Sudan University of Science and Technology

College of Graduate Studies

Sero-prevalence and Assessment of Factors for the Spread of Paste des Petites Ruminants among Cattle in River Nile State, Sudan

الإنتشارالمصلي وتحديد عوامل إنتشارمرض طاعون المجترات الصغيرة في الابقار 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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November, 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 November 2018 to November 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
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Dr. Nussieba Ahmed Osman Elhag
Supervisor
November, 2020

Dedication

To my beloved ones

My Dear mother

My Dear Husband

My daughter and my son

My dear friends

Thank you for supporting me in order to succeed in this work

Rabaa

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Abstract

The present study was conducted between April and October 2019 to investigate the presence of peste des petits ruminant virus (PPRV) antibodies among cattle populations in the River Nile State where PPR is known as endemic in small ruminant populations. In order to investigate the sero-prevalence of PPRV antibodies, sera were collected from four localities in the River Nile State namely Atbara, Ad-Damar, Berber and Elmatama and tested by the haemagglutination-inhibition (HI) test for detection and quantification of PPRV antibodies. Results revealed that from the total 517 cattle sera tested, 267 sera were reacted positive in the HI test with an overall antibodies sero-prevalence of 51.7% whereas 250 sera (48.3%) were reacted negative. Moreover, the HI titres for the tested sera ranged from 2 and 128 haemagglutination-inhibition units (HAI) with mean titre of 9.2 HAI. Sera with HI titers higher than or equal to 8 were considered as positive and protective. Of note, one sera (0.2%) only showed high haemagglutination-inhibition titre of 128 HAI, 135 (26.1%) sera achieved titre of 8 HAI, 89 (17.2%) sera achieved titre of 16 HAI, 35 (6.8%) sera achieved titre of 32 HAI, 7 (1.4%) sera achieved titre of 64 HAI. Obviously, most of the samples (135, 26.1%) achieved HI titre of 8. Within localities of the River Nile State, the highest overall sero-prevalence of PPRV antibodies among cattle was demonstrated in Atbara locality (67.9%, 214/315 sera) whereas the least sero-prevalence was demonstrated in Berber locality (20.3%, 12/59 sera).

Moreover, factors that affecting sero-prevalence of PPRV antibodies among cattle such as presence of close contact with sheep and goats, cattle age and breed, were investigated. Considering the effect of contact with sheep and goats, the highest sero-prevalence of PPRV antibodies was demonstrated among cattle came in close contact with sheep and goats (45.3%) compared to cattle not in contact with sheep and goats (6.4%). Considering the effect of cattle age, the highest sero-prevalence of PPRV antibodies was demonstrated among adult cattle (42.9%) compared to young cattle (8.7%). Considering the effect of cattle breed, the highest sero-prevalence of PPRV antibodies was demonstrated among Friesian cattle (41.8%) followed by Butana cattle (9.4%) and lastly Holstein cattle (0.4%).

ملخص البحث

أجريت الدراسة الحالية في الفترة من ابريل إلى أكتوبر2019م للتقصي عن وجود الأجسام المضادة لفيروس مرض طاعون المجترات الصغيرة بين قطعان الأبقارفي ولاية نهر النيل حيث يعتبر مرض طاعون المجترات الصغيرة مستوطن. للتقصي عن الإنتشار المصلي للأجسام المضادة لفيروس مرض طاعون المجترات الصغيرة ، تم جمع عينات المصل من أربعة محليات في ولاية نهر النيل وهي عطبرة ، الدامر، بربر والمتمةوتم إختبارها بإستخدام اختبار تثبيط تلازن كريات الدم الحمراء للكشف عن وجود وحساب كمية الأجسام المضادة لفيروس طاعون المجترات الصغيرة.

اوضحت النتائج أن من مجموع 517 عينة مصل ابقار والتي تم إختبارها فإن 267 عينة قد أعطت نتائج إيجابية في إختبار تثبيط تلازن كريات الدم الحمراء بنسبة إنتشار مصلي عام 51.7% ، فيحين أن 250عينة مصل (48.3%) أعطت نتائج سلبية. بالإضافة فقد تراوح حساب معيار الفيروس للعينات الموجبة بين 2و128 وحدة تثبيط لتلازن الدم بمتوسط 9.2 وحدة تثبيط لتلازن الدم. عينات المصل والتي لها حساب معيار الاجسام المضادة اكثر من او مساوياً ل8 فتعتبر موجبة وتشكل حماية. الجدير بالذكر أن عينة مصل واحدة فقط (0.2%) قد أعطت معيار فيروس عالي وصل إلي 128 وحدة تثبيط لتلازن الدم،بينما 135 عينة مصل (26.1%)أعطت معيار فيروس وصل حتى 8 وحدة تثبيط لتلازن الدم ، 89 عينة مصل (17.2%) أعطت معيار فيروس وصل حتى 16 وحدة تثبيط لتلازن الدم ، و35 عينة مصل (6.8%) أعطت معيار فيروس وصل حتى 32 وحدة تثبيط لتلازن الدم ، و7 عينات مصل (1.4%) أعطت معيار فيروس وصل حتى 64 وحدة تثبيط لتلازن الدم. واتضح جلياً أن معظم العينات قد أعطت معيار فيروس 8 وحدة تثبيط لتلازن الدم. بين محليات ولاية نهر النيل فقد أوضح الفحص المصلي عن وجود اعلي معدل عام للإنتشار المصلي للأجسام المضادة لفيروس طاعون المجترات الصغيرة في محلية عطبرة (67.9% ، 214/315) بينما كانت اقل نسبة في محلية بربر (20.3% ، 12/59).

بالإضافة فقد تم التقصي عن العوامل التي تؤثر علي الإنتشار المصلي لفيروس طاعون المجترات الصغيرة بين الابقار كتأثير الإحتكاك اللصيق مع الضأن والماعز ، عمر الابقار وسلالتها. باعتبار تأثير احتكاك الأبقار مع الضأن والماعز فقد وجدت اعلي نسبة لوجود الأجسام المضادة لفيروس طاعون المجترات الصغيرة في الأبقار التي لها احتكاك مباشر مع الضأن والماعز (45.3%) مقارنة مع الأبقار التي ليس لها احتكاك (6.4%). وباعتبار تأثير العمر فقد وجدت اعلي نسبة لوجود الأجسام المضادة لفيروس طاعون المجترات الصغيرة في الأبقار الكبيرة العمر (42.9%) مقارنة مع الأبقار الصغيرة العمر (8.7%). وباعتبار تاثير نوع السلالة فقد وجد أن نسبة وجود الأجسام المضادة لفيروس طاعون المجترات الصغيرة كانت عالية في الأبقار الفريزيان (41.8%) تليها أبقار البطانة (9.4%) واخيرا أبقار الهوليشتاين (0.4%).

Introduction

Peste des petits ruminants (PPR) is a highly contagious, notifiable and transboundary viral disease (OIE, 2019; Amarasinghe *et al.,* 2019) caused by peste des petits ruminants virus (PPRV), a member of the *Small Ruminant Morbillivirus* (SRMV)species, *Morbillivirus* genus, within the *Orthoparamyxovirinae* subfamily, the *Paramyxoviridae* family in the order *Mononegavirales*(Amarasinghe *et al.,* 2019; Maes *et al.,* 2019).

PPR is characterized by severe morbidity and mortality rates leading to high economic impact in endemic areas of Africa, the Middle East, and Asia, where small ruminants contribute to guaranteeing livelihoods (Banyard *et al.,* 2014; OIE, 2019). Affected animals presented high fever, depression, along with eye and nose discharges, animals cannot eat, as the mouth becomes covered in painful erosive lesions and the animals suffer from severe pneumonia, diarrhoea and finally death (Ozkul *et al.,* 2002; Pope *et al.,* 2013; Wernike *et al.,* 2014).

The disease was first reported in the Ivory-Coast in 1942 (Gargadennec and Lalanne, 1942). Since then, the disease had fast dissemination towards the east, till it becomes present in over 70 countries across Asia, Africa, the Middle-East and few parts of Europe (OIE, 2019).

Small ruminants "sheep and goats" are the natural usual/typical hosts for PPRV (Lefevre and Diallo 1990; Parida *et al.,* 2015) beside some wild relatives of domesticated small ruminants (Rahman *et al.,* 2018; 2020). However, various species of domesticated large ruminants "camels, cattle and buffaloes" and wild large ruminants are recognized as unusual or atypical hosts for PPRV (Rahman *et al.,* 2020). Cattle are known as naturally subclinically infected by PPRV with demonstrated seroconversion (Lembo *et al.,* 2013; Abubakar *et al.,* 2017; Ali *et al.,* 2019). Cattle experimentally infected with the wild-type PPRV did not develop any clinical signs and did not transmit PPRV to susceptible in-contact animals (Sen *et al.,* 2014; Couacy-Hymann *et al.,* 2019; Schulz *et al.,* 2019). Therefore, cattle are recognized as dead-end hosts for PPRV and do not play any potential role in the epidemiology and spread of PPRV (Couacy-Hymann *et al.,* 2019; Schulz *et al.,* 2019).

