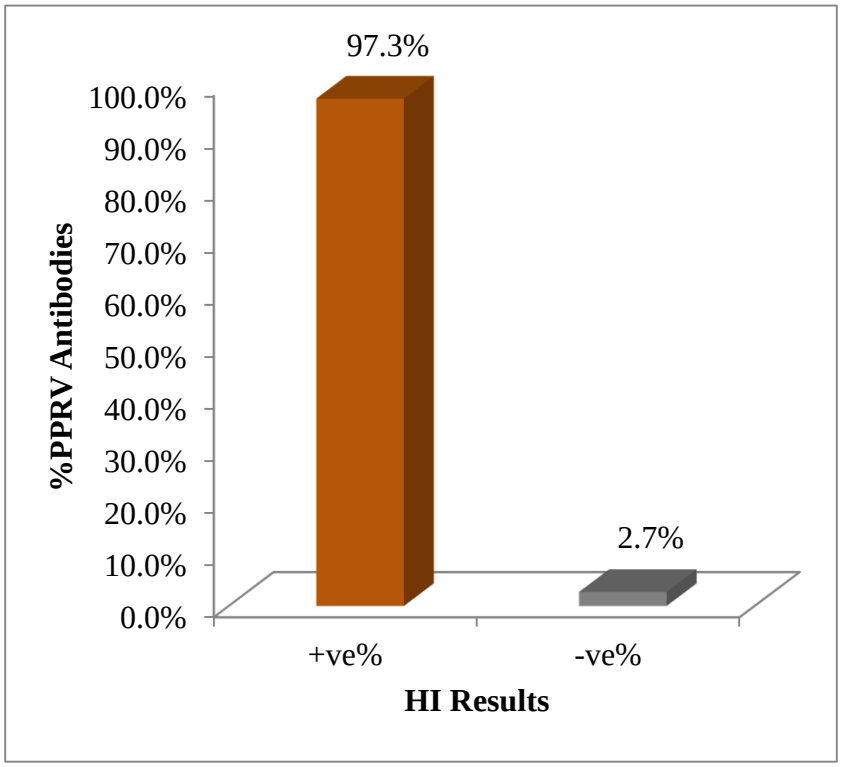

Figure 6. Positive (brown) and negative (dark grey) sheep and goats serum samples from River Nile State using haemagglutination-inhibition (HI) test for detection of PPRV antibodies.

