Sudan University of Science and Technology
College of Graduate Studies

Sero-prevalence of Peste des Petits Ruminants Virus (PPRV) Antibodies in Sheep and Goats from North and Central Sudan

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Fulfillment of the Requirements for the Degree of
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September, 2018
DECLARATION OF THE STATUS OF THESIS

BY STUDENT

The work described in this master degree thesis was carried out in the Virology laboratory in the Department of Pathology, Parasitology and Microbiology at the College of Veterinary Medicine, Sudan University of Science & Technology from August 2016 to March 2018 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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BY SUPERVISOR

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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
September, 2018
DEDICATION

To soul of my father

To my kind unfailing support mother

To my brothers

For those who were in my heart and are still there

Thank you for your support
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First of all, thanks to Allah to whom would be ascribed all perfection and majesty, and praise to Almighty Allah again for giving me the strength and stamina to finish this work. I would like to express my grateful thanks to my supervisor Dr. Nussieba Ahmed Osman Elhag for her keen supervision and unlimited help.

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ABSTRACT

Peste des petits ruminants (PPR), goat plague, is an infectious, fatal viral disease affecting mainly sheep and goats and some wild small ruminants. Peste des petits ruminants virus (PPRV), the causative agent of PPR, is classified as a member of the Morbillivirus genus in the Paramyxoviridae family in the Order Mononegavirales.

In the Sudan, sheep and goats are the major known hosts of PPR, however, lately PPR was reported to cause a fatal respiratory syndrome in camels in the Sudan. Between 2016 and 2017, suspected PPR outbreaks were occurred in different parts of the country. A total of 320 tested sera (258 sheep and 62 goats) were collected from sheep and goats from PPR suspected outbreaks. In 2016, the sera were from Dongola in Northern State (n= 2); Garie in River Nile State (n= 72); Kab Elgidad in Gezira State (n= 64); and Raboob-East Nile in Khartoum State (n= 64). During 2017, the sera were from Hajar Elasal in River Nile State (n= 104); and Elgadid Elthaora in Gezira State (n= 14). None of the animals was known to have been vaccinated against PPR before or at the time of sampling.

All sera (n = 320) were examined for the presence of PPRV antibodies by competitive ELISA (C-ELISA) using monoclonal antibodies (MAbs) directed against PPRV nucleoprotein. Results revealed an overall sero-prevalence of 80.9% (259/320). On the species basis, sheep sera yielded a higher sero-prevalence of 84.5% (218/258) while goat’s sera yielded a lower overall sero-prevalence of 66.1% (41/62).

The highest overall sero-prevalence of PPRV antibodies was demonstrated in River Nile State 90.3% (159/176 sera) followed by Gezira State 88.5% (69/78 sera), then Khartoum State 48.4% (31/64 sera) and finally the lowest incidence was present in Northern State 0.0% (0/2 sera).

The findings indicated that PPR is currently circulating widely in the Sudan and still is a leading cause to a clinical disease and higher fatalities. The continual presence of PPR outbreaks, despite the application of PPRV vaccine, might be due to that the vaccination program is not reaching herds of animals in some areas. Therefore, the effective PPR vaccine is recommended to be used with a plan for covering all parts of the Sudan to prevent the occurrence of the disease outbreaks.
منتصف البحث

مرض طاعون المجترات الصغيرة أو طاعون الاغنام هو مرض فيروسي معد وقاتل يصيب الاغنام والماعز. بصورة أساسية، يصيب بعض المجترات الصغيرة الوراثية. فيروس طاعون المجترات الصغيرة هو المسبب للمرض والذى تم تصنيفه في جنس الموربيليفيروس وفي عائلة البراميكوزفيروسات وفي فصيلة المونونيكافيراليز.

الطاعون والماعز هما العائل الأساسي للإصابة بالمرض في السودان، رغم أنه مؤخراً اتضح أن طاعون المجترات الصغيرة يمكن أن يصيب الجمال والإصابة تكون في شكل مرض تنفسي قاتل.

خلال عامي 2016-2017م، ظهر المرض في شكل وبائي في مناطق مختلفة من البلاد. حوالي 320 عينة مصل تم اختبارها من الضان (258 عينة مصل) والماعز (62 عينة مصل) من المناطق التي ظهر بها الوباء بالممرض. في عام 2016م، العينات كانت (2 عينة مصل) من دنقلا في الولاية الشمالية، (2 عينة مصل) من قري في ولاية نهر النيل، (64 عينة مصل) من كاب الحداد في ولاية الجزيرة، و(64 عينة مصل) من ريبوب شرق النيل في ولاية الخرطوم. في عام 2017م، عينات المصل كانت (104 عينة مصل) من حجر العسل في ولاية نهر النيل، (14 عينة مصل) من الجديد الثورة في ولاية الجزيرة. لم يتم إعطاء لقاح ضد المرض لهذه الحيوانات قبل أو أثناء جمع العينات.

كل الأحصائيات (320 عينة) تم اختبارها للكشف عن وجود الأجسام المضادة للفيروس بواسطة اختبار الإليزا التنافسي، والذي يتم فيها استخدام الأجسام المضادة وحيدة النسيلة مجهزة ضد البروتين النووي للفيروس. النتائج وضحت أن النسبة المئوية لتواجد المرض بصورة عامة هي 80.9% (259/320). عند الاعتبار حسب الاعتقاد جنس الحيوان فإن النسبة المئوية لتواجد المرض في الضان كانت عالية (84.5%, 258/320) وفي الماعز كانت أقل (66.1%, 41/62).