PPR is endemic in the Sudan since it first emergence as a rinderpest-like outbreak in sheep and goats, in the southern part of Gedarif near Dinder River, Eastern Sudan in 1971 (Elhag Ali, 1973) followed by other outbreaks in Sinnar and El Mieliq, central

Sudan in 1972 (Elhag Ali and Taylor, 1984). The virus caused these outbreaks was confirmed as PPRV (Elhag Ali and Taylor, 1984).

In recent years, outbreaks of PPR had been reported from all over the country with at least 52 outbreaks had been reported from the River Nile State during 2007 to 2018 (OIE-WAHIS, 2019). These outbreaks indicated that PPR is endemic in small ruminants in the River Nile State. Sero-prevalence of PPRV antibodies was reported earlier in cattle (Haroun *et al.,* 2002; Saeed *et al.,* 2017). Recently, higher sero-prevalence of PPRV antibodies in apparently healthy cattle was demonstrated in five states of the Sudan during 2015-2016 (Ali *et al.,* 2019).

Objectives:

The present study was designed to achieve the following aims:

1. To investigate the presence of peste des petits ruminants (PPR) among cattle population in the River Nile State, Northern Sudan.
2. To perform serological detection for antibodies against PPRV to demonstrate the sero-prevalence among cattle in the River Nile State, Northern Sudan.
3. To assess the sero-prevalence of PPRV antibodies in cattle in four localities in the River Nile State namely Atbara, Ad-Damar, Berber and Elmatama.
4. To assess some factors that affecting the sero-prevalence of PPRV antibodies in cattle.

Chapter I
Literature Review

1.1. Definition of PPR:

Peste des petits ruminants (PPR), is an acute, contagious and fatal disease affects mainly sheep, goats, camels and wild small ruminants (Kwiatek *et al.,* 2011). The disease has also been reported on a few occasions in cattle and buffaloes (Balamurugan *et al.,* 2012). PPR usually causes high rates of illness and deaths (Diallo, 2003; Wang *et al.,* 2009).

1.2. Economic importance of PPR:

PPR is considered as one of the main constraints in improving the productivity of the small ruminants in endemic developing areas. The disease has a high economic importance because of its high mortality (100%) and morbidity (90%) rates among susceptible animals, especially young animals, leading to restrictions on the livestock trading (Rossiter and Taylor, 1994).

1.3. History of the disease:

Peste des petits ruminants virus (PPRV) was identified for the first time in West Africa in the early 1940s (Gargadennec and Lalanne, 1942). Based on the clinical picture, at first the causative virus was thought to be a variant of rinderpest virus (RPV) adapted to small ruminants, however, it was later known as PPRV, a distinct virus in the *Morbillivirus* genus (Gibbs *et al.,* 1979; Diallo *et al.,* 1989). The disease was thereafter spread to neighbouring African countries including Nigeria, Senegal and Ghana (Balamurugan *et al.,* 2014). The distribution of the disease was clearly understood starting from the late 1980s onwards after the development of specific diagnostic tools (Diallo *et al.,* 1995). PPR first appeared as a recognized disease in Asia in the late 1980s (Shaila *et al.,* 1989; 1996). In 1987, the disease appeared in the Middle East and has since then been confirmed in Jordan (Lefevre *et al.,* 1991), Pakistan (Amjad *et al.,* 1996), Southern India (Shaila *et al.,* 1989; Nanda *et al.,* 1996), and Israel (Perl *et al.,* 1994). PPR outbreaks occurred between 2007 and 2009 in Uganda, Kenya and Tanzania (OIE-WAHIS, 2009).

In North Africa, outbreaks of PPRV were reported for the first time in Morocco (Sanz-Alvarez *et al.,* 2008) followed by Tunisia (Sghaier *et al.,* 2014) and Algeria (De Nardi *et al.,* 2012). In recent years PPRV has extended southwards in Africa as far as southern Tanzania, Zambia, the Democratic Republic of Congo and Angola (Parida *et al.,* 2016).
In East Asia, the virus reported in Tibet, China in 2007 and has recently been reported all over China (Liu *et al.,* 2018).
In Europe, PPR has described early in Turkey and recently in Bulgaria (OIE-WAHIS, 2018; OIE, 2019).

1.4. Causative agent:

1.4.1. Virus classification:

All members of the genus *Morbillivirus*, within the family *Paramyxoviridae* in the order *Mononegavirales*, are responsible for some of the most devastating humans and animals diseases (Gibbs *et al.,* 1979; Maes *et al.,* 2019). Measles virus (MV) is considered the type-virus for this genus, the name of which is derived from *morbilli*, the diminutive from of the Latin word of plague (morbus). This name was originally used to distinguish measles from smallpox and scarlet fever, which in former times were considered more serious diseases (Barrett *et al.,* 2006). In addition to MV other morbilliviruses that infect terrestrial mammals include rinderpest virus (RPV), which causes cattle plague, peste des petits ruminants virus (PPRV), the cause of sheep and goat plague, and canine distemper virus (CDV) which infects many carnivore species, including domestic dogs, mink and ferrets and can have serious consequences when endangered wildlife species are threatened (Barrett *et al.,* 2006).
In clinical terms, PPR is very similar to bovine rinderpest (RP). Both viruses are antigenically closely related and belong to the *Morbillivirus* genus of the family *Paramyxoviridae* (Gibbs *et al.,* 1979).

1.4.2. Virus replication:

The viral infection begins with the liberation of the nucleo-capsid into the cytoplasm of the cell due to the fusion of the cellular and viral membranes. Then viral RNA polymerase

starts to transcribe the genome into messenger RNA (mRNA) in the order of arrangement of the genes, N_P/C/V_M_F_H_L (from the 3 extremity of the genomic RNA to its 5 extremity) (Bailey*et al.,*2005). These mRNAs are translated into different proteins by the enzymes of the target cell, each messenger gives rise to one protein, the second one, however, is translated into two reading frames resulting in structural P protein and non-structural C and V proteins (MacLachlan and Dubovi, 2011). Once the concentration of the viral proteins reaches a sufficient amount, polymerase turns to the synthesis of new genomes, each of which is surrounded by N, P and L proteins to form nucleo-capsids. These migrate to a region of the membrane where M, F and H proteins are already present. At this site a bud forms and expands until it detaches from the target cell as a mature virus (Lefevre and Diallo, 1990). The budding process starts at 12 h after infection of sheep cells by PPRV and continues until the seventh day. Its importance drops with the development of syncytia (Laurent, 1968).

1.4.3. Virus properties: size and morphology:

Morbilliviruses are enveloped pleomorphic particles, with single-stranded negative sense RNA genome (Haffar *et al.,* 1999) with virion sizes ranging from 350 to 600 nm.

1.4.4. Genome organization and viral proteins:

Morbilliviruses have two known non-structural proteins (C) and (V) beside six structural proteins (Haffar *et al.,* 1999) namely the large protein (L), the haemagglutinin (H), the phosphoprotein (P), the nucleo-capsid protein (N), the fusion (F), and the matrix protein (M) (Bailey *et al.,* 2005). Their genome, is a single-stranded RNA, about 16000 nucleotides (nt) long, that has to be transcribed by their own polymerase into at least six mRNAs and each mRNA species is translated into one structural protein, while a part of P which is read in two different frames leads to P, V and C non-structural proteins (MacLachlan and Dubovi, 2011).

1.5. Epidemiology of the disease:

1.5.1. Geographic distribution:

PPR was first reported in the Ivory Coast, West Africa (Gargadennec and Lalanne, 1942) and later from other parts of the world namely sub-Saharan Africa, the Middle East and the Indian subcontinent (Banyard *et al.,* 2010; OIE, 2019). PPR is reported as endemic in several countries in Africa (except southern countries), the Arabian Peninsula, throughout most of the Near East and Middle East, and in the Central and South East Asia (Banyard *et al.,* 2010; OIE, 2019) (Figure 1).

1.5.2. Lineage identification of PPRV:

PPRV is genetically grouped into four lineages (I, II, III, and IV) based on the F and N gene sequences analyses (Shaila *et al.,* 1996; Dhar *et al.,* 2002; Kerur *et al.,* 2008; Balamurugan *et al.,* 2010). Lineages (I-III) were known to circulate in Africa, while lineage IV was generally found in Asia with recent incursion in Africa (Banyard *et al.,* 2010; Kwiatek *et al.,* 2011). The spread of disease to a number of new countries in Africa and Asia with involvement of various lineage of PPRV is a cause of global concern especially recent introduction of Asian lineage IV in some African countries and presence of PPR in Europe through Western Turkey and Bulgaria (Albina *et al.,* 2013; Badnyard *et al.,* 2010; Kwiatek *et al.,* 2011; OIE-WAHIS, 2018).