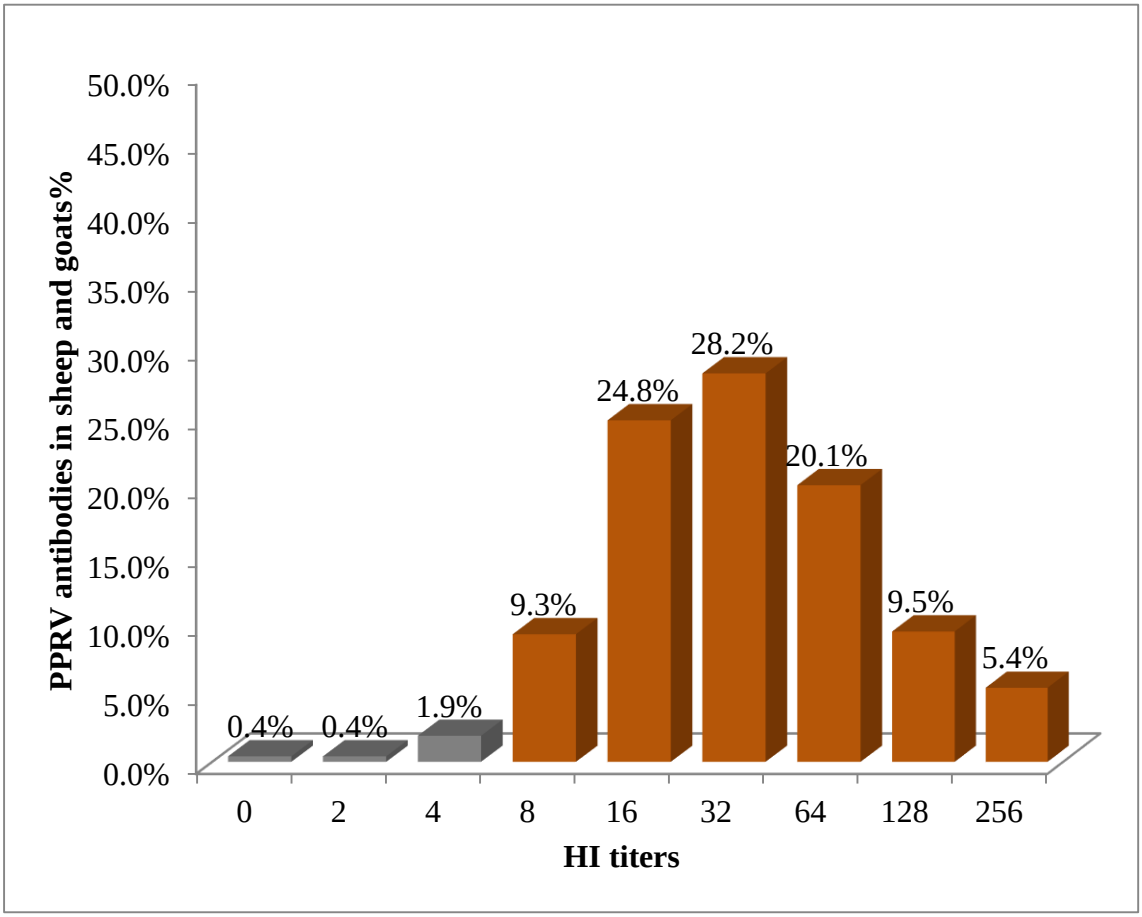

Figure 7. PPRV antibodies detected in sheep and goats sera at different haemagglutination-inhibition titers by haemagglutination-inhibition (HI) test. Positive (brown) and negative (dark grey) sheep and goats sera were indicated.

3.4. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in serum samples of sheep:

Considering the species under study, of the tested sheep sera 324/337 (96.1%) were positive while only 13/337 (3.9%) were negative (Table 5). Within sheep, results of the haemagglutination-inhibition test revealed 324 positive sera with HI titers ranged from 8 to 256 with mean titer of 48.9, apparently most of the samples (95/337, 28.2%) achieved HI titer of 16 (Table 5) and 16/337 (4.7%) samples achieved the highest HI titer of 256. Of the 13 negative sera, 9/337 (2.7%) samples achieved HI titer of 4, 2 (0.6%) samples achieved HI titer of 2 and 2 (0.6%) samples achieved HI titer of 0 (Table 5, Figure 8).

Table 5. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in sheep or goats serum samples from River Nile State. Number of positive and negative sheep sera, positive and negative goats sera at different HI titers were presented.

End-point dilution	HI Titer (HAI)	Animal Species			
		Sheep		Goats	
		+ve%	-ve%	+ve%	-ve%
0	0	0 (0.0%)	2 (0.6%)	0 (0.0%)	0 (0.0%)
1/2	2	0 (0.0%)	2 (0.6%)	0 (0.0%)	0 (0.0%)
1/4	4	0 (0.0%)	9 (2.7%)	0 (0.0%)	0 (0.0%)
1/8	8	36 (10.7%)	0 (0.0%)	9 (6.2%)	0 (0.0%)
1/16	16	95 (28.2%)	0 (0.0%)	25 (17.1%)	0 (0.0%)
1/32	32	89 (26.4%)	0 (0.0%)	47 (32.2%)	0 (0.0%)
1/64	64	56 (16.6%)	0 (0.0%)	41 (28.1%)	0 (0.0%)
1/128	128	32 (9.5%)	0 (0.0%)	14 (9.6%)	0 (0.0%)
1/256	256	16 (4.7%)	0 (0.0%)	10 (6.8%)	0 (0.0%)
Total		324 (96.1%)	13 (3.9%)	146 (100%)	0 (0.0%)

*Sera with HI titers ≥8 were considered as positive.