اعلي نسبة مئوية لتواجد الأجسام المضادة بالمرض كانت في ولاية نهر النيل (90.3%, 176/159) تليها ولاية الجزيرة (88.5%, 78/70) ثم ولاية الخرطوم (48.4%, 64/69) وآخراً فإن أقل نسبة مئوية لتواجد المرض كانت في الولاية الشمالية (0.0%, 2/0).
المعطيات دلت على أن مرض طاعون المجترات الصغيرة منتشر حالياً في السودان وأنه السبب المؤذي لظهور المرض السريري ونسبة الوفاة العالية في الحيوانات. الظهور المستمر لوبائيات المرض بالرغم من استخدام اللقاح ضد المرض يبدو أنه بسبب ان حملات التطعيم لا تصل لكل قطعان الحيوانات في بعض المناطق. لذلك يوصى بإعطاء اللقاح الفعال للمرض مع ضمان ان تشمل خطة التطعيم كل مناطق السودان لمنع حدوث وبائيات المرض.
INTRODUCTION

Peste des petits ruminants (PPR), goat plague, is an acute and highly contagious viral disease contracted by small ruminants and camels. PPR was firstly described in Cote d’Ivoire (the Ivory Coast) in West Africa in 1940. Recently, PPR is considered as an emerging disease which is extended over most of Africa, the Arabian Peninsula, The Middle East, Asia and Turkey.
PPR is caused by peste des petits ruminant virus (PPRV), a member of the Morbillivirus genus of the Paramyxoviridae family in the order Mononegavirales.
The disease is characterized by fever, ocular and nasal discharges, erosive and necrotic stomatitis, conjunctivitis, gastroenteritis, diarrhea and pneumonia. PPR has an economic importance due to its highly mortality and morbidity rates.
PPR is primarily a disease of goat and sheep, however, goats are usually more severely affected than sheep. Several outbreaks of PPR were occurred in different species of wild small ruminants. PPRV infection of camels was recognized as a fatal respiratory disease. Transmission of PPR requires close contact between susceptible and infected animals and outbreaks occurs mostly by introduction of PPRV infected animals into a herd.
In the Sudan, peste des petits ruminants (PPR) was known since the beginning of the 1970th where outbreaks in sheep and goats were reported in Elgadarif, Sinnar and Melieq. PPR viruses were isolated and the existence of PPR in the country was documented.
A tentative diagnosis of PPR can be made depending on disease history, epidemiology, high morbidity and mortality rates, characteristic clinical signs and postmortem lesions. Detection of antibodies to PPRV is generally carried out using virus neutralization test and competitive ELISAs (C-ELISA) based on monoclonal antibodies (MAbs) against the N or H proteins which are recommended by the World Organization for Animal Health (OIE).
Currently the PPRV live attenuated cell culture „homologous vaccine“ is used for control of PPR in small ruminants in the Sudan which confers protective immune response for at least 3 years. Despite the application of the effective vaccine, outbreaks of the disease were reported in different parts of the country over the years.
The present study was designed to achieve the following goals:

1. To perform sero-surveillance of peste des petits ruminants virus (PPRV) antibodies.

2. To investigate the distribution and the current situation of the disease in some parts in North and Middle Sudan.

3. To demonstrate the use of a competitive ELISA assay for detection of antibodies against PPRV in areas of outbreaks.
1.1. Definition:

Peste des petits ruminants (PPR) or plague of small ruminants, is an acute, extremely infectious and lethal viral disease of domestic and wild small ruminant (Diallo, 2003; Ozmen et al., 2009). PPR is characterized by pyrexia, oculo-nasal discharge, mouth sloughing, necrotic stomatitis, conjunctivitis, pneumonia, gastroenteritis leading to diarrhea, and death (OIE, 2013).

Other names of PPR commonly used include: goat plague; contagious pustular stomatitis; pest of sheep and goat; pseudo-rinderpest of small ruminants; pest of small ruminants; stomatitis pneumoenteritis syndrome and pneumoenteritis complex (Merck, 2010).

2.1. Economic Importance of PPR:

PPR has an economic importance due to its high rates of mortality which may reach 100% and morbidity rates ranging from 50 to 80% (Losos, 1986; Kitching, 1988; OIE, 2013). It is recognized as the most critical disease and the number one killer disease of small ruminants in endemic countries in Africa and Asia.

3.1. History of PPR:

The primary description of PPR incidence was in the Ivory Coast (Cote D’Ivoire) in West Africa (Gargadennec and Lalanne, 1942). After that the disease was further distributed to other countries in West-Africa then spread further towards middle and East Africa. For several years it was thought that PPR was limited to West Africa, until the appearance of the disease in middle Africa followed by East-Africa (Elhag Ali and Taylor, 1984). Following that PPR was reported in several countries in the Middle East and Asia (Shaila et al., 1996; Banyard et al., 2010).
4.1. Etiology:

4.1.1. Classification:

PPR is caused by peste des petits ruminant virus (PPRV), a member of the *Morbillivirus* genus of the *Paramyxovirinae* subfamily of the *Paramyxoviridae* family in the order *Mononegavirales* (Gibbs et al., 1979; Parida et al., 2015). The *Morbillivirus* genus contains several viruses which are closely related to PPR, these viruses include rinderpest virus (RPV) of cattle and buffaloes, the canine distemper virus of dogs (CDV), the measles virus (MV) of humans, phocine distemper virus (PDV) of seals, Cetacean morbillivirus (CMV) which include dolphin (DMV) and porpoise (PMV) morbilliviruses of aquatic mammals (Gibbs et al., 1979; Parida et al., 2015).

5.1. Virus Properties:

5.1.1. Morphology of PPRV:

The virions are enveloped, pleomorphic particles with a size ranging from 350 to 600 nm. The virion contains single-stranded RNA genome which is enclosed into the ribonucleoprotein (RNP) core (Durojaiye et al., 1985; Diallo, 1990).

5.1.2. Genomic Structure:

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 RNA genome encodes 6 structural proteins namely: the nucleoprotein (N), the matrix protein (M), the phosphoprotein (P), the haemagglutinin (H), the fusion protein (F), and the large protein or polymerase (L) in addition to two non-structural proteins (C and V) (Bailey et al., 2005; Chard et al., 2008) (Figure 1).

