بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection of Peste des Petits Ruminants Virus Antigen in Pneumonic Lungs from Camels at Al Sahafa Slaughterhouse, Khartoum State

الكشف عن انتجين فيروس طاعون المجترات الصغيرة في انسجة رئوية من إبل مصابة بالإلتهاب الرئوي بمسلخ الصحافة ، ولاية الخرطوم

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

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January, 2021

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 and the Virology department at the Central Veterinary Research Laboratory (CVRL), Soba, from January 2019 to January 2021 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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Dr. Nussieba Ahmed Osman Elhag

Supervisor

January 2021

Dedication

To my mother

To my brothers & my sisters

For the deceased soul my father

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Abstract

The present study was conducted to determine the presence and antigenic-prevalence of PPR among camels slaughtered at Al-Sahafa slaughterhouse, Khartoum State, Sudan. During June to December 2019, 78 lung samples, showing pneumonic lesions at post-mortem, were collected from camels from Al-Sahafa slaughterhouse, Khartoum State. Camels slaughtered at Al-Sahafa slaughterhouse were from Darfur and Butana breeds and their ages ranged mostly from 1 to 3 years but also camels up to 7 years old. No obvious apparent clinical signs were observed in camels during ante-mortem. However, many pneumonia lesions and changes in the lung colour and shape were observed during post-mortem.

Camel lung samples prepared as 20% lung tissue homogenate were screened for the presence of PPRV antigen using the haemagglutination (HA) test using 1% chicken red blood cells (RBCs) suspension, PBS of pH 6.8 and 4°C for incubating the HA plates in order to achieve highest haemagglutination titres. After 17-18 minutes incubation, results of the HA indicated that 76/78 samples were positive for PPRV antigen with an overall antigenic prevalence of 97.4% and only 2/78 (2.6%) samples were found negative. The HA titres for the positive samples ranged from 2 to 32 haemagglutination units (HAU) with mean titre of 10 HAU. Of note, two samples only showed a very high haemagglutination titre of 32 HAU whereas apparently most of the samples achieved HA titre of 8 HAU. The results showed that PPRV could be detected in pneumonic lungs of camels indicating their role in causing pneumonia in camels. Further studies are needed to investigate whether camels may transmit the virus to other susceptible animal species, or not, and to determine their role in the epidemiology of the disease in the Sudan.

ملخص البحث

أجريت الدراسة الحالية لتحديد وجود وانتشار المعدل الانتجيني لفيروس طاعون المجترات الصغيرة بين الإبل المذبوحة في مسلخ الصحافة بولاية الخرطوم ، السودان. خلال الفترة من يونيو إلى ديسمبر 2019 ، تم جمع 78 عينة من رئة الابل والتي تظهر فيها التهابات رئوية خلال فحص ما بعد الذبح ، هذه العينات تم جمعها من مسلخ الصحافة بولاية الخرطوم. هذه الإبل التي تم ذبحها في مسلخ الصحافة كانت من سلالات دارفور والبطانة وتراوحت أعمارها في الغالب ما بين 1 إلى 3 سنوات مع وجود بعض الإبل التي يصل عمرها حتى 7 سنوات. لم يلاحظ ظهور اي علامات سريرية واضحة في الإبل خلال فحص ما قبل الذبح. ومع ذلك ، لوحظت العديد من آفات الالتهاب الرئوي والتغيرات في لون الرئة وشكلها أثناء فحص مابعد الذبح.