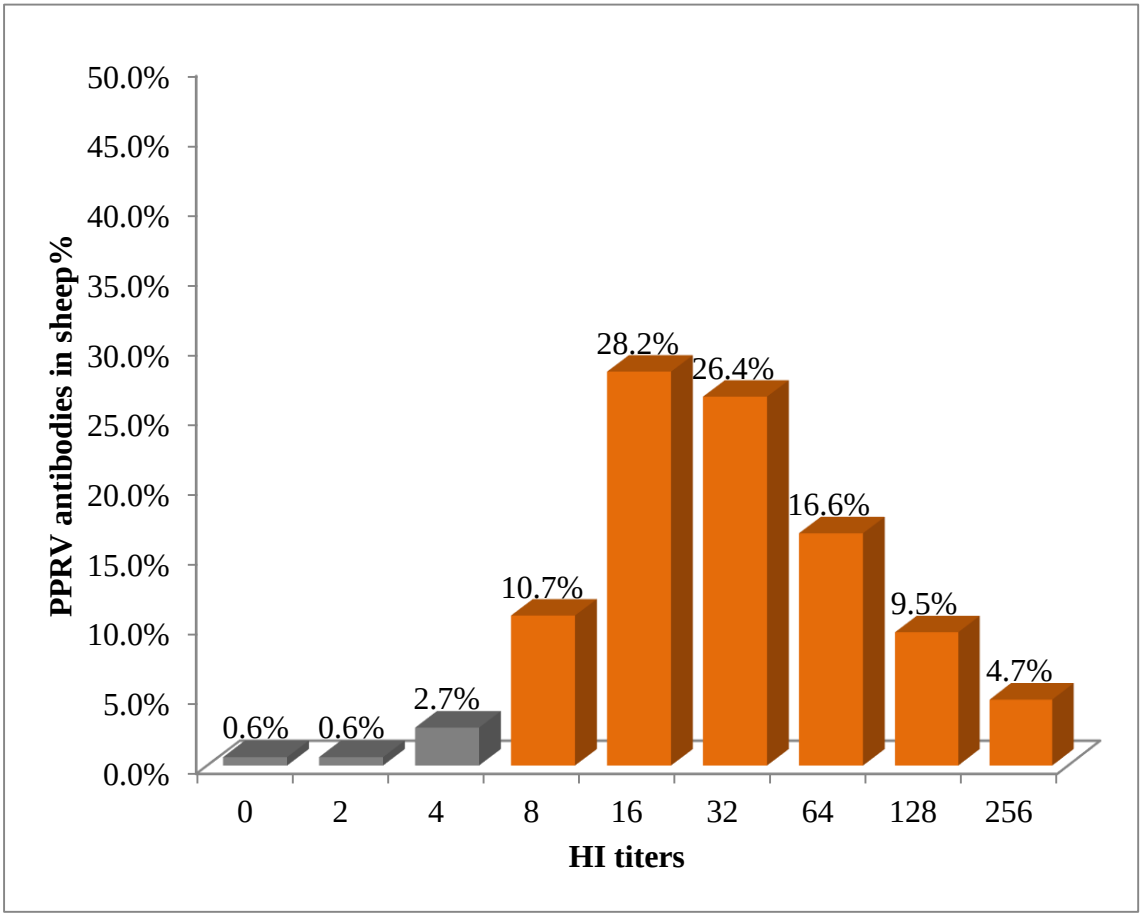

Figure 8. PPRV antibodies detected in sheep sera at different haemagglutination-inhibition titers by haemagglutination-inhibition (HI) test.

3.5. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in serum samples of goats:

Considering the species under study, all the tested goats sera 146/146 (100%) were positive (Table 5).

Within goats, results of the haemagglutination-inhibition test revealed HI titers ranged from 8 to 256 with mean titer of 61.3, apparently most of the samples (47/146, 32.2%) achieved HI titer of 32 (Table 5) and 10/146 (6.8%) samples achieved the highest HI titer of 256 (Table 5, Figure 9).