The N protein plays an important role in the replication of PPRV (Servande Almeida et al., 2007). The N protein is the most collected protein in infected cells and is antigenically most preserved between morbilliviruses (Libeau et al., 1995).

The phosphoprotein (P) is very susceptible to proteolysis. The P protein of PPRV is the longest among morbilliviruses and is the very important element of the viral L–polymerase complex, and it is assumed to be a key determinant of cross-species morbillivirus pathogenicity (Yoneda et al., 2004).
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 Fusion (F) protein, the first viral glycoprotein, is 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 haemagglutinin (H) is the second viral glycoprotein. It serves attaching the virus to the host cell (Diallo, 2003).

H and F proteins are playing a role in the pathogenesis of all Paramyxovirus infections. H and F protein are greatly immunogenic and confer protecting immunity (Diallo, 2003).

*The Large (L) protein* is considered as the largest protein in PPR virions. The polymerase or large protein (L) is the third viral protein involved in the ribonucleoprotein structure. The L protein of PPRV carries a length identical to that of RPV, MV and DMV. In all morbilliviruses, the L protein acts as RNA-dependent RNA polymerase and performs transcription and replication of the viral genomic RNA. Furthermore, the L protein is also responsible for capping, methylation and polyadenylation of viral mRNA (Murphy *et al.*, 1999).

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

![Figure 1](image-url)
6.1. Epidemiology of PPR:

6.1.1. Geographic Distribution:

PPR was initially described in Ivory Coast in West Africa in 1940 (Gargadennec and Lalanne, 1942). Afterwards, it was reported in Benin (Mornet et al., 1956). Subsequently, the disease distributed further to Senegal, Ghana, Togo, and Nigeria (Merck, 2010). At the beginning, reports of PPR were limited to West Africa. It is only later that it is known geographical distribution has gradually prolonged through a lot of African countries, the Middle and Near East, and Asian countries expanding from West Asia to China (Libeau et al., 2014). Currently, PPR is endemic in most of African countries excluding a small number of countries in South Africa, most of Asia, the Arabian Peninsula, the Middle East and in Turkey (Banyard et al., 2010; OIE, 2013; Jones et al., 2016) (Figure 2).

![Figure 2. Worldwide distribution of peste des petits ruminants (Jones et al., 2016)](image)
6.1.2. Transmission:

Transmission of PPR requires close contact between susceptible and infected animals. The virus is shed in oral, nasal, and ocular secretions and in feces which are considered rich sources of the virus, thus it can be transmitted to close in-contact susceptible animals (OIE, 2013).

PPR is generally regarded as a seasonal disease, peak infections usually occurring in the dry, cool season in endemic areas of Africa (Abubakar et al., 2009). Experimentally, the virus has been transmitted parenterally through different routes: intraocular, intratracheal and intravenous nasal, oral, subcutaneous, or by contact (Durtnell, 1972; Durojaiye, 1980; Osman et al., 2009).

6.1.3. Host Range:

PPR is mainly a disease of small ruminants. It affects sheep and goat, sheep and goats are probably equally susceptible to the virus, but sheep may be somewhat more resistant to its clinical effects. While sero-surveillance of affected flocks often shows higher prevalence levels in sheep, this may reflects the fact that a higher proportion of the affected goats have died, cattle are only subclinically infected, and humans are not at risk (Merck, 2010). Goats are severely affected whereas sheep usually undertake a mild form. Species of the family Caprinae are predominantly susceptible to disease including some endangered mountain and desert ungulates (Munir, 2014). A few large ruminants including wildlife and cattle show seroconversion for antibodies specific to PPR virus (Lembo et al., 2013), the virus has been isolated from experimentally infected cattle (Sen et al., 2014) and the disease has been reported in camels (Khalafalla et al., 2010). It caused a high mortality and severe disease in Dorcas Gazelles (Gazelladorcas), Nubian Ibex (Capra ibex nubiana), Laristan sheep (Ovisorientalislaristani) and gemsbok (Oryx gazella). Subclinical involvement of Nigale (Tragelaphinae) was suspected. In another report from Saudi Arabia, Antelope and other small wild ruminant species can also be severely affected (Abu Elzein et al., 2004). Several species of gazelle, Oryx, and white-tailed deer are fully susceptible; these and other wild ruminants may play a role in the epidemiology of the disease, but little or no data are available for infection in wild small ruminants. Pigs are dead-end hosts and do not transmit the disease to susceptible pigs or goats, and it is unlikely that they play a role in PPR epidemiology. Although cattle and domestic buffalo are
susceptible to infection, there is no evidence that they exhibit clinical signs following natural or experimental infection or that they transmit the disease to susceptible species (Merck, 2010).

7.1. Clinical Signs:
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 three to five days; the animals become depressed, anorexic and develop a dry muzzle (OIE, 2013). Serous oculo-nasal discharges become progressively mucopurulent and, if death does not arise, continue for around 14 days. Within four days of the beginning of fever, the gums become hyperemic, and erosive lesions develop in the oral cavity with excessive salivation. These lesions may become necrotic (OIE, 2013). Watery blood-stained diarrhea is common in the final phase. Coughing, Pneumonia, pleural rales and abdominal breathing also occur. The morbidity rate can be up to 100% with very high case fatality in severe cases. However, morbidity and mortality may be much lower in milder outbreaks, and the disease may be overlooked (OIE, 2013).