تم تحضير مستخلص بتركيز 20% من عينات رئة الإبل ثم تم فحص هذا المستخلص للكشف عن وجود انتجين فيروس طاعون المجترات الصغيرة باستخدام إختبار تلازن كريات الدم الحمراء وذلك باستخدام محلول خلايا كريات الدم الحمراء للدجاج بتركيز 1٪ ، ومحلول الفوسفات المتعادل بدرجة حموضة 6.8 و تم حفظ اطباق الإختبار في درجة حرارة تعادل 4 درجات مئوية وذلك لضمان الحصول علي أعلى معدلات انتجينيه للفيروس. بعد مرور 17-18 دقيقة من حفظ الاطباق ، اوضحت نتائج اختبار تلازن كريات الدم الحمراء إلى أن 78/76 عينة كانت إيجابية لفيروس طاعون المجترات الصغيرة مع وجود انتشار انتجيني عام بنسبة 97.4٪ وفقط 78/2 عينة (2.6٪) كانت سلبية. تراوح معدل تلازن كريات الدم الحمراء للعينات الإيجابية من 2 إلى 32 وحدة لتلازن الدم بمتوسط 10 وحدة لتلازن الدم. من الجدير بالذكر أن عينتين فقط أظهرتا معدلًا عاليًا جدًا من التلازن الدموي يبلغ 32 وحدة لتلازن الدم بينما يبدو أن معظم العينات حققت معدل 8 وحدة لتلازن الدم. وأظهرت النتائج أن فيروس طاعون المجترات الصغيرة يمكن كشفه في عينات الرئة المصابة بالإلتهاب الرئوي للإبل مما يشير إلى دورها كمسبب للإلتهاب الرئوي في الإبل. هناك حاجة إلى مزيد من الدراسات لمعرفة ما إذا كانت الإبل قد تنقل الفيروس إلى أنواع أخرى من الحيوانات القابلة للإصابة بالمرض ، ام لا ، ولتحديد دورها في وبائيات المرض في السودان.

Introduction

Peste des petits ruminants, known recently as a transboundary animal disease, affects basically small ruminants "sheep and goats" with goats being more sensitive to PPR viral infection than sheep (Lefevre and Diallo 1990; Parida *et al.,* 2015) with several outbreaks had been reported in camels, other large ruminants and some wildlife species (OIE, 2019; Rahman *et al.,* 2018; 2020a).

Peste des petits ruminants (PPR), is caused by peste des petits ruminants virus (PPRV), a member of the *Small Ruminant Morbillivirus* (SRMV) species, *Morbillivirus* genus, *Orthoparamyxovirinae* subfamily, *Paramyxoviridae* family, *Mononegavirales* order alongside with other important viral pathogens (Gibbs *et al.,* 1979; Amarasinghe *et al.,* 2019; Maes *et al.,* 2019).

Nowadays, PPR is known to be present and endemic in huge parts of Africa, except the southern countries, the Middle East, most of Asia, and only in few countries "Turkey and Bulgaria" in Europe (Banyard *et al.,* 2010; OIE-WAHIS, 2018; OIE, 2019). PPRV generally causes fever, conjunctivitis, gastroenteritis, and pneumonia in its susceptible host species (OIE, 2019). Many cases of co-infections of PPRV with other viruses such as orf (Saravanan *et al.,* 2007), goatpox virus (Malik *et al.,* 2011) and bluetongue virus (Mondal *et al.,* 2009) had been reported.

First outbreaks of PPR in the Sudan were reported during 1971 in flocks of sheep and goats in Gedarif, eastern Sudan and during 1971 and 1972 in Sinnar and Meiliq in Central Sudan (Elhag Ali, 1973). The disease was firstly diagnosed as a rinderpest-like disease of sheep and goats. However, many years later PPRV was confirmed as the cause of these outbreaks (Elhag Ali and Taylor, 1984). At the beginning of the 2000th antibodies against PPRV had been detected in sera of camels in Sudan using C-ELISA (Haroun *et al.,* 2002). Interestingly, the first outbreak of PPR in camels with clinical signs of the disease was reported in Kassala, Eastern Sudan in 2004 (Khalafalla *et al.,* 2010). Many respiratory viral diseases of camels such as bovine herpes virus-1 (BHV-1) (Saeed *et al.,* 2009), bovine viral diarrhea virus (BVDV) (Saeed *et al.,* 2010a), respiratory syncytial virus (RSV) (Saeed *et al.,* 2010b), parainfluenza virus 3 (PIV3) (Saeed *et al.,* 2010c), and adenovirus (Saeed *et al.,* 2010d) had been detected in pneumonic camel lungs in the Sudan. A recent study demonstrated a mixed infection of PPRV with many respiratory viruses "Parainfluenza virus 3 (PIV 3), respiratory