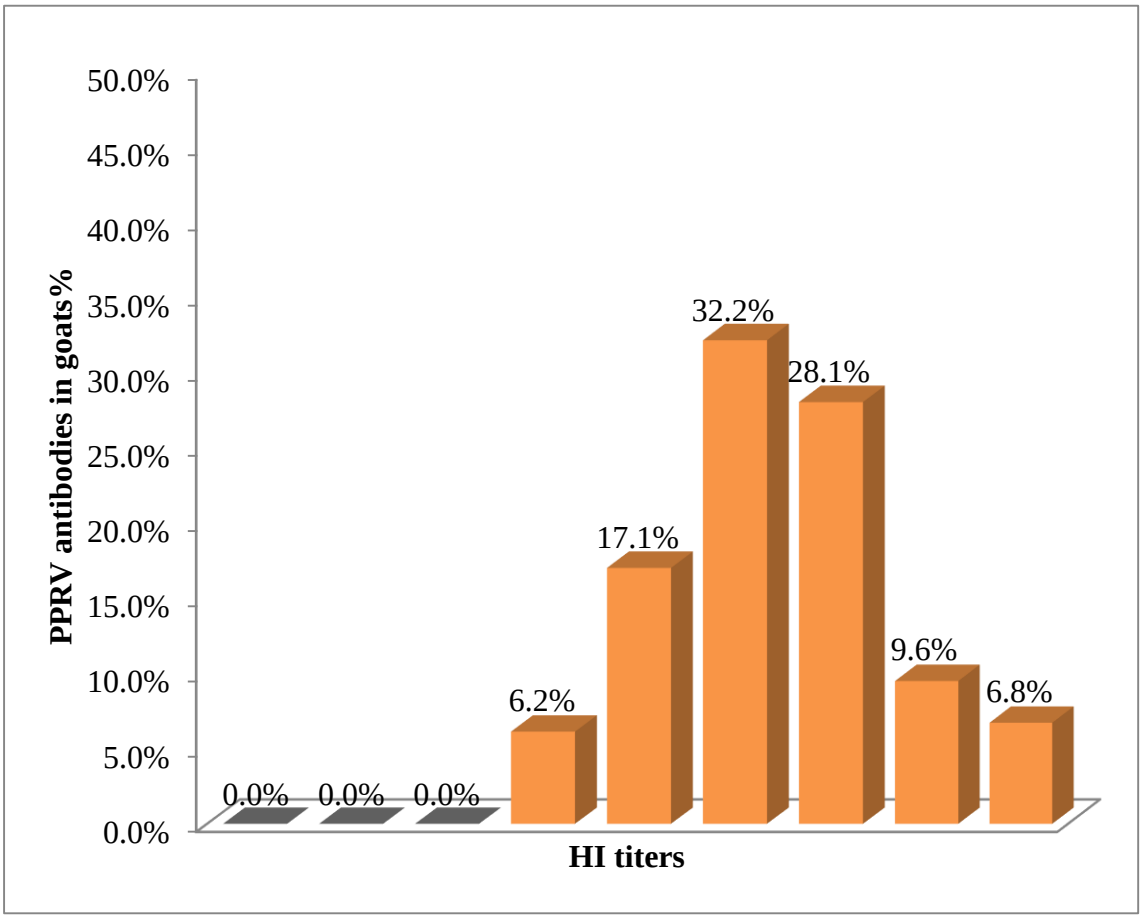

Figure 9. PPRV antibodies detected in goats sera at different haemagglutination-inhibition titers by haemagglutination-inhibition (HI) test.

3.6. Sero-prevalence of PPRV antibodies in both sheep and goats sera within localities of River Nile State:

Within localities of the River Nile State, the highest overall sero-prevalence of PPRV antibodies was demonstrated in Shendi (100%) and Berber (100%), with relatively lower sero-prevalence demonstrated in Ad-Damar (98.8%), Abu Hamad and Elbohira (97.4%), Atbara (94.8%) and the least sero-prevalence was demonstrated in Elmatama (90.0%) (Table 6).

Considering the species under study, within sheep the highest sero-prevalence of PPR antibodies (100%) was demonstrated in Shendi and Berber whereas the least sero-prevalence (87.5%) was demonstrated in Elmatama (Table 7).