8.1. Post-Mortem Findings:
At necropsy, the lesions are very similar to those viewed in cattle affected with rinderpest, except that distinct crusty scabs over the superficial lips and severe interstitial pneumonia commonly occur with PPR (OIE, 2013). Erosive lesions may extend from the mouth to the reticulo–rumen junction. Characteristic linear red areas of congestion or hemorrhage may occur along the longitudinal mucosal folds of the large intestine and rectum (zebra stripes), but they are not a consistent finding. Erosive or hemorrhagic enteritis is usually present and the ileocecal junction is commonly involved. Peyer’s patches may be necrotic. Lymph nodes are enlarged, and the spleen and liver may show necrotic lesions (OIE, 2013).

9.1. Diagnosis:
PPR and RP are both diseases that can infect small ruminants. Differential diagnosis clinically is not possible as similar diseases is produced by both viruses in small ruminants. Therefore, tentative clinical diagnosis may have to be confirmed by laboratory analysis. Diagnosis of PPR may be performed by virus isolation, antigens
detection methods, nucleic acid sequencing and detection of specific antibody in serum (OIE, 2013).

9.1.1. Clinical Diagnosis:

A tentative diagnosis of rinderpest-like disease can be made based on high morbidity and mortality rates, clinical signs, postmortem lesions, history and epidemiology of the disease. A provisional diagnosis can be made by laboratory confirmatory tests of PPR and differential diagnosis with other diseases which have the same clinical signs (OIE, 2013).

9.1.2. Laboratory Diagnosis:

9.1.2.1. Samples Required for Diagnosis:

To perform a laboratory diagnosis of PPR, samples which can be from live animals 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. Samples which can be collected 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, 2013).

9.1.2.2. Antigen Detection Methods:

Many methods can be applied for PPRV antigen detection including: Agar gel immunodiffusion test (AGID) (Durojaiye, 1982), Counter immune-electrophoresis (CIEP) (Osman et al., 2009; OIE, 2013), Immunocapture ELISA (IC-ELISA) and Sandwich ELISA (S-ELISA) using two monoclonal antibodies (MAb) raised to PPRV N protein (Libeau et al., 1995; Singh et al., 2004) and Haemagglutination Test (HA) (Wosu, 1991; Osman et al., 2008).

9.1.2.3. Serological Techniques:

Many diagnostic techniques have been applied for the demonstration of PPR antibodies in serum such as competitive ELISA (C-ELISA) and virus neutralization test (VNT) which are recommended by the OIE(OIE, 2013; Balamurugan et al., 2014).
9.1.2.3.1. **Competitive ELISA (C-ELISA):**

Competitive ELISA is a reliable, sensitive and specific and has a high diagnostic specificity (99.8%) and sensitivity (90.5%), because it is better choice for PPR antibody detection. It is one of the most extensively used tests for serological screening and diagnosis of PPR in infected animals. This type of ELISA distinguished between PPR and RP. It was used to determine the presence of PPR specific antibodies both pre and post- vaccination. This assay is based on a monoclonal antibody that is specific for PPRV N protein or H protein (Saliki et al., 1993; Anderson and McKay, 1994; Libeau et al., 1995; Singh et al., 2004; OIE, 2013).

9.1.2.3.2. **Virus Neutralisation Test (VNT):**

VNT is the only obtainable precise serological test for PPRV and RPV. Though it can differentiate between antibodies to both viruses, it is rigid and complicated when the samples size is large. Virus neutralization test 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, 2013).

9.1.2.3.3. **Haemagglutination Inhibition (HI) Test:**

Haemagglutination inhibition (HI) test for detection of PPRV antibodies being cheaper, uncomplicated and comparatively sensitive- HI test can be used for routine screening purposes in control programmes. This test is widely used for the quantitative measurement of PPRV antibodies (Wosu and Ezeibe, 1992).

10.1. **Control of PPR:**

Methods applied for rinderpest eradication may be appropriate for PPR. Control of PPR outbreak depend on restriction and control of animal movement, slaughter, disposal carcasses properly and avoidance of fomites contact, disinfectant of facilities and equipment to remove the contamination, restriction of sheep and goats on the import from affected areas combined with animal vaccination (OIE, 2013). The disease can be prevented by not introducing new stock from unknown sources, especially animals bought at livestock markets. In addition animals returned unsold from markets should be segregated unless the entire herd or flock has been vaccinated (Radostitis, 2007).
regions and countries where the disease is endemic in nature, the most commonly employed control method is controlling the disease by increasing the immunity level through extensive vaccination campaigns.

### 10.1.1. Vaccination for the Control of PPR:

A live attenuated PPR vaccine was developed by serial attenuation of PPRV Nigeria 75/1 strain, belong to the lineage 1, in Vero cells. Other attenuated PPR vaccines that have been shown to produce strong immunity too (OIE, 2013; Sen et al., 2010). The vaccine is effective for the control of PPR and can provide protection against PPR in small ruminant for at least 3 years (Diallo et al., 2007; Sen et al., 2010), this Homologous PPR vaccine are available, and have been used extensively in Africa and Middle East to suppress outbreaks (Diallo et al., 1989; Sen et al., 2010). Full protection is achieved from standard doses of vaccine at three weeks post vaccination (OIE, 2013). Vaccination in animals aged 4-6 months is recommended (Balamurugan et al., 2012a). A number of vaccine manufacturers in Africa and the Middle East produce vaccines based on the original Nigeria 75/1 vaccine, and their effectiveness has been tested in several studies (Saravanan et al., 2010). Although different attenuated strains are in use in vaccination activities, the attenuated Nigeria 75/1 is the only vaccine strain approved by OIE (OIE, 2013).

Capripox virus strains expressing the homologous peste des petits ruminants virus H or F (Diallo et al., 2002; Berhe et al., 2003) proteins have also been shown to protect goats or sheep against infection by PPRV (Chenet et al., 2010).