syncytial virus (RSV), bovine herpes virus-1 (BHV-1), bovine viral diarrhea (BVD), and adenovirus in pneumonic lungs of dromedary camels in Sudan (Saeed *et al.*, 2015). Camels can be infected with PPRV and expressing the characteristic clinical signs of the diseases associated with high fatalities and abortion in pregnant females (Roger *et al.*, 2000; 2001; Khalafalla *et al.*, 2010; Omani *et al.*, 2019; Zakian *et al.*, 2016; Rahman *et al.*, 2020a). In some occasions, PPRV antibodies had been detected in dromedary camels (El-Dakhly, 2015; Woma *et al.*, 2015; Shabbir *et al.*, 2020; Rahman *et al.*, 2020b). Other studies reported presence of PPRV antigens in pneumonic lung tissues of camels in slaughterhouses (Ayelet *et al.*, 2013; Saeed *et al.*, 2015; Abdalla, 2019). Camels can be infected and are dead-end host for PPRV as there is no evidence of virus shedding or transmission to other susceptible species (Schulz *et al.*, 2019).

Objectives:

The present study is designed to accomplish the following goals:

1. To investigate the presence and the antigenic prevalence of PPRV antigen in pneumonic lung tissues collected from camels slaughtered at Al-Sahafa slaughterhouse in Khartoum State, Sudan.
2. To demonstrate the usefulness of the haemagglutination (HA) test for diagnosis of PPR.

Chapter I
Literature Review

1.1. Definition of PPR:

Peste des petits ruminants (PPR) is an acute, contagious, and frequently fatal disease of sheep and goats causing high morbidity and mortality rates (Wang *et al.*, 2009; Parida *et al.*, 2015; OIE, 2019). Outbreaks of the disease also have been reported recently in camels (Roger *et al.*, 2000; 2001; Khalafalla *et al.*, 2010; OIE, 2019).

The disease is characterized by depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing and cough, diarrhoea and death (Roeder and Obi, 1999; OIE, 2019).

1.2. Synonymous of PPR:

PPR is also known as sheep and goat plague, goat plague, Kata 'syndrome of stomatitis pneumo-enteritis', pseudo-rinderpest of small ruminants, pest of small ruminants, pest of sheep and goat, contagious pustular stomatitis, pneumo-enteritis complex, stomatitis pneumo-enteritis complex and ovine rinderpest (Merck, 2010).

1.3. Economic importance of PPR:

Peste des petits ruminants (PPR) is an important factor which influences the productivity and economy of an animal farm (Kulkarni *et al.*, 1996) particularly in the inter-tropical regions of Africa, in the Arabian Peninsula, the Middle East and Asia (Wang *et al.*, 2009). The economic impacts of the disease are due to the direct losses through the high morbidity of 50% to 100% and mortality rates that reached 100% (Roeder and Obi, 1999; Singh and Prasad, 2008). The economic losses associated with PPR occur indirectly due to restrictions on animal movements like limiting trade, export, import of new breeds and the development of intensive livestock production (Kitching, 1988; OIE, 2019).

1.4. History of the disease:

In the early 1940's, a fatal disease of goats with high mortality, was first described as peste-des-petits-ruminants (PPR) in the Ivory Coast (Cote d'Ivoire) in West Africa

(Gargadennec and Lalanne, 1942). Following that the disease continued to spread to other African countries. PPR was first appeared as a recognized disease in Asia in the late 1980s (Shaila *et al.*, 1989; 1996). PPRV has continued to spread across middle, and East Africa and Asia (Dhar *et al.*, 2002). In East Africa, the first outbreak of the disease in sheep and goats in Sudan was firstly diagnosed as rinderpest and later confirmed to be PPR (Elhag Ali and Taylor, 1984). PPR outbreaks occurred in 2007 in Uganda and Kenya and in 2009 in Tanzania (WAHIS, 2009). Currently, PPR is known as endemic and occurred across most of Africa and Asia, the Middle East and the Indian subcontinent (Banyard *et al.*, 2010; OIE, 2019). Recently, the disease has been described only in Turkey and Bulgaria from Europe (OIE-WAHIS, 2018; OIE, 2019).