Within goats, the highest sero-prevalence of PPRV antibody (100%) was demonstrated in all six localities surveyed, except of Shendi (Table 7).

Table 6. Overall sero-prevalence of PPRV antibodies in sheep and goats sera in localities of River Nile State.

	Sheep and goats sera		
Locality	No. tested (%)	No. +ve (%)	No. -ve (%)
Atbara	96 (100.0%)	91 (94.8%)	5 (5.2%)
Ad-Damar	254 (100.0%)	251 (98.8%)	3 (1.2%)
Shendi	15 (100.0%)	15 (100.0%)	0 (0.0%)
Berber	39 (100.0%)	39 (100.0%)	0 (0.0%)
Elmatama	40 (100.0%)	36 (90.0%)	4 (10.0%)
Abu Hamad and Elbohira	39 (100.0%)	38 (97.4%)	1 (2.6%)
Total	483 (100.0%)	470 (97.3%)	13 (2.7%)

Table 7. Sero-prevalence of PPRV antibodies in sheep or goats sera in localities of River Nile State.

	Animal species					
	Sheep sera			Goats sera		
Locality	No. tested (%)	No. +ve (%)	No. -ve (%)	No. tested (%)	No. +ve (%)	No. -ve (%)
Atbara	51 (100.0%)	46 (90.2%)	5 (9.8%)	45 (100.0%)	45 (100.0%)	0 (0.0%)
Ad-Damar	195 (100.0%)	192 (98.5%)	3 (1.5%)	59 (100.0%)	59 (100.0%)	0 (0.0%)
Shendi	15 (100.0%)	15 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Berber	26 (100.0%)	26 (100%)	0 (0.0%)	13 (100.0%)	13 (100.0%)	0 (0.0%)
Elmatama	32 (100.0%)	28 (87.5%)	4 (12.5%)	8 (100.0%)	8 (100.0%)	0 (0.0%)
Abu Hamad and Elbohira	18 (100.0%)	17 (94.4%)	1 (5.6%)	21 (100.0%)	21 (100.0%)	0 (0.0%)
Total	337 (100.0%)	324 (96.1%)	13 (3.9%)	146 (100.0%)	146 (100.0%)	0 (0.0%)

Chapter IV
Discussion

Outbreaks of peste des petits ruminants (PPR), in sheep and goats, were first reported in south Gedarif, Eastern Sudan in 1971 (Elhag Ali, 1973). Initially, these outbreaks were misdiagnosed as rinderpest, due to their clinical similarities, but later confirmed as PPR (Elhag Ali and Taylor, 1984). Nowadays, PPR became an endemic disease in the Sudan. The study aimed to investigate the presence of PPR in the River Nile State, Northern Sudan. During 2018 to 2019, many suspected PPR outbreaks occurred mainly in sheep and goats in seven different localities of the River Nile State. Small ruminants of both species showed clinical signs suggestive of PPR, high morbidity and mortalities rates are similar to that described earlier (Parida *et al.,* 2015; OIE, 2019).

Sheep and goats sera were screened using the haemagglutination-inhibition (HI) assay for detection of PPRV antibodies. Results of the present study indicated that 470/483 sera were positive with an overall antibodies sero-prevalence of 97.3% among both sheep and goats while only 13 (2.7%) sera were negative. A previous sero-surveillance of PPR in River Nile and Northern States in North Sudan demonstrated a very lower sero-prevalence of 36.5% (Saeed *et al.,* 2017). A recent study conducted during 2016-2017 showed a lower sero-prevalence (90.3%) of PPRV antibodies in the River Nile State in Northern Sudan (Osman *et al.,* 2018). A similar study performed during 2018-2019 in Kordofan States, used C-ELISA assay, showed a slightly lower sero-prevalence (99.5%) of PPRV antibodies in sheep and goats sera (Ahmed, 2019). Another sero-prevalence study of PPRV antibodies demonstrated an overall sero-prevalence of 78.9% among both sheep and goats in White Nile State, Sudan (Ali Ahmed, 2019).