### 11.1. PPR in the Sudan:

In the Sudan, PPR was known since the starting of the 1970th where outbreaks of a rinderpest-like disease in sheep and goat were reported in three areas in south Gedarif (Located in Eastern Sudan) in 1971 (El Hag Ali, 1973). Following that the disease was reported in 1971-1972 in Sinnar and in Mieliq in 1972. At that time the disease was wrongly diagnosis as rinderpest and ten year later it is confirmed to be PPR (Elhag Ali and Taylor, 1984). PPRV was firstly isolated by Elhagali and the isolate were named PPRV SUD72/1 (Sinnar strain) and SUD72/2 (Mieliq strain) (Elhag Ali and Taylor, 1984). Subsequently, outbreaks of the disease were reported during the years 1989 and
1999 in Elhilalia in Gezira State, the virus isolated was named PPR VHL (Elhassan et al., 1994).

Several studies on PPR in the Sudan was carried out including virus isolation (Osman et al., 2008); virus characterization using agar gel precipitation test (AGPT); haemagglutination test (HA); reverse passive haemagglutination test (HA); immunocapture ELISA (IC-ELISA) and reverse transcription polymerase chain reaction (RT-PCR) (Ishag et al., 2015).

Serological surveillance using agar gel precipitation test (AGPT); counter immune electrophoresis (CIEP) and competitive ELISA (C-ELISA) confirmed the presence of the disease in different regions of the country such as Gezira, White Nile, Blue Nile, Khartoum, River Nile, Northern, Red Sea, Kordofan and Darfur States (Osman et al., 2009; Abdalla et al., 2012; Ali et al., 2014; Saeed et al., 2017). A per acute fatal respiratory disease of camels in Eastern Sudan and the first report for PPR infection in camels in the Sudan was reported by Khalafalla et al., (2010).
CHAPTER II
MATERIALS AND METHODS

2.1. Materials

2.1.1. Blood collection and preparation of serum:

The following equipments were used for collection of blood sample from sheep and goats and preparation of serum samples:

1. Syringes.
2. Vacutainers and needles.
3. Eppendorf tubes (1-1.5 ml).
4. Centrifuge (Thermo Scientific, USA).
5. Single channel micropipette 1000 mL.
6. Tips.

2.1.2. Competitive-Enzyme Linked Immunosorbent Assay (C-ELISA):

2.1.2.1. Kit:

ID Screen® PPR Competition kit for the detection of antibodies against the Peste des Petits Ruminants (PPR) virus in sheep and goat serum and plasmaby competitive screening ELISA (IDVet Innovative diagnostics, France).

2.1.2.2. Reagents for ID Screen® PPR Competition ELISA:

1. Microplates coated with PPR recombinant nucleoprotein.
2. Anti-NP-HRP concentrated conjugate (10X).
3. Positive control.
4. Negative control.
7. Wash Concentrate (20X).
8. Substrate Solution.
9. Stop Solution (0.5M)
10. Deionized Distilled Water.
2.1.2.3. Equipments:

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single channel micropipette 10 µl, 100 µl and 200µl</td>
<td>Biohit and Labtech, Germany</td>
</tr>
<tr>
<td>Multichannel micropipettes 50-300 µl</td>
<td>Biohit and Labtech, Germany</td>
</tr>
<tr>
<td>Disposable Tips</td>
<td></td>
</tr>
<tr>
<td>Incubator Shaker</td>
<td>Awareness Technology, USA</td>
</tr>
<tr>
<td>96 well Microplate ELISA Reader</td>
<td>Thermo Electron Corporation, USA</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Thermo Scientific, USA</td>
</tr>
<tr>
<td>Parafilm DispensePM-996</td>
<td>Pechiney Plastic Packaging</td>
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<td>Glassware</td>
<td>Pyrex</td>
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<tr>
<td>Plasticware</td>
<td></td>
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<tr>
<td>Large Analogue Timer w/ handle</td>
<td>Jencons</td>
</tr>
<tr>
<td>pH Meter</td>
<td>Corning Incorporation, USA</td>
</tr>
</tbody>
</table>

2.2. Methods

2.2.1. Study area:

A total of 320 serum samples were collected from recently suspected PPR out breaks reported in different regions of the Sudan during 2016 and 2017. These sera were collected from 258 sheep and 62 goats with different ages and breeds (Table 1). Of these, sera which were collected during the year 2016 include: 2 sheep serum samples from Dongola, Northern Statein Northern Sudan; 64 sera (48 sheep sera and 16 goats sera) were from Kab Elgidad, Gezira State in the middle of Sudan; 64 sera (42sheep sera and 22from goat sera of which 3 are from goat Kids) were collected from Raboob a region in East Nile-, Khartoum State; 72 sheep sera were collected from Garie, River Nile, Northern Sudan. During 2017 serum samples were collected from suspected PPR outbreaks include: 104 sera (80 sheep sera and 24 goats sera) were collected from Hajar Elasal region, River Nile State in addition to 14 sheep sera were collected from Elgadid Elthaora, Gezira State (Table 1).
Table 1. Sheep and goats sera collected from PPR outbreaks in the Sudan during 2016-2017

<table>
<thead>
<tr>
<th>Date of Collection</th>
<th>Place of Collection</th>
<th>Animal Species</th>
<th>Total No. of Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sheep</td>
<td>Goats</td>
</tr>
<tr>
<td>2016</td>
<td>Dongola/ Northern State/ Sudan</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>2016</td>
<td>Kab Elgidad/ Gezira State/ Sudan</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>2016</td>
<td>Raboob, East Nile/ Khartoum State/ Sudan</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>2016</td>
<td>Garie/ River Nile State/ Sudan</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td>2017</td>
<td>Hajar Elasal/ River Nile State/ Sudan</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>2017</td>
<td>Elgadid Elthaora/ Gezira State/ Sudan</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>258</td>
<td>62</td>
</tr>
</tbody>
</table>
2.2.2. Collection of blood samples:

Blood samples were collected from sheep and goat from suspected PPR outbreaks from different regions of the country.