OIE Members' official peste des petits ruminants status map

Last update May 2020

Click on a specific region to zoom in

© OIE 2020

Members and zone recognised as free from PPR

Suspension of PPR free status

Countries and zone without an OIE official status for PPR

Figure 1.Official peste des petits ruminants status map. World Organization for Animal Health (OIE, 2020):http://www.oie.int/en/animal-health-in-the-world/ppr-portal/distribution/

1.6. PPR in the Sudan:

The first report of an outbreak of a rindepest-like disease in sheep and goats in Sudan was in 1971 in the southern part of Gedarif State near Dindir River (Elhag Ali, 1973). The disease was diagnosed as Rinderpest (RP) based on clinical signs; however, RPV preciptinogens were demonstrated by agar gel precipitation test (AGPT) (Elhag Ali, 1973). Later virus isolation from these samples with its ability to cause a disease in sheep and goats confirmed that the causative agent was PPRV (Elhag Ali and Taylor,1984). The disease was then reported from the Sinnar area in central Sudan during 1971-1972 and in Mieliq areas in western Sudan in 1972, from goats and sheep, respectively (Elhag Ali and Taylor, 1984). The PPRV isolates were termed as SUD 72/1 (Sinnar) and SUD 72/2 (Meilig) (Elhag Ali and Taylor, 1984). After these reports, the disease remained endemic in Sudan. Other outbreaks of PPR were reported during 1989-1990 in Elhilalia in Gezira State in central Sudan (El Hassan *et al.,* 1994), and Elfashir in North Darfur (Rasheed, 1992).

Sero-surveillance results demonstrated the prevalence of the disease in Khartoum (Zeidan, 1994; Saeed *et al.,* 2010), Southern Sudan, Khartoum, Gezira, River Nile, Kordofan Kassala and Gedarif States (Osman, 2005; Osman *et al.,* 2009; Saeed *et al.,* 2010; Shuaib *et al.,* 2014). Outbreaks of PPR in sheep and goats were reported between 2008 and 2009 in White Nile State (Ishag *et al.,* 2015). The existence of PPR in camels in the Sudan was reported (Khalafalla *et al.,* 2010) and they successfully isolated the virus from a camel population. During the years 2016-2017, several suspected PPR outbreaks involved mainly sheep and to lesser extent goats were reported in different parts of the country including Northern, River Nile, Gezira and Khartoum States (Osman *et al.,* 2018).

The prevalence of PPRV antibodies among cattle in five different states in the Sudan was investigated during 2015–2016. Of note, higher overall sero-prevalence of PPRV antibodies (42.0%) was reported among cattle populations in the Sudan (Ali *et al.,* 2019).

PPRV outbreaks were reported in Dorcas gazelles (*gazelle dorcas*) in the Sudan during 2016-2017 which may influence the epidemiology of PPR in the Sudan (Asil *et al.,* 2019).

1.7. Host range:

PPR affects small ruminants and it is not a zoonosis disease. Goats have been reported to be more susceptible than sheep (Nanda *et al.,* 1996), but this has not been confirmed in other outbreaks (Singh *et al.,* 2014). West African goats have been found to be more susceptible than European varieties, and within the former group, the dwarf varieties were most susceptible to the disease (Couacy-Hymann *et al.,* 2007).

PPRV was isolated from an outbreak of a rinderpest-like disease in buffaloes in India (Govindarajan *et al.,* 1997). Cattle and pigs can be infected with the virus, but this is only detected by the presence of specific antibodies, while the animals show no clinical signs (Nawathe and Taylor, 1979; Anderson and Mckay, 1994; Lembo *et al.,* 2013). It is not known if these species could spread the virus or have viraemia.

Camels are considered susceptible to PPR but this is still to be clarified by experimental infection. Antibodies to PPRV as well as PPRV antigen and nucleic acid were detected in some samples from an epizootic disease that affected one humped camels in Ethiopia and Sudan (Abraham *et al.,* 2005; Roger *et al.,* 2000; 2001; Khalafalla *et al.,* 2010).

PPR can infect wild sheep such as bharals in Tibet and in wild goats in Kurdistan (Bao *et al.,* 2011; Hoffmann *et al.,* 2012). Cases of a clinical disease have been reported in wildlife resulting in deaths of wild small ruminants (Kinne *et al.,* 2010; Abubakar *et al.,* 2011; Bao *et al.,* 2011). Recently, an outbreak of PPR in truly free-ranging Sindh ibex was confirmed in Pakistan with many deaths possibly associated with a presumed infected goat herd (Abubakar *et al.,* 2011). The role of wild species as a reservoir has not been studied. However, it might be possible considering the role of wildlife in the epidemiology of rinderpest (Couacy-Hymann *et al.,* 2005; Kock *et al.,* 2006; Rossiter *et al.,* 2006).

1.8. Transmission of PPR:

Virus transmission is essentially by direct contact with infected animals often through the inhalation of infective droplets, however, the virus is known to be excreted by large amount in eye and nasal discharges but to a lesser extent in urine and faecal matter (Lefevre and Diallo, 1990; OIE, 2019).Fine infected droplets are released into the air when infected animals cough and sneeze (Taylor, 1984; Bundza *et al.,* 1988). Animals in close contact inhale the droplets then become infected (cited in Khan *et al.,* 2008). The virus may be spread over large distances through movement of infected animals

specially those animals incubating the disease without any observed clinical signs (Parida *et al.,* 2015).

1.9. Clinical signs:

The clinical disease is usually acute and characterized by pyrexia, serous ocular and nasal discharges, erosive lesions on different mucous membranes particularly in the mouth, diarrhoea and pneumonia (OIE, 2019).
The incubation period is typically 4-6 days, but may range between 3 and 10 days. The clinical disease is acute, with pyrexia up to 41°C that can last for 3-5 days, the animals become depressed, anorexia and develop a dry muzzle (Parida *et al.,* 2015). Serous oculo-nasal discharges become progressively mucopurulent and, if death dose not ensue, persist for around 14 days (Parida *et al.,* 2015). Within 4 days of the onset of fever, the gums become hyperaemic, and erosive lesions develop in the oral cavity with excessive salivation. These lesions may become necrotic. Watery blood-stained diarrhoea is common in the later stage of the disease, pneumonia, coughing, pleural rales and abdominal breathing may also occur. The morbidity rate can be up to 100% with very high case fatality in severe cases (Radostitis *et al.,* 2007).

1.10. Post-mortem lesions:

The lesions are very similar to those observed in cattle affected with rinderpest, except that, prominent crusty scabs along the outer lips and severe interstitial pneumonia frequently occur with PPR (Chauhan *et al.,* 2009). Erosive lesions may extend from the mouth to the reticulo-rumen junction. Characteristic linear red areas of congestion or haemorrhage, zebra stripes, may occur along the longitudinal mucosal folds of the large intestine and rectum but they are not a consistent finding(Chauhan *et al.,* 2009). Erosive or haemorrhagic enteritis is usually present and the ileo-caecal junction is commonly involved. Peyer’s patches may be necrotic. Lymph nodes are enlarged, and the spleen and liver may show necrotic lesions (Lefevre and Diallo, 1990; OIE, 2019).

1.11. Diagnosis of PPR:

For an effective implementation of control measures for PPR, the diagnosis of the disease should be made as quickly as possible, and requires specific and sensitive methods for diagnosis to contain outbreaks and minimize economic losses (Rahman *et*

al., 2004; Bruning-Richardson *et al.,* 2011). Peste des petits ruminants can be confused with other diseases including rinderpest, bluetongue and contagious caprine pleuropneumonia, due to the similarity of these diseases in clinical signs. Diagnosis of the disease may also be complicated, as the result of secondary bacterial infections specifically caused by Mannheimia haemolytica (OIE, 2019).

Diagnosis of PPR is tentatively done based onclinical examination, gross pathology, and histological findings and confirmed by laboratory tests (Rahman *et al.,* 2004; Bruning-Richardson *et al.,* 2011; OIE, 2019).

For the diagnosis of PPR, the laboratory tests currently available can be grouped into those detecting the PPR virus or viral antigen such as virus isolation, immuno-capture ELISA and lateral flow devices (Brindha *et al.,* 2001; OIE, 2019), those detecting the genetic material of the virus such as RT-PCR, real-time PCR and LAMP PCR (OIE, 2019) and those detecting antibodies against the virus like virus neutralization test, immuno-capture enzyme-linked immunosorbent assay (IC-ELISA) which include competitive ELISAs and indirect ELISAs (Libeau *et al.,* 1994; Khan *et al.,* 2007; Singh *et al.,* 2004; Abubakar *et al.,* 2008; Saravanan *et al.,* 2008).

1.11.1. Methods for serological diagnosis:

There are various serological tests that applied in detection of PPRV antibodies include virus neutralization and competitive enzyme-linked immunosorbent assay (c-ELISA) (Singh *et al.,* 2004; Ezeibe *et al.,* 2008; OIE, 2019). Both tests can distinguish PPRV from rinderpest. In addition, older serological tests for diagnosis of PPR include haemagglutination-inhibition (HI) test (Wosu and Ezeibe, 1992).