In contrast, a study previously demonstrated an overall antibodies sero-prevalence of 59.15% among both sheep and goats in the Sudan, the prevalence of PPRV antibodies in different States of the Sudan detected by C-ELISA had shown the lowest incidence of PPRV antibodies (33.96%) in the River Nile State (Osman *et al.,* 2009). A cross-sectional study performed during 2013 in Libya showed the lowest overall antibodies sero-prevalence of 33% (Dayhum *et al.,* 2018). Also the study show higher prevalence of PPR in sheep and goat and revealed that sheep and goats are susceptible to PPRV infection.

In the present study and considering the species basis, of the tested sheep sera 324/337 (96.1%) were positive while 13/337 (3.9%) were negative. In contrast, all the tested goats sera 146/146 (100%) were positive. Indeed, goat's sera yielded higher sero-

prevalence values compared to sheep sera. Results revealed that goats are more susceptible to PPRV infection which is supported by the fact that most of PPR outbreaks occurred in the River Nile State were associated with higher morbidity rates among goats (62.0%, ranged between 80.0% and 33.3%) than sheep (21.7%, ranged between 58.3% and 10.5%). Similarly, the mortality rates is higher among goats (38.0%, ranged between 43.3% and 13.3%) than sheep (12.8%, ranged between 36.7% and 8.0%) at age category 1-2 months. This study is in disagreement with a recently performed study in the Sudan reported that sheep sera yielded the higher (84.5%) antibodies sero-prevalence compared to goats sera which yielded a lower (66.1%) antibodies sero-prevalence (Osman *et al.,* 2018). A study conducted during 2008-2012, showed lower sero-prevalences of PPR in the Sudan in sheep (67.1%) and goats (48.2%) (Saeed *et al.,* 2017). In contrast, a relatively similar sero-prevalence of PPRV antibodies in sheep (62.9%) and goats (59.7%) were reported in different States (Blue Nile, Gedarif and North Kordofan) of the Sudan (Abdalla *et al.,* 2012). The least sero-prevalence in sheep (25.6%) and goats (18.8%) of PPRV antibodies were detected during 2008 in Marawi, Northern State (Enan *et al.,* 2013).

Dhar *et al.* (2002) and Ozkul *et al.* (2002) showed higher sero-prevalence of PPR in goats than in sheep which is in agreement with our observation. Similarly, Swai *et al.* (2009) reported a sero-prevalence of 49.5% and 39.8% in goats and sheep, respectively, in northern part of Tanzania. Abraham *et al.* (2005) also confirmed that goats react more severely to PPR virus exposure compared to sheep and suffer severe clinical cases than sheep. The same trend was observed by Khan *et al.* (2007) in Pakistan, where goats had higher sero-positivity (51.2%) than sheep (39.0%). These results agree with a study in Ethiopia which reported a sero-prevalence of 67% in goats and 33% in sheep (Roeder *et al.,* 1994), but disagree with another study a decade later found the sero-prevalence to be 9% in goats and 13% in sheep (Abraham *et al.,* 2005).

In fact, 52 outbreaks had been reported in the River Nile State during 2007 to 2018 (OIE-WAHIS, 2019). The disease may be introduced from neighboring States by mixing with animal flocks from others states during seasonal animal movement to Al-Butana rangelands were they spend a long time yearly. Appearance of PPR outbreaks might be due to lack of vaccination in some areas.

Our findings revealed that haemagglutination-inhibition (HI) test is useful in detecting PPRV antibodies showing its simplicity and rapidity. Thus, HI test can be used, as an alternative to the highly expensive competitive ELISA, for serological diagnosis of PPR