Blood samples were collected aseptically from the jugular vein of sheep and goat by the aid of needles and plain vacutainers. Vacutainers containing blood were placed in a diagonal position into test tube racks. After delivery to the laboratory, blood collected for serum samples were left at room temperature for 1-2 hours then were kept overnight in a refrigerator at 4°C.

2.2.3. Preparation of serum samples:

On the following day, serum were separated from blood by centrifugation at 3000 rpm for 5 minutes then transferred into 1-1.5 ml Eppendorf tubes by the aid of a 1 ml single channel micropipette and blue tips, sera were stored frozen at -20°C till used.

2.2.4. Competitive-Enzyme Linked Immunosorbent Assay (C-ELISA):

In order to perform a serological surveillance of PPR, a competitive ELISA (C-ELISA) assay was used for detection of PPRV antibodies in the suspected sera directed against PPRV nucleoprotein. The reagents were supplied in the form of a competitive screening ELISA kit “ID Screen® PPR Competition kit for the detection of antibodies against PPR in sheep and goats serum and plasma” developed by CIRAD-EMVT, FAO reference laboratory for PPR in Montpellier, France and purchased from IDVet Innovative Diagnostics, France.

2.2.4.1. Principle of the C-ELISA:

The wells are coated with the purified recombinant PPR nucleoprotein (NP). The sample to be tested and the controls are added to the micro wells. Anti-NP antibodies, if present, form an antibody-antigen complex which masks the NP epitopes. An anti-NP-peroxidase (HRP) conjugate is added to the micro wells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested that means firstly in the absence of antibodies, a blue solution appears which
becomes yellow after addition of the stop solution, secondly in the presence of antibodies no coloration appears. The microplate is read at 450 nm using ELISA reader.

**2.2.4.2. C-ELISA procedure:**

The procedure for the C-ELISA was performed following the manufacturer’s instructions (IDVet, France). Before starting the assay, reagents were cooled down to room temperature (25°C), reagents were homogenized by inversion or using vortex. The procedure was initiated by addition of 25µl of Dilution Buffer 13 to each well of the plate. Addition of controls was carried out by addition of 25µl of the positive control to wells A1 and B1 of the plate, followed by addition of 25µl of the Negative Control to well C1 and D1 (Figure 3). Afterwards, addition of 25µl of each test sample to one of the remaining wells from E1 to H12 (Figure 3). The plates were incubated for 45-50 minutes at 37°C. The wells of the ELISA plate were washed 3 times each using approximately 300 µl of the washing solution, the 1X conjugate was prepared by diluting the 10X conjugate 1:10 in Dilution Buffer 4, then 100µl of the 1X conjugate was added to each well of the ELISA plate. The plates were incubated at room temperature (21-25°C) for 30 minutes. Wash as before. 100 µl of the Substrate Solution (TMB) was added to each well then plate was incubated for 15 minutes at 21-25°C in the dark. In order to stop the reaction 100 µl of the 0.5 M Stop Solution was added to each well. Finally the plate was read using an ELISA Reader (Thermo Electron Corporation, USA) and the optical density (O.D.) of the controls and samples were recorded at 450 nm.

**Calculation and interpretation of the ELISA results:**

For each sample, the competition percentage (S/N %) was calculated as follow:

\[ \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{NC}}} \times 100 \]

Samples yielded equal to or less than 50% were considered positive whereas samples yielded greater than 60% were considered negative.
**MATERIALS AND METHODS**

**Controls**
(Column 1A-1D)

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**Serum Samples**
(Column 1-12)

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</table>

Figure 3. Layout of the C-ELISA plate.

**Notes:**

**Controls:**

- **PC** = Positive control serum (A1, B1)
- **NC** = Negative control serum (C1, D1)
- **TS** = Test serum(E1-H12)
Table 2. Steps of the C-ELISA

<table>
<thead>
<tr>
<th>Assay Steps</th>
<th>Incubation Time</th>
<th>Incubation Temperature</th>
<th>Plate Shaking</th>
<th>Wash Step</th>
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</thead>
<tbody>
<tr>
<td>Add dilution Buffer 13</td>
<td>45-50 min</td>
<td>37°C</td>
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<td>3X</td>
</tr>
<tr>
<td>Add Positive (+ve) and Negative (-ve) Controls</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Add Test Samples</td>
<td></td>
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<tr>
<td>Add Conjugate</td>
<td>30 min</td>
<td>21-25°C</td>
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<td>3X</td>
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<tr>
<td>Add Substrate Solution</td>
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<td>21-25°C</td>
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<tr>
<td>Add Stop Solution</td>
<td>none</td>
<td>None</td>
<td>none</td>
<td>None</td>
</tr>
<tr>
<td>Read Reaction (O.D.)</td>
<td></td>
<td>450 nm filter must be in plate reader</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER III

RESULTS

3.1. Description of the areas of PPR outbreaks:

During 2016, PPR suspected outbreaks involved sheep and goats, were occurred in different locations of the Sudan namely: Dongola in Northern State and Garie in River Nile State (northern Sudan); Kab Elgidad in Gezira State and Raboob in East Nile region in Khartoum State (middle Sudan). Additional suspected PPR outbreaks occurred during 2017 were in Hajar Elasal in River Nile State (northern Sudan) and Elgadid Elthaora in Gezira State (middle Sudan).

3.2. PPR clinical disease in sheep and goats:

PPR outbreaks were characterized by characteristic and typical clinical signs of the disease including pyrexia, anorexia, restless accompanied by severe depression, dull coat, emaciation, profuse serous nasal and ocular discharges changes gradually to mucopurulent discharges due to secondary bacterial infections or co-infection with other diseases, dry muzzle, respiratory signs and diarrhea. The cases were associated with a higher morbidity and mortality rates particularly in young animals than in the adults and sudden death especially in the neonates.