1.11.1.1. Competitive enzyme-linked immunosorbent assay (C-ELISA):

For PPR antibody detection, competitive ELISA is a better choice because it is sensitive, specific, reliable, and has a high diagnostic specificity of 99.8% and sensitivity of 90.5% (Brindha *et al.,* 2001; Choi *et al.,* 2005). C-ELISA is based on the use of the monoclonal antibosies (MAbs) raised against the nucleo-capsid protein and a recombinant nucleo-capsid protein produced in the baculovirus as the antigen (Libeau *et al.,* 1995) and the other one that based on the monoclonal antibosies (MAbs) raised against the viral attachment protein (H) and antigen consisting of purified or part-purified PPRV (vaccine strain) (Saliki *et al.,* 1993; Anderson and McKay, 1994). All

the assays work on the principle that antibodies to PPRV in the test sera can block the binding of the MAbs to the antigen.

1.11.1.2. Virus neutralization (VN) test:

Virus neutralization is considered to be the ‘gold standard’ test for diagnosis of the disease (OIE, 2019). This test is sensitive and specific, but it is time-consuming. The standard neutralization test is now usually carried out in 96-well microtitre plates using Vero cells or primary lamb kidney cells (OIE, 2019).

1.11.1.3. Haemagglutination-inhibition (HI) test:

Haemagglutination inhibition (HI) test can be employed for detection and titration of PPRV antibodies in serum samples (Osman *et al.,* 2008). The HI test is more significant for detecting PPR viral antibodies and for diagnosis of PPR in field conditions where there is no available sophisticated facilities and equipment. Moreover, the HI test is also simple, cheap and less time consuming compared with competitive ELISA (Mahajan *et al.,* 2013; Islam *et al.,* 2018). It is quick, easy, simple, reliable confirmatory test for the diagnosis of PPR and differential diagnosis of PPRV and RPV that can be used for routine screening purposes in control programs (Osman *et al.,* 2008; Mahajan *et al.,* 2013; Islam *et al.,* 2018). The HI test can be performed using red blood cells (RBCs) from different animal species such as chicken, goat and pig, however, chicken RBCs were found to be the most sensitive for detection of PPRV antigen, followed by goat then pig RBCs (Osman *et al.,* 2008).

1.12. Immunity against PPR:

Maternal passive immunity resulted in protection of the newborn for a certain period of time. Previous exposure to PPRV infection or development of protective immunity due to vaccines in pregnant dams decides the level of maternal antibodies in the colostrum. The suckling lambs acquire this passive immunity via the colostrum, which is protective for 3-4 months (Libeau *et al.,* 1992). Therefore, vaccination of newborns should be started at the age of 3 months in both kids and lambs in PPRV endemic areas (Bodjo *et al.,* 2006).

Active immunity frequently comprises both the cellular and humoral immunity that can be acquired by contracting PPRV infection or by vaccination via immunization with PPRV vaccine (Sinnathamby *et al.,* 2001).

1.13. Control of PPR:

For the proper control of PPR, there is a need of strong support of diagnostic methods and proper, timely vaccination of the susceptible populations which is the only way for control of PPR (OIE, 2019). PPRV vaccine was successfully used to control PPR in West African and other African countries. Considering the close antigenic relationship between RPV and PPRV, the live attenuated RP vaccine was used in the past for vaccination against PPR and provided a protection for a period of one year (Taylor, 1979). The first homologous PPR vaccine was developed using the live-attenuated Nigerian strain PPRV Nig 75/1 after 63 passages in Vero cells which provide a solid immunity for three years (Diallo *et al.,*1989; 2007). Several vaccine trials had been conducted during 1989-1996 which demonstrated the efficacy of this vaccine in 98,000 sheep and goats in the field. The vaccine was safe under field conditions even for pregnant animals and induced immunity in 98% of the vaccinated animals (Diallo *et al.,* 1989). The vaccinated animals did not develop any disease following challenge with virulent PPRV strains and thereby this vaccine was used worldwide, in Africa, the Middle East and Sothern Asia, for the effective control of PPR (Saravanan *et al.,* 2010).
The control of PPR can be also achieved by the implementation of the effective prophylactic measures. All the sheep and goats of the affected flocks should be placed under quarantine for at least one month after the last clinical case. Animal movements have to be strictly controlled in the area of the infection (Rossiter and Taylor, 1994). Some of other control measures or sanitary prophylaxis are strict quarantine and control of animal movements, quarantine of newly purchased or newly arriving goats/sheep for at least 2-3 weeks and know the health status and the source of any new animal(s) brought into the flock, migratory flocks are threat to local sheep and goat therefore contact may be avoided, effective cleaning and disinfection of contaminated areas of all premises with lipid solvent solutions of high or low pH and disinfectants including physical perimeters, equipment and clothing, dead animal carcasses should be burnt or buried deeply, monitor animals closely and frequently for any developing illness or signs of the disease, isolate any sick animals from the flock and contact the Veterinarian

immediately to examine the sick animals in the herd/flock, use separate facilities and staff to handle isolated animals, educate and train the employees about PPR and the signs of illness and monitoring of wild and captive animals, especially in contact with sheep and goats (OIE, 2019).

Chapter II
Materials and Methods

2.1. Materials:

Study area:

The study was conducted in River Nile State in Northern Sudan which located at the coordinates 16-22 north and longitudes 32-35east. The present study was carried out in 4 localities of the River Nile State namely Atbara, Ad-Damar, Berber and Elmatama **(Figure 2)** in order to investigate presence of of peste des petits ruminants virus (PPRV) antibodies among cattle populations.

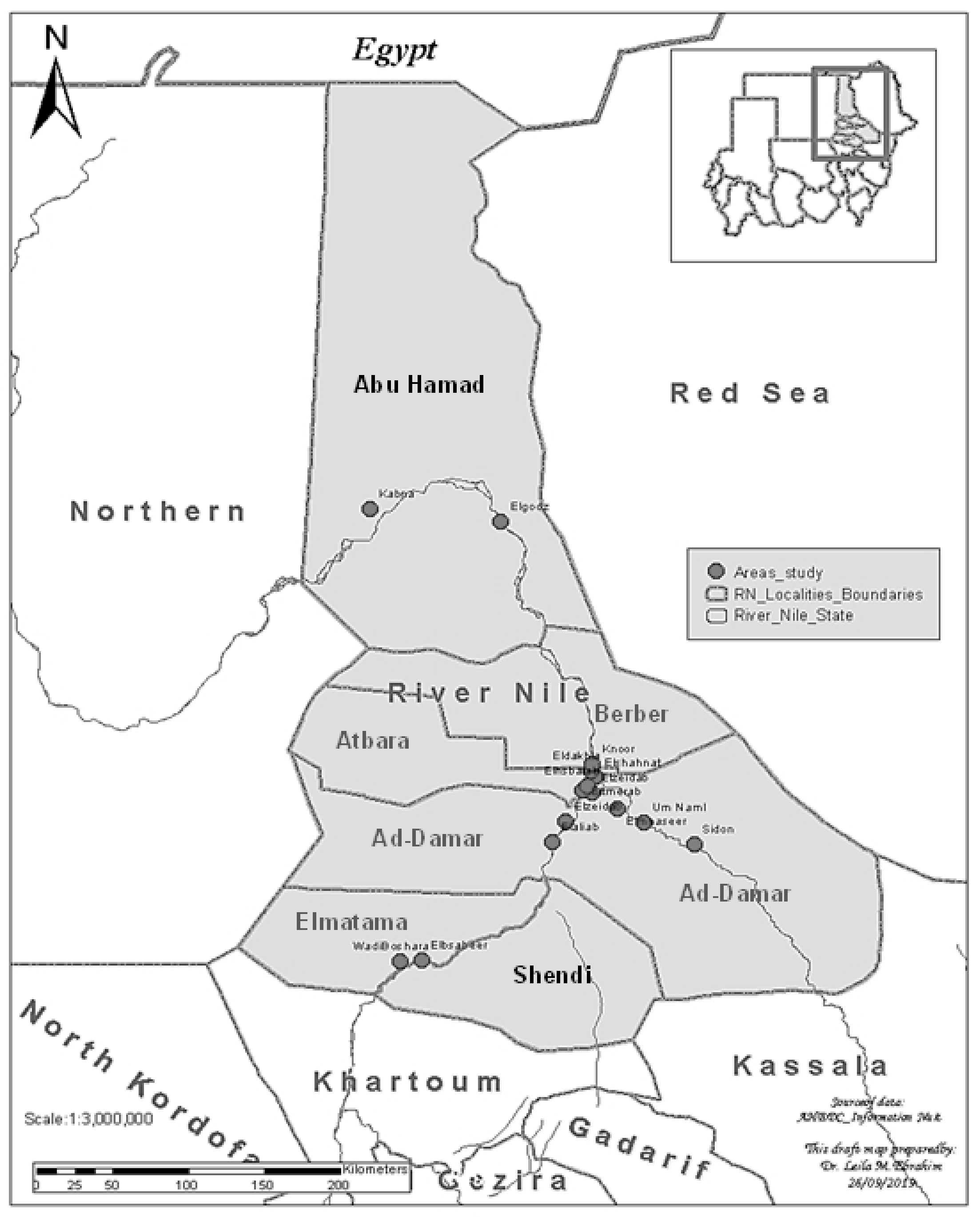

Figure 2. Map of the Sudan showing the study area in River Nile State, Ad-Damar, Atbara, Berber, and Elmatama localities were shown in dark grey colour font.