in developing countries as had been described in the only report by Wosu and Ezeibe (1992). A study for comparative evaluation of HI test with C-ELISA for sero-diagnosis of PPR showed higher degrees of relative sensitivity (76.8%) and specificity (95.4%) (Mahajan *et al.*, 2013). They suggested HI test as an alternative for C-ELISA for detecting PPRV antibodies and for screening studies. In contrast to C-ELISA, HI test has a higher sensitivity for detection of PPRV antibodies in goats (78.7%) compared to sheep (75.0%). Therefore, the relative sensitivity and specificity of the HI test was species-specific (Mahajan *et al.*, 2013). A recent study compared between HI and C-ELISA, showed 81.22% and 77.69% relative sensitivity and 94.44% and 95.73% relative specificity of HI and C-ELISA (Islam *et al.*, 2018), respectively. Islam *et al.* (2018) and Mahajan *et al.*, (2013) confirmed that the HI test is cheaper, less time consuming and more significant than the C-ELISA for serological diagnosis of PPR especially in developing countries where sophisticated facilities and laboratory equipment are not available.

Conclusion and Recommendations

Conclusion:

Outbreaks of the disease were reported in the River Nile State, Northern Sudan, although it is considered as a disease free area. Screening of sheep and goats sera from these suspected outbreaks revealed higher sero-prevalence of PPRV antibodies among both sheep and goats. Using the haemagglutination-inhibition (HI) test, high titers of PPRV antibodies were detected in sheep and goats sera in all localities in the River Nile State where outbreaks occurred. The higher sero-prevalence values achieved in this study indicated the wide exposure of these animals to PPRV and the wide distribution of the disease in the River Nile State.

Haemagglutination-inhibition (HI) test, is a very cheap, simple, useful, and quick method for detection and determining the antibody titers of PPRV antibodies in animals sera. HI test can be used, as an alternative to the highly expensive competitive ELISA, for serological diagnosis of PPR in developing countries.

Recommendations:

1- To prevent occurrence of PPR, it is recommended to use the PPRV vaccination programs using a plan for covering all parts of the River Nile State.
2- Perform effective specific sero surveillance systems and monitoring of animals in the country.
3- Control of animal movement from endemic areas by established more veterinary check points.
4- Improve diagnostic techniques and veterinary services.
5- Launch an effective extension and communication campaign.
6- Provide the necessary needs for researchers to expand their studies in this field.

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Appendix

Preparation of buffers and solutions:

Deionized Distilled Water (DDW):

Water was distilled by passing through the water distillation device. Then the distilled water was deionized by passing through the Ionic equation device. Deionized Distilled Water (DDW) was sterilized by autoclave at 120°C for 30 minutes.

Phosphate Buffer Saline (PBS), pH 7.2-7.4:

One PBS tablet was dissolved in 100 ml of deionized, distilled, sterilized water. The solution was sterilized by autoclave at 120°C for 30-60 minutes then stored at 4°C. Antibiotics (Penicillin and Gentamycin) and antifungal (Mycostatin) were added just before use, stored at 4°C.

Phosphate Buffer Saline (PBS), pH 6.8:

After preparation of PBS, the pH was adjusted to 6.8 using drops of diluted HCl, stored at 4°C.

Alsever's Solution (Anticoagulant):

D-glucose	20.5 g
NaCl	4.2 g
Na citrate	8.0 g
Citric acid	0.55 g
DDW completed to	1 L

The solution was sterilized by autoclave at 120°C for 30 minutes.

Preparation of antibiotic for buffers:

Penicillin/Streptomycin (Final concentration 200,000 IU/ml / 100µg/ml):

Benzyle Penicillin powder	2,000,000 I.U (2 vials)
Streptomycin powder	1 gm (1 vial)
DDW completed to	10 mL

The antibiotics powder was dissolved in DDW, transferred into universal bottle then mixed well by shaking. The prepared solution contains 200,000 IU/ml Penicillin and 100 µg/ml Streptomycin and stored at -20°C.

Gentamycin (Final concentration 10,000 µg/ml)

Gentamycin	2 mL (80 mg) (1 ampule)
DDW completed to	6 mL

Gentamycin solution, in one ampule, was transferred into universal bottle then mixed well by shaking with DDW. The prepared solution contains 10,000 µg Gentamycin per 1 ml, stored at -20°C.

Mycostatin (Final concentration 50,000 IU/ml):

One vial of Mycostatin containing 500,000 units was dissolved in 10 ml of Sterile DDW, stored at -20ºC.