There was no previous history of vaccination of sheep and goats against PPR in herds where the disease outbreaks occurred.

3.3. Competitive ELISA (C-ELISA) for detection of PPRV antibodies

A total of 320 sera collected from herds of sheep and goats from suspected PPR outbreaks. All sera were examined for the presence of PPRV antibodies by C-ELISA, using monoclonal antibodies directed against PPRV nucleoprotein gene. The positive serum samples were indicated by colorless wells whereas negative samples appeared as blue colored wells (Figure 4).
Figure 4. Competitive ELISA (C-ELISA) assay for detection of PPRV antibodies in sheep and goats sera: test plate showing the results of the C-ELISA for detection of PPRV antibodies where colourless wells indicate a positive serum samples and conversely blue wells refer to a negative serum samples.

Notes:

Controls:
PC (A1, B1): Positive Control Serum
NC (C1, D1): Negative Control Serum
TS (E1-H12): Test Sera

Results:
Positive (+ve) serum samples: Colourless wells.
Negative (-ve) serum samples: Blue coloured wells.
3.4. Prevalence of PPRV antibodies in sheep and goats sera

When C-ELISA was performed for detection of PPRV antibodies, of the 320 total sera tested, 259 (80.94%) sera were positive while 61 (19.1%) sera were negative. Results of the C-ELISA yielded an overall sero-prevalence of 80.9% (259/320) (Table 3; Figure5).

On the species basis, of the 258 sheep sera analyzed, 218/258 sera were found positive (+ve) with an overall sero-prevalence of 84.5% whereas only 40/258 (15.5%) sera were found negative (-ve). Additionally, of the 62 goats sera tested by the C-ELISA, 41/62 sera were positive (+ve) with an overall sero-prevalence of 66.1% whereas 21/62 (33.9%) sera were negative (+ve) (Table 3; Figure 5). On the species basis, sheep sera yielded the higher sero-prevalence of 84.5% while goats’ sera yielded a lower overall sero-prevalence of 66.1%.

Table 3. The overall sero-prevalence of PPRV antibodies in sheep and goats sera tested by C-ELISA assay

<table>
<thead>
<tr>
<th></th>
<th>Sheep Sera(%)</th>
<th>Goats Sera(%)</th>
<th>Total No. tested Sera(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Sera</td>
<td>218 (84.5%)</td>
<td>41 (66.1%)</td>
<td>259 (80.9%)</td>
</tr>
<tr>
<td>Negative Sera</td>
<td>40 (15.5%)</td>
<td>21 (33.9%)</td>
<td>61 (19.1%)</td>
</tr>
<tr>
<td>Total No. tested Sera</td>
<td>258 (100%)</td>
<td>62 (100%)</td>
<td>320 (100%)</td>
</tr>
</tbody>
</table>

Figure 5. The prevalence of PPRV antibodies in sheep and goats sera.
3.5. Prevalence of PPRV antibodies in sheep and goats sera in different States of the Sudan:

The overall sero-prevalence of PPRV in different States of the Sudan that reflected by the presence of antibodies against PPRV in sheep and goats sera as demonstrated by C-ELISA assay is 80.94% (Tables 3 and 4). Results indicated that the highest overall sero-prevalence of PPRV antibodies was demonstrated in River Nile State 90.3% (159/176 sera) followed by Gezira State 88.5% (69/78 sera), then Khartoum State 48.4% (31/64 sera) and finally the lowest incidence was present in Northern State 0.0% (0/2 sera) (Table 4).

The sero-prevalence of PPRV in sera in the four different States of the Sudan considering species level is demonstrated in Table 5 and Figure 6. Within sheep, the highest sero-prevalence of PPRV was demonstrated in Gezira State 90.3% (56/62 sera), followed by River Nile State 88.8% (135/152), then Khartoum State 64.3% (27/42 sera) and finally the lowest incidence was present in Northern State 00.0% (0/2 sera) (Table 5; Figure 6A).

Within goats, the highest sero-prevalence of PPRV antibody was demonstrated in River Nile State 100% (24/24) followed by Gezira State 81.2% (13/16 sera) and finally the lowest incidence was present in Khartoum State 18.2% (4/22 sera) (Table 5; Figure 6B).
### Table 4. The overall sero-prevalence of PPRV antibodies in both sheep and goats sera in different States of the Sudan

<table>
<thead>
<tr>
<th>Location</th>
<th>Total No. tested (%)</th>
<th>No. +ve (%)</th>
<th>No. –ve (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern State</td>
<td>2 (100%)</td>
<td>0 (00.0%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>River Nile State</td>
<td>176 (152 sheep + 24 goats)(100%)</td>
<td>159 (135 sheep + 24 goats) (90.3%)</td>
<td>17 (17 sheep + 0 goats) (9.7%)</td>
</tr>
<tr>
<td>Khartoum State</td>
<td>64 (42 sheep + 22 goats) (100%)</td>
<td>31 (27 sheep + 4 goats) (48.4%)</td>
<td>33 (15 sheep + 18 goats) (51.6%)</td>
</tr>
<tr>
<td>Gezira State</td>
<td>78 (62 sheep + 16 goats) (100%)</td>
<td>69 (56 sheep + 13 goats) (88.5%)</td>
<td>9 (6 sheep + 3 goats) (11.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>320 (258 sheep + 62 goats) (100%)</td>
<td>259 (218 sheep + 41 goats) (80.9%)</td>
<td>61 (40 sheep + 21 goats) (19.1%)</td>
</tr>
</tbody>
</table>
Table 5. Sero-prevalence of PPRV antibodies in sheep and goats sera in different States of the Sudan