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

Antibiotics and antifungal:

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

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	-

Disposables:

Name	**Company**
Vacutainers and needles/sterile Vacuum blood collection system	**-**
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 (Sterile)	Nunc, 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	-

Preparation of buffers and solutions:

2.1.6.1. Preparation of PBS:

One tablet of phosphate buffer saline (PRB) was dissolved into 100 ml of sterilized deionized distilled water, the pH of the prepared PBS solution ranged from 7.2 to 7.4. For preparation of BPS solution of pH 6.8, the pH was adjusted using pH meter by addition of drops of diluted HCl.

Software and websites:

www.google.com
www.ncbi.nlm.nih.gov
Microsoft Office Word 2010 software
Microsoft Office Excel 2010 software was used for data analysis.

Methods:

Sample collection:

A total of 517 serum samples were collected from cattle, with different ages, sex and breeds, from River Nile State during the period from April to October 2019in order to investigate the presence of PPRV infection among cattle species. These samples were collected from 4 localities of the River Nile State including, Atbara (315 sera), Ad-Damar (66 sera), Berber (59 sera) and Elmatama (77 sera) (Table 1).

Blood samples were collected from the jugular vein of cattle by the aid of needles and plain vacutainers. Vacutainers containing blood were placed in a diagonal position into tube racks. The racks were left at room temperature for 1-2 hours then were kept overnight in a refrigerator at 4°C.

Preparation of serum samples:

On the next day, serum was separated from blood by centrifugation at 3000 rpm for 5 minutes and transferred into plain tubes by aid of single channel micropipette and blue tips, sera were stored frozen at -20°C till used.

Table1. Cattle seracollected in the River Nile State during 2019.

Date of Collection	Place of Collection		No. of Sera	Total No. of Sera
	Locality	District/Location		
April-July 2019	Atbara	Alamn Algezaye	80	315
May-June 2019		Nawawey	94	
July 2019		Alfadlab	24	
October 2019		Almawrada	15	
June-July 2019		Atbara Slaughter House	102	
July 2019	Ad-Damar	Almogran	11	66
July-September 2019		Alamn Algezaye	15	
September 2019		Alesheer	14	
September 2019		Alhassanaab	26	
July 2019	Berber	Dar Maly	9	59
October 2019		Algadawab	50	
August 2019	Elmatama	Alnorab	77	77
Total No. of Sera				517

Preparation of antigen samples:

The virus antigen was obtained by preparing of 50% tissue suspension/homogenate from the pneumonic sheep lung samples in PBS (pH 7.2-7.4) supplemented with antibiotics and anti-fungal, into mortar and pestle using sterile scissors to grind and mince the suspected samples under cold sterile conditions inside the laminar flow safety cabinet. The minced preparation was then evacuated into 15 ml bluecap tubes and centrifuged at 4ºC at 3000 round per minutes (rpm) for 5 min. Then the supernatant was aspirated into new tubes, frozen 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.

Detection of PPRV antibodies in cattle sera:

In order to establish the haemagglutination-inhibition (HI) test for detection and quantification of PPRV antibodies in cattle sera, the haemagglutination (HA) test was performed for detection and quantification of 4 HAU of PPRV in the prepared tissue homogenate.

2.1.3.1. Collection of blood for red blood cells:

The blood used in the test was collected from the wing vein of a healthy non-immunized chicken. A volume of 2.5 ml of blood was collected into a syringe containing Alsever's solution.

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

For preparation of red blood cells (RBCs) suspension, the collected chicken whole blood was centrifuged at 1500 rpm for 10 minutes at 25°C (RT), then the plasma and buffy coat were aspirated and discarded, the remaining RBCs were washed after addition of 10 ml of PBS (pH 6.8) and gentle mixing then centrifugation at 1500 rpm for 10 minutes, the supernatant PBS was discarded and washing was repeated 3 times. Subsequently, 0.8% RBCs suspension was prepared by addition of 1 ml of washed RBCs to 125 ml of PBS (pH

6.8), the suspension was used as indicator in the HA and HI tests. The RBCs suspension was stored at 4°C and used forthe next following 5 days.

2.1.3.3. Haemagglutination (HA) test:

The haemagglutination test is used to quantify the amount of the virus in the tissue homogenate which is considered the PPRV antigen by determining its end-point dilution and viral titre. This result of the HA test for the antigen is expressed as HA titre. The end-point will be the last well that show a complete haemagglutination of RBCs and contains one haemagglutination unit (the minimum amount of the virus that will cause complete agglutination in the well). The haemagglutination titre is the reciprocal of the end-point dilution that produced 1 HA unit.

The haemagglutination (HA) test was performed as described recently (Abdalla, 2019; Alhussein, 2019). Firstly, 50 µl of 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 PPRV antigen was dispensed into well A1 then 2-fold serial dilution of the virus was prepared. Secondly, 50 µl of 0.8% RBCs suspension was added to column 1 and to column 2 which served as RBCs control. Shaking was performed using the Orbital Shaker SO3 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 results were indicated by formation of the haemagglutination sheet in the virus dilution wells while negative results in the RBCs control wells appeared as a sharp red button of sedimented RBCs.

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

2.1.3.4. Haemagglutination-inhibition (HI) test:

The procedure of the HI test was performed as described recently by Eltahir (2020). Initially, 0.25 µl of the diluent Phosphate Buffer Saline (PBS) with pH 6.8 was distributed into all wells of the V-bottom 96-well microtiter plate(Figure 3), then 0.25 µl of PPRV positive serum was added to well A1, 0.25 µl of the cattle sera to be tested were added to each well of row A from A2 to A11, Column 12 served as RBCs negative control and

received only PBS diluent and RBCs. 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 and continue mixing and transferring to the next row till row H then 25 µl volume was discarded away. After that, 0.25 µl of PPRV antigen containing 4 HAU was added to all wells of the plate except column 12 (RBCs control)(Figure 3), then shaking of the plate for mixing the contents in the wells was performed using the Orbital Shaker SO3. The HI plate was incubated overnight in a refrigerator at 4°C. 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 (15-20°C) for 14 minutes, results of the HI test were recorded by the naked eye.The end-point dilution was determined as the last well containing the red button then the HI titres of the control and tested sera were calculated as the reciprocal of the end-point dilutions and expressed as HAI.

2.1.3.4.1. Calculation and interpretation of the HI results:

The settling patterns of single and agglutinated red blood cells are different. Single cells roll down the side of the V-bottom well and settle as red button while the agglutinated cells settle as agglutinated sheet of RBCs.

Accordingly the interpretations of the HI test results were as follows:

- Positive HI result = sharp red button.
- Negative HI result = sheet.
- Red blood cells control = sharp red button.

	Control Sera (Column 1)	Tested Sera (Columns 2-11)									RBCs Control (Column 12)	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Ab dilution**
A	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:2
B	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:4
C	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:8
D	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:16
E	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:32
F	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:64
G	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:128
H	cAb	sAb1	sAb2	sAb3	sAb4	sAb5	sAb6	sAb7	sAb8	sAb9	sAb10	RBCs	1:256

Figure 3.Layout of the HI plate.

Notes:

cAb = Positive control sera (Column 1).

sAbx (1-10) = Tested Sera (Columns 2-11).

RBCs = Red blood cells (RBCs) control (Column 12).

Chapter III
Results

3.1. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in cattle sera:

The aim of this study was to investigate the presence and sero-prevalence of PPRV antibodies among cattle populations in the River Nile State where PPR is known as endemic in small ruminant populations. The presence of PPRV-specific antibodies was demonstrated in cattle by using the haemagglutination-inhibition (HI) test. The HI test was employed to assess the antibody sero-prevalence of PPRV in four localities in the River Nile State namely Atbara, Ad-Damar, Berber and Elmatama. Positive cattle sera caused inhibition of RBCs agglutination which is indicated by presence of sharp red button whereas negative cattle sera is indicated by presence of a diffused sheet or film in the HI plate(Figure 4).

Figure 4. Haemagglutination-inhibition (HI) test for detection of PPRV antibodies in cattle sera. HI test plate showing positive HI result as a red button (samples are in vertical position, columns 1-6; 8-12, red blood cells (RBCs) control (column 7) appeared as a sharp red button.

3.2. Overall antibodies sero-prevalence of PPRV by the haemagglutination-inhibition (HI) test in cattle sera:

A total of 517 cattle sera were screened for presence of PPRV antibodies using the haemagglutination-inhibition (HI) test, 267/517 cattle sera were positive with an overall antibodies sero-prevalence of 51.7% while 250/517 of cattle sera were negative with an overall antibodies sero-prevalence of 48.3% (Table 2, Figure 5).

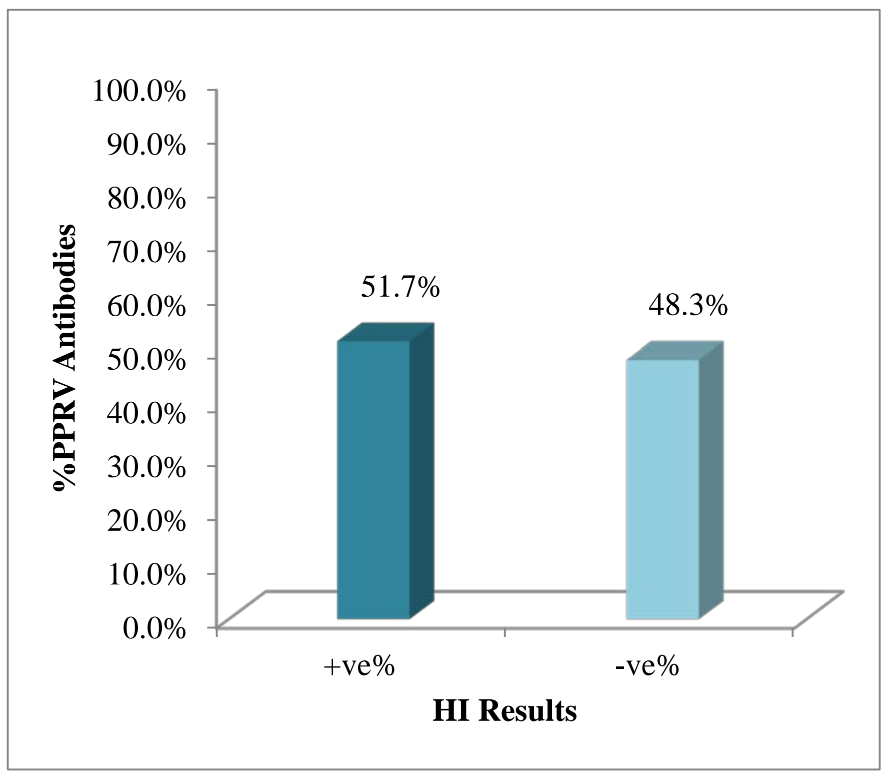

Figure 5. Positive and negative cattle sera from River Nile State using haemagglutination-inhibition (HI) test for detection of PPRV antibodies.

3.3. Positive PPRV antibodies detected in cattle sera at different haemagglutination-inhibition (HI) titres by HI test:

Results of the HI test indicated that HI titres achieved ranged between 2 and 128 haemagglutination-inhibition units (HAI) with an average titre of 9.2 HAI (Table 2). Sera with HI titers higher than or equal to 8 were considered as positive and protective. Of note, one sera (0.2%) only showed high haemagglutination-inhibition titre of 128 HAI (Table 2, Figure 6). Moreover, 135 (26.1%) sera achieved titre of 8 HAI, 89 (17.2%) sera achieved titre of 16 HAI, 35 (6.8%) sera achieved titre of 32 HAI, 7 (1.4%) sera achieved titre of 64 HAI (Table 2, Figure 6). Obviously, most of the samples (135, 26.1%) achieved HI titre of 8.

Table 2. Detection of PPRV antibodies in cattle sera by the Haemagglutination-inhibition (HI) test. Number of positive and negative sera for PPRV antibodies, end-point dilutions and number of samples at different HI titers expressed as HAI were shown.

End-point dilution	HI Titers (HAI)	Positive sera%	Negative sera%
0	0	0 (0.0%)	87 (16.8%)
1/2	2	0 (0.0%)	40 (7.7%)
1/4	4	0 (0.0%)	123 (23.8%)
1/8	8	135 (26.1%)	0 (0%)
1/16	16	89 (17.2%)	0 (0%)
1/32	32	35 (6.8%)	0 (0%)
1/64	64	7 (1.4%)	0 (0%)
1/128	128	1 (0.2%)	0 (0%)
Total		267 (51.7%)	250 (48.3%)

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

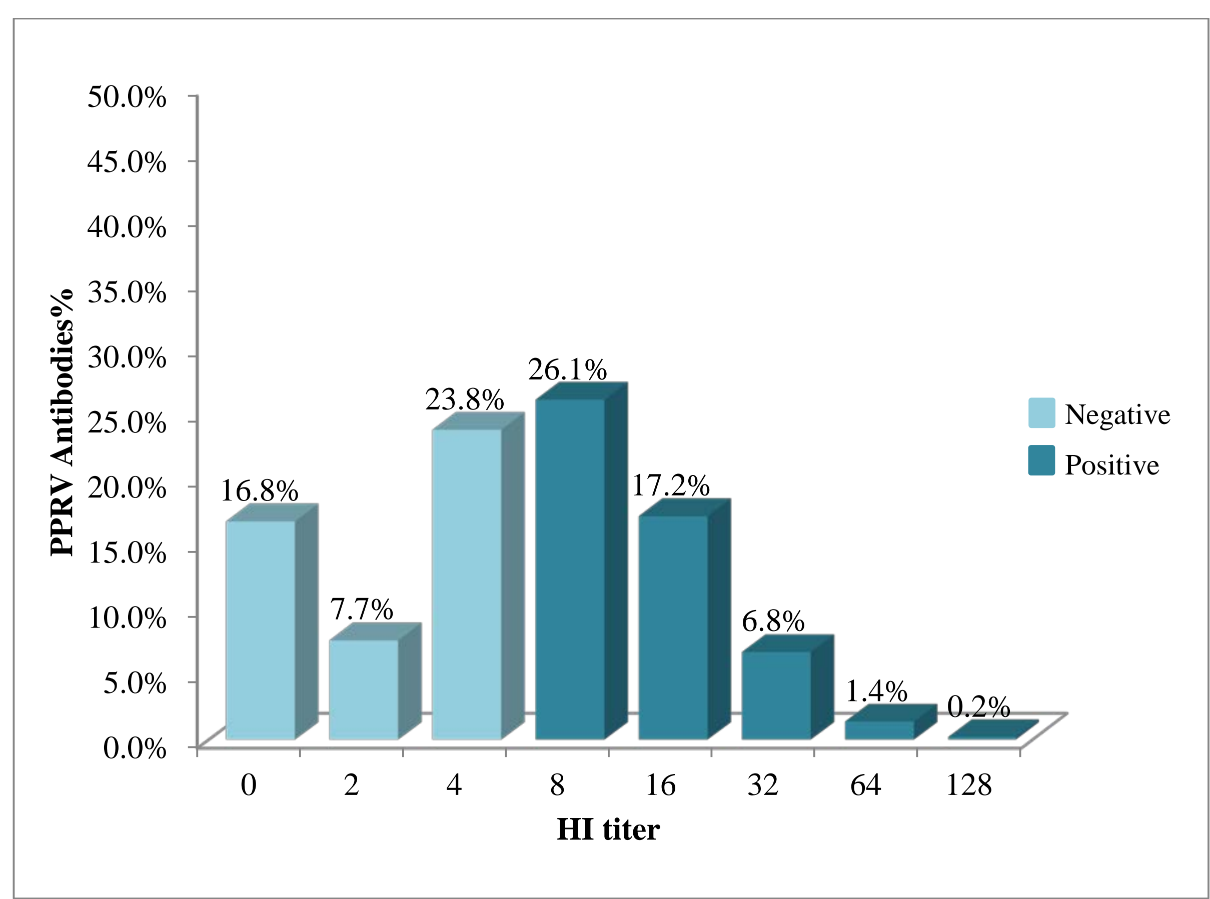

Figure 6. PPRV antibodies detected in cattle sera at different haemagglutination-inhibition titers by haemagglutination-inhibition (HI) test. Positive results are in dark color and negative results are in light color.

3.4. Sero-prevalence of PPRV antibodies in cattle sera within localities of RiverNileState:

Within the 4 localities under study of River Nile State, results indicated that the highest overall sero-prevalence of PPRV antibodies (67.9%, 214/315 sera) was detected in Atbara locality, followed by Elmatama locality (35.1%, 27/77 sera), Ad-Damar locality (21.2%, 14/66 sera), and finally Berber locality (20.3%, 12/59 sera) (Table 3, Figure 7).

Table 3.The overall sero-prevalence of PPRV antibodies in cattle sera in localities of River Nile State.