<table>
<thead>
<tr>
<th>Location</th>
<th>Animal Species</th>
<th>Sheep Sera</th>
<th>Goats Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested (%)</td>
<td>No. +ve (%)</td>
<td>No. -ve (%)</td>
</tr>
<tr>
<td>Northern State</td>
<td>2 (100%)</td>
<td>0 (0.0%)</td>
<td>2 (0.0%)</td>
</tr>
<tr>
<td>River Nile State</td>
<td>152 (100%)</td>
<td>135 (88.8%)</td>
<td>17 (11.2%)</td>
</tr>
<tr>
<td>Khartoum State</td>
<td>42 (100%)</td>
<td>27 (64.3%)</td>
<td>15 (35.7%)</td>
</tr>
<tr>
<td>Gezira State</td>
<td>62 (100%)</td>
<td>56 (90.3%)</td>
<td>6 (9.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>258 (100%)</td>
<td>218 (84.5%)</td>
<td>40 (15.5%)</td>
</tr>
</tbody>
</table>
RESULTS

Figure 6. The prevalence of PPRV antibodies in sheep or goats sera as demonstrated by C-ELISA in four different States of the Sudan:

(A) Sheep sera.

(B) Goats sera.
PPR was firstly recognized in the Sudan in early 1970\textsuperscript{th} (Elhag Ali, 1973), now it is considered endemic in Africa as whole and particularly in the Sudan (OIE, 2013; Kwiatek \textit{et al.}, 2011). The live attenuated cell culture PPRV vaccine derived from Nigeria 75/1 strain is used for control of PPR in small ruminants in the Sudan since 1989 which confers protective immune response for at least 3 years (Diallo \textit{et al.}, 1989; Fadol and ElHussein, 2004; Sen \textit{et al.}, 2010). Despite the application of the effective vaccine, outbreaks of the disease were reported in different parts of the country over the years. Outbreaks of the disease continued to occur covering a huge part and is described in almost all parts of the Sudan. During the years 2016-2017, several suspected PPR outbreaks were reported in different parts of the country including Northern, River Nile, Gezira and Khartoum States. Sera were collected from herds of sheep and goats in the four mentioned States and tested by competitive ELISA for the presence of PPRV antibodies.

Results indicated that an overall sero-prevalence of 80.9\% (259/320) was demonstrated among both sheep and goats. A much lower overall antibodies sero-prevalence of 50.67\% among both sheep and goats was previously demonstrated in the Sudan (Osman \textit{et al.}, 2009). On the species basis, indeed sheep sera yielded the higher antibodies sero-prevalence of 84.5\% (218/258) compared to goats sera which yielded a lower antibodies sero-prevalence of 66.1\% (41/62). In contrast, a much lower PPRV antibodies sero-prevalence of 51.9\% and 56.2\% in sheep and goats, respectively, were reported earlier in the country (Haroun \textit{et al.}, 2002). A recently performed sero-prevalence study revealed lower PPRV antibodies sero-prevalence of 67.1\% and 48.2\% among sheep and goats, respectively, in the Sudan (Saeed \textit{et al.}, 2017). Of note, higher sero-prevalence values were achieved in this study than previously documented. These findings revealed higher sero-prevalence percentages in sheep compared to goats which are in accordance with previous comparable studies performed in the country (Haroun \textit{et al.}, 2002; Saeed \textit{et al.}, 2017). The results indicated the presence of PPR in the country and that the disease is responsible of the outbreaks and the reported clinical cases keeping in mind that infected animals were never vaccinated before against PPR.
DISCUSSION

In the four States under study, where sera were tested, results revealed sero-prevalence of PPRV antibody in River Nile State, Gezira State, and Khartoum State while no incidence at all in Northern State. Indeed, previous studies for sero-surveillance of PPR in the country indicated the distribution of PPR in most of the country including the States under study (Osman et al., 2009; Saeed et al., 2017).

In the four States under study where sera were tested, the highest prevalence of PPRV antibody was demonstrated in River Nile State 90.3% (159/176 sera) followed by Gezira State 88.5% (69/78 sera), then Khartoum State 48.4% (31/64 sera) and the lowest prevalence was present in Northern State 0.0% (0/2 sera) with no incidence at all. Concerning areas where outbreaks occurred, the higher incidence represents areas where there is a huge number of animals and where animal movement occurs regularly and could leads to the exposure of naïve animals to PPRV causing infection and disease.

In the Northern State of the Sudan, the animal movement is restricted due to the location of the area in the far north of the Sudan and it is occupied mostly by the desert. The findings indicated that PPR is currently circulating widely in the Sudan and still is a leading cause to a clinical disease and higher fatalities. The continual presence of PPR outbreaks, despite the application of PPRV vaccine, might be due to that the vaccination program is not reaching herds of animals in some areas. Therefore, the effective PPR vaccine is recommended to be used with a plan for covering all parts of the Sudan to prevent the occurrence of the disease and the appearance of outbreaks.

Competitive ELISA can detect PPRV antibodies in the sera of animals after the first week of infection. C-ELISA represents a fast, sensitive specific and accurate assay for detection of PPRV antibodies in the sera of affected sheep and goats (Anderson and Mckay, 1994; Saliki et al., 1993; Libeau et al., 1995; Singh et al., 2004). Finally these finding indicated that competitive ELISA appears suitable as a serological diagnostic tool for PPR.
CONCLUSION AND RECOMMENDATION

Conclusion:

1- PPRV antibodies were detectable in sheep and goats in each region under study where outbreaks were reported.

2- This indicated the wide spread distribution of PPR in the Sudan despite vaccination program against PPR and the ongoing eradication program organized by the FAO and OIE.

3- C-ELISA can be used effectively for sero-epidemiological studies on PPR.

Recommendations:

1- PPR was distributed widely in the Sudan, thus it is recommended to use the PPRV vaccine in all part of the country.

2- Sero-prevalence study should be established in other States of the Sudan with high animal population.

3- Control of animal movement from endemic areas.
REFERENCES


REFERENCES


REFERENCES