	Cattle sera		
Locality	No. tested (%)	No. +ve (%)	No. -ve (%)
Atbara	315 (100.0%)	214 (67.9%)	101 (32.1%)
Ad-Damar	66 (100.0%)	14 (21.2%)	52 (78.8%)
Berber	59 (100.0%)	12 (20.3%)	47 (79.7%)
Elmatama	77 (100.0%)	27 (35.1%)	50 (64.9%)
Total	517 (100.0%)	267 (51.7%)	250 (48.3%)

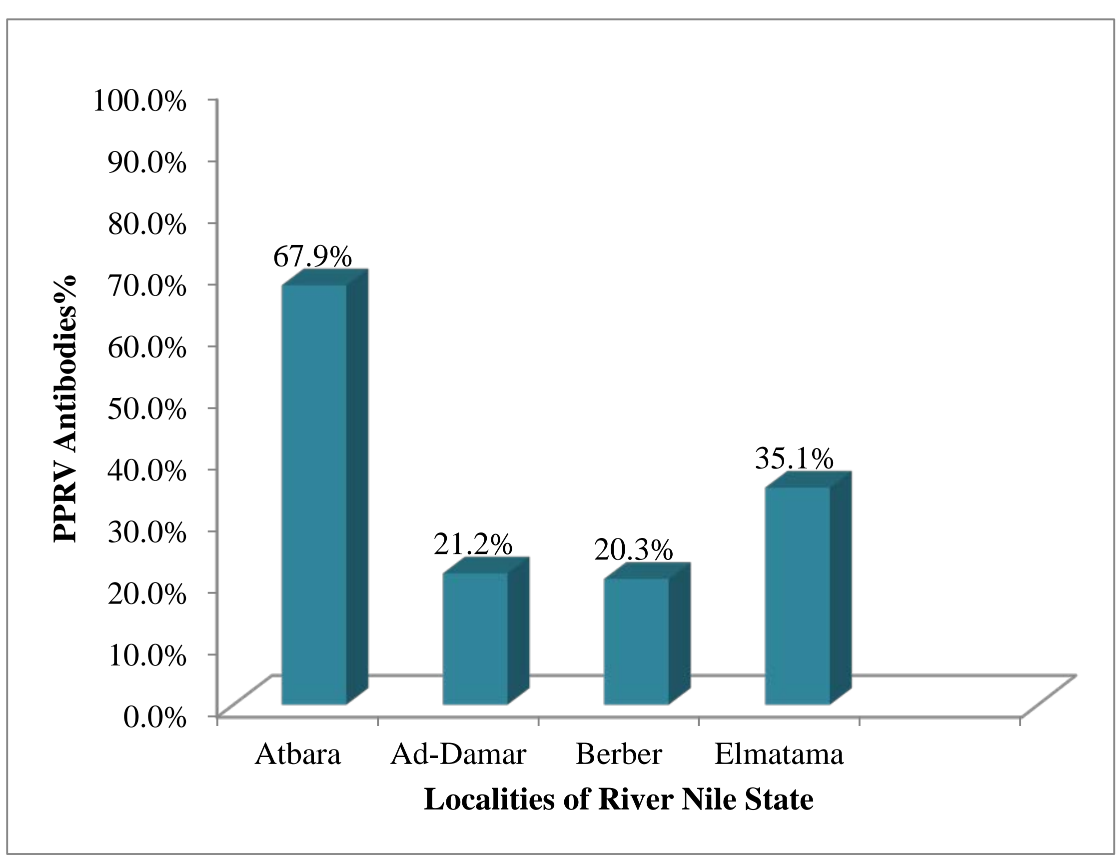

Figure 7.Sero-prevalence of PPRV antibodies in cattle sera in localities of River Nile State.

3.5. Factors affecting sero-prevalence of PPRV antibodies in cattle sera:

3.5.1. Sero-prevalence of PPRV antibodies in cattle sera, considering presence or absence of close contact with sheep and goats, in River Nile State:

Cattle in close contact with sheep and goats constitute 413 (79.9%)whereas that had no previous contact with sheep and goats constitute 104 (20.1%) of the 517 cattle tested (Table 4).

Considering the effect of cattle close contact with sheep and goats, the highest sero-prevalence of PPRV antibodies was demonstrated among cattle in close contact with sheep and goats (45.3%, 234/517 sera), compared to cattle not in contact with sheep and goats which demonstrated the lowest sero-prevalence of PPRV antibodies (6.4%, 33/517 sera) (Table 4).

In conclusion, presence of close contact of cattle with sheep and goats has a significant effect on sero-prevalence of PPRV antibodies.

Within localities of the River Nile State, the highest sero-prevalence of PPRV antibodies among cattle in close contact with sheep and goats was detected in Atbara locality (63.5%, 200/315 sera). In contrast, the highest antibodies sero-prevalence among cattle with no contact with sheep and goats was detected in Elmatama locality (19.5%, 15/77 sera) (Table 4).

Table 4.Sero-prevalence of PPRV antibodies in cattle sera, considering presence or absence of close contact with sheep and goats, in localities of River Nile State.

Locality	Total sera tested	Close contact			No contact		
		Total No.	No. +ve (%)	No. -ve (%)	Total No.	No. +ve (%)	No. -ve (%)
Atbara	315	277 (87.9%)	200 (63.5%)	77 (24.4%)	38 (12.0%)	14 (4.4%)	24 (7.6%)
Ad-Damar	66	56 (84.8%)	11 (16.6%)	45 (68.2%)	10 (15.2%)	3 (4.5%)	7 (10.7%)
Berber	59	50 (84.7%)	11 (18.6%)	39 (66.1%)	9 (15.3%)	1 (1.7%)	8 (13.6%)
Elmatama	77	30 (39.0%)	12 (15.6%)	18 (23.4%)	47 (61.0%)	15 (19.5%)	32 (41.5%)
Total	517	413 (79.9%)	234 (45.3%)	179 (34.6%)	104 (20.1%)	33 (6.4%)	71 (13.7%)

3.5.2. Sero-prevalence of PPRV antibodies in cattle sera, considering age of cattle, in River Nile State:

Regarding their age, adult cattle constitute 374 (72.3%) whereas young ones constitute 143 (27.7%) of the 517 cattle tested (Table 5).

Considering the effect of cattle age, the highest sero-prevalence of PPRV antibodies was demonstrated among adult cattle (42.9%, 222/517 sera) compared to young cattle which demonstrated the lowest overall sero-prevalence of PPRV antibodies (8.7%, 45/517 sera) (Table 5).

From these findings, it is appeared that highest sero-prevalence of PPRV antibodies was demonstrated among adult cattle rather than young ones.

Within localities of the River Nile State, the highest sero-prevalence of PPRV antibodies among adult cattle was demonstrated in Atbara locality (57.1%, 180/315 sera). Similarly, the highest sero-prevalence of PPRV antibodies among young cattle was demonstrated in Atbara locality (10.8%, 34/315 sera) (Table 5).

Table 5.Sero-prevalence of PPRV antibodies in cattle sera, considering age of cattle, in localities of River Nile State.

		Cattle age					
	Total sera	Adult Cattle			Young Cattle		
Locality	tested	Total No.	No. +ve (%)	No. -ve (%)	Total No.	No. +ve (%)	No. -ve (%)
Atbara	315	250 (79.3%)	180 (57.1%)	70 (22.2%)	65 (20.6%)	34 (10.8%)	31 (9.8%)
Ad-Damar	66	43 (65.2%)	14 (21.2%)	29 (44.0%)	23 (34.8%)	0 (0.0%)	23 (34.8%)
Berber	59	42 (71.2%)	9 (15.3%)	33 (55.9%)	17 (28.8%)	3 (5.1%)	14 (23.7%)
Elmatama	77	39 (50.6%)	19 (24.7%)	20 (25.9%)	38 (49.4%)	8 (10.4%)	30 (39.0%)
Total	517	374 (72.3%)	222 (42.9%)	152 (29.4%)	143 (27.7%)	45 (8.7%)	98 (19.0%)

3.5.3. Sero-prevalence of PPRV antibodies in cattle sera, considering breed of cattle, in RiverNileState:

In the present study, the 517 cattle tested in the River Nile State belongs to three different breeds included Friesian Cattle, Butana Cattle and Holstein Cattle which constitute 415 (80.3%), 100 (19.3%) and 2 (0.4%), respectively (Table 6).

Considering the effect of breed of cattle, the highest sero-prevalence of PPRV antibodies was demonstrated among Friesian cattle (41.8%, 216/517 sera) followed by Butana cattle (9.4%, 49/517 sera) and lastly Holstein cattle (0.4%, 2/517 sera) which demonstrated the lowest sero-prevalence of PPRV antibodies (Table 6).

From these findings, it is appeared that highest sero-prevalence of PPRV antibodies was demonstrated among Friesian cattle rather than other breeds. However, the breed of cattle has no clear effect on sero-prevalence of PPRV antibodies due to the variable number of the cattle sera tested from different breeds.

Within localities of the River Nile State, the highest sero-prevalence of PPRV antibodies among Friesian cattle was demonstrated in Atbara locality (54.3%, 171/315 sera). Among Butana cattle, the highest sero-prevalence of PPRV antibodies was demonstrated in Elmatama locality (15.6%, 12/77 sera). Of note, Holstein cattle were tested only in Atbara locality and demonstrated (0.6%, 2/315 sera) sero-prevalence of PPRV antibodies (Table 6).

Table 6.Sero-prevalence of PPRV antibodies in cattle sera, considering breed of cattle, in localities of River Nile State.

		Breed of Cattle								
		Friesian Cattle			Butana Cattle			Holstein Cattle		
Locality	Total sera tested	Total No.	No. +ve (%)	No. -ve (%)	Total No.	No. +ve (%)	No. -ve (%)	Total No.	No. +ve (%)	No. -ve (%)
Atbara	315	238 (75.6%)	171 (54.3%)	67 (21.3%)	75 (23.8%)	41 (13.0%)	34 (10.8%)	2 (0.6%)	2 (0.6%)	0 (0.0%)
Ad-Damar	66	61 (92.4%)	14 (21.2%)	47 (71.2%)	5 (7.6%)	0 (0.0%)	5 (7.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Berber	59	59 (100%)	12 (20.3%)	47 (79.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Elmatama	77	57 (74.0%)	19 (24.7%)	38 (49.3%)	20 (26.0%)	8 (10.4%)	12 (15.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	517	415 (80.3%)	216 (41.8%)	199 (38.5%)	100 (19.3%)	49 (9.4%)	51 (9.9%)	2 (0.4%)	2 (0.4%)	0 (0.0%)

Chapter IV
Discussion

This study aimed to investigate the presence of PPR and its sero-prevalence among cattle populations in the River Nile State, Northern Sudan. Furthermore, it aimed to assess some factors affecting PPRV sero-prevalence among cattle populations in regards to its different age, different breed, and presence or absence of close contact with sheep and goats, the usual hosts of PPRV. The type of animal housing there, constitute from mixed animal farming in which the breeding practices occurred enable close interactions between small ruminants (sheep and goats) and large ruminants (cattle) species.

During 2007 and 2019, at least 52 outbreaks of PPR in small ruminants had been officially reported in the River Nile State (OIE-WAHIS, 2019). Haroun *et al.* (2002) and Saeed *et al.* (2017)detected PPRV antibodies in cattle sera. In a recent report from the Sudan, higher sero-prevalence of PPRV antibodies was demonstrated when analyzing 1000 sera from apparently healthy cattle regardless of their age, sex and breed (Ali *et al.,* 2019).

Haemagglutination-inhibition (HI) testdetected PPRV protective antibodies in 267/517 (51.7%) cattle sera whereas 250/517 (48.3%) cattle sera were negative. In this study, results of the HI test revealed a significantly higher sero-prevalence of PPRV antibodies of 51.7% among cattle populations in the River Nile State. A recent study for assessment of the sero-prevalence of PPRV antibodies, using competitive ELISA (C-ELISA), revealed an overall sero-prevalence of 42.0% (420/1000) among apparently healthy cattle in five different States in the Sudan during 2015 and 2016 (Ali *et al.,* 2019) which is obviously lower than the sero-prevalence achieved in this study. In the same study, 45.5% (91/200) sero-prevalence of PPRV antibodies was demonstrated among cattle populations in the River Nile State (Ali *et al.,* 2019) which is lower than the values achieved in this study. Furthermore, 49.431% overall sero-prevalence was detected from 176 serum samples obtained from cattle imported from Sudan and tested by C-ELISA (Hekal *et al.,* 2019) which is relatively near to that achieved in the present study. Interestingly, it was found that the sero-prevalence of PPRV is higher in hot summer season (57%, 56/98 serum samples) compared to cold winter season (39.75%, 31/78 serum samples) (Hekal *et al.,* 2019). Likewise, 41.86% sero-prevalence of PPRV antibodies was detected earlier in cattle sera in Pakistan (Khan *et al.,* 2008).

In contrast, previous studies detected 11.4% (Haroun *et al.,* 2002) and 25.8% (Saeed *et al.,* 2017) sero-prevalence of PPRV antibodies among cattle populations in the Sudan, using C-ELISA, which is much lower than reported in this study. In several studies conducted through other African countries, lower sero-prevalence of PPRV antibodies had been reported in Ethiopia (9%) (Abraham *et al.,* 2005) and Tanzania (26.7%) (Lembo *et al.,* 2013).

The result of the present study demonstrated presence and higher prevalence of PPRV antibodies in cattle sera as had been documented previously in the country (El Amin and Hassan 1999; Haroun *et al.,* 2002; Saeed *et al.,* 2017; Ali *et al.,* 2019) and reflects the wide spread of PPRV among cattle populations in the River Nile State where PPR is endemic in small ruminants. This indicated that cattle are susceptible to PPRV infection with absence of any observable clinical signs. Although a clinical disease had not been reported in cattle, pyrexia and oral lesions observed in experimentally infected calves could be of significance (Mornet *et al.,* 1956). Therefore, it can be concluded that higher sero-prevalence values of PPRV had been detected in cattle although these animals did not vaccinated against PPRV or RPV which suggested exposure of these animals to PPRV. The relatively high sero-prevalence of PPRV in cattle in the present study reflects the continuous exposure of these animals to PPRV via their close interactions with small ruminants (sheep and goats) sharing the same grazing area. Khan et al. (2008) attributed thehigher sero-prevalence of PPRV in cattle to a natural field infection with PPR virus when contact occurs with small ruminants in shared grazing areaespecially in the watering points. A study in Ethiopia suggest that cattle can be used as sentinel species for PPR surveillance in cattle-small ruminant mixed farming areas and also for monitoring the impacts of interventions and disease freedom in high risk areas (Agga *et al.,* 2019). In addition, cattle are known as unusual hosts of PPRV, so presence of PPRV protective antibodies in the sera of unvaccinated cattle might suggest presence of subclinical infection after exposure to PPRV. Cattle either naturally or experimentally infected by PPRV developed subclinical infection with demonstrated seroconversion (Lembo *et al.,* 2013; Sen *et al.,* 2014; Abubakar *et al.,* 2017; Ali *et al.,* 2019; Couacy-Hymann *et al.,* 2019; Schulz *et al.,* 2019). The studies of Couacy-Hymann *et al.* (2019) and Schulz *et al.* (2019) confirms cattle as dead-end hosts for PPRV that do not play an epidemiological role in the maintenance and spread of PPRV but can serve as indicators of PPRV infection during a PPR surveillance programme.

For assessment of the effect of presence or absence of close contact with sheep and goats, a significantly highest overall sero-prevalence of PPRV antibodies was demonstrated among cattle living in close contact with sheep and goats (35.4%, 183/517 sera) compared to a least sero-prevalence (6.4%, 33/517 sera) in cattle not living in close contact with sheep and goats. It is obvious that the sero-prevalence value is increased upon contact with infected small ruminants suggested the potential exposure of cattle to PPRV and occurrence of natural transmission via contact with infected small ruminants as had been documented by Lembo *et al.* (2013) and Sen *et al.* (2014).

For assessment of the effect of the age of cattle on the sero-prevalence of PPRV antibodies, it is found thata significantly highest overall sero-prevalence of 42.9% (222/517 sera) was demonstrated among adult cattle rather than young ones (8.7%, 45/517 sera). In conclusion, the sero-prevalence of PPRV antibodies is significantly increased with cattle growing old. Similarly, a cross-sectional sero-survey for the estimation of the age cohorts responsible for PPRV transmission among small and large ruminants in Tanzania, found that an apparent sero-prevalence of PPRV for cattle was increased with age (Herzog *et al.,* 2020) and this also applied for small ruminants. Of note, these results were typical with a study in Sudan confirmed that sheep and goats at the age of 12 months or more had the highest prevalence of PPRV antibodies (Salih *et al.*, 2016).

When assessing the effect of different breeds of cattle on the sero-prevalence of PPRV antibodies, it was found a significantly highest sero-prevalence of PPRV antibodies was demonstrated among Friesian cattle (41.8%, 216/517 sera) whereas other cattle breeds like Butana cattle (9.4%, 49/517 sera) and Holstein cattle (0.4%, 2/517 sera) demonstrated the lowest sero-prevalence keeping in mind that the sample size of the Friesian cattle (80.3%, 415/517) was much large than Butana (19.3%, 100/517) and Holstein (0.4%, 2/517) breeds.In can be concluded that, in this case the breed of cattle has no clear effect on sero-prevalence of PPRV due to the variable number of the cattle sera tested from different breeds.

Conclusion and Recommendations

Conclusion:

The higher sero-prevalence of PPRV antibodies among cattle population in the River Nile State indicated the wide-spread of the disease in this area in Northern Sudan. Cattle are unusual or atypical hosts for PPRV thus absence of clinical signs accompanied with development of antibodies indicated subclinical infection. Detection of PPRV antibodies in sera of apparently healthy cattle indicated exposure of these animals to PPRV via contact with infected small ruminants.

The findings of this study concluded that factors such as presence of close contact of cattle with small ruminants and cattle age had a significant effect on sero-prevalence of PPRV antibodies. However, the effect of the cattle breed needs more investigation.

Recommendations:

1. Perform more sero-survey among cattle populations in other areas of the country especially in areas were mixing animal grazing is practiced.
2. Other genetic, epidemiologic and viral transmission studies should be planned for providing more information on the situation of the disease in usual and also unusual hosts.
3. Additional studies to determine the factors that affecting the spread of the disease is needed.
4. Implement an effective vaccination programs and other control measures for reducing losses of the animal populations due to PPR and controlling the disease in small ruminants in the River Nile State.
5. Restrict animal movement from endemic areas by establishing veterinary check points to prevent possible exposure of usual and unusual hosts to PPRV.

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Appendix

Preparation of buffers and solutions:

Deionized Distilled Water (DDW):

For preparation of Deionized Distilled Water (DDW), water was distilled by passing through the water distillation device then deionized by passing through the De-ionizer device. DDW was sterilized by autoclaving at 120°C for 30 minutes.

Preparation of antibiotic:

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

One ampule of Gentamycin was transferred into universal bottle, DDW was added then the solution was mixed well by shaking. 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.The prepared solution contains 50,000 IU Mycostatin per 1 ml.

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

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

Phosphate Buffer Saline (PBS), pH 6.8:

After preparation of PBS with pH 7.2-7.4, 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 autoclaving at 120°C for 30 minutes.