1.5. Etiology:

1.5.1. Virus classification:

PPR is caused by peste des petits ruminants virus (PPRV) which has been classified under the species *Small Ruminant Morbillivirus* in the genus *Morbillivirus* in the subfamily *Orthoparamyxovirinae* in the family *Paramyxoviridae* in the order *Mononegavirales* (Gibbs *et al.*, 1979; Amarasinghe *et al.*, 2019; Maes *et al.*, 2019). The *Morbillivirus* genus contains several viruses which are closely related to PPRV and include rinderpest virus (RPV) of cattle and buffaloes, canine distemper virus (CDV) of carnivores, the measles virus (MeV) of humans, phocine distemper virus (PDV) of seals, cetacean morbillivirus (CeMV) which include dolphin (DMV) and porpoise (PMV) morbilliviruses of aquatic mammals, and feline morbillivirus (FeMV) (Amarasinghe *et al.*, 2019; Maes *et al.*, 2019). Morbilliviruses were known as restricted in their ability to infect different species, however, recently its host range has been expanded. PPRV was initially believed to be restricted to small ruminant hosts, but currently the virus has many hosts from domesticated ruminants and wildlife species (Rahman *et al.*, 2018; 2020a).

1.5.2. Size and morphology:

The negative-stain electron microscopy showed the structure of morbillivirus virions as pleomorphic, enveloped particles (Bourdin and Laurent-Vautier, 1967; Gibbs *et al.*, 1979). The size of PPR virus particle ranges between 400 and 500 nm (Gibbs *et al.*, 1979). During virus budding, the viral envelope is derived from host infected cell

membrane, and is studded with glycoprotein peplomers consisting of the viral fusion (F) and haemagglutinin (H) glycoproteins (cited in Parida *et al.,* 2015).

1.5.3. Genomic Structure and viral proteins:

The genome of PPRV is a single-stranded, negative-sense, enveloped RNA with 15,948 Kbp in length. It has 6 transcription units encodes for six structural proteins and two non-structural proteins in the order: 3´-N-P(C/V)-M-F-H-L-5´ (Bailey *et al.,* 2005; Mahapatra *et al.,* 2006).

All members of the *Morbillivirus* genus possess six structural proteins namely, the nucleo-capsid protein (N), which encapsulates the virus genomic RNA; the phosphoprotein (P), which associates with the polymerase or large protein (L); the matrix (M) protein which is intimately associated with the internal face of the viral envelope making a link between the nucleo-capsid and the virus external glycoproteins (Bailey *et al.,* 2005; Chard *et al.,* 2008). The viral envelope glycoproteins are the fusion (F) protein and the haemagglutinin (H) which are responsible for the attachment and fusion, respectively, which allows the penetration and entry of the virus into the cell in order to be infected. There are two more non-structural proteins, C and V, which are produced during the infection phase from the phosphoprotein transcription unit (Bailey *et al.,* 2005; Mahapatra *et al.,* 2006).

1.6. Epidemiology of PPR:

1.6.1. Geographic distribution:

PPR was first described in 1940 in the Ivory Coast (Cote d'Ivoire) in West Africa (Gargadennec and Lalanne, 1942). The disease has since been described in other West African countries like Senegal (Mornet *et al.,* 1956), Ghana and Nigeria (Johnson and Ritchie, 1968). Currently, the disease is present in all countries in most of Africa, the Middle East with the extension to Europe in Turkey and Bulgaria, and in most of Asia (Banyard *et al.,* 2010; OIE, 2019) (Figure 1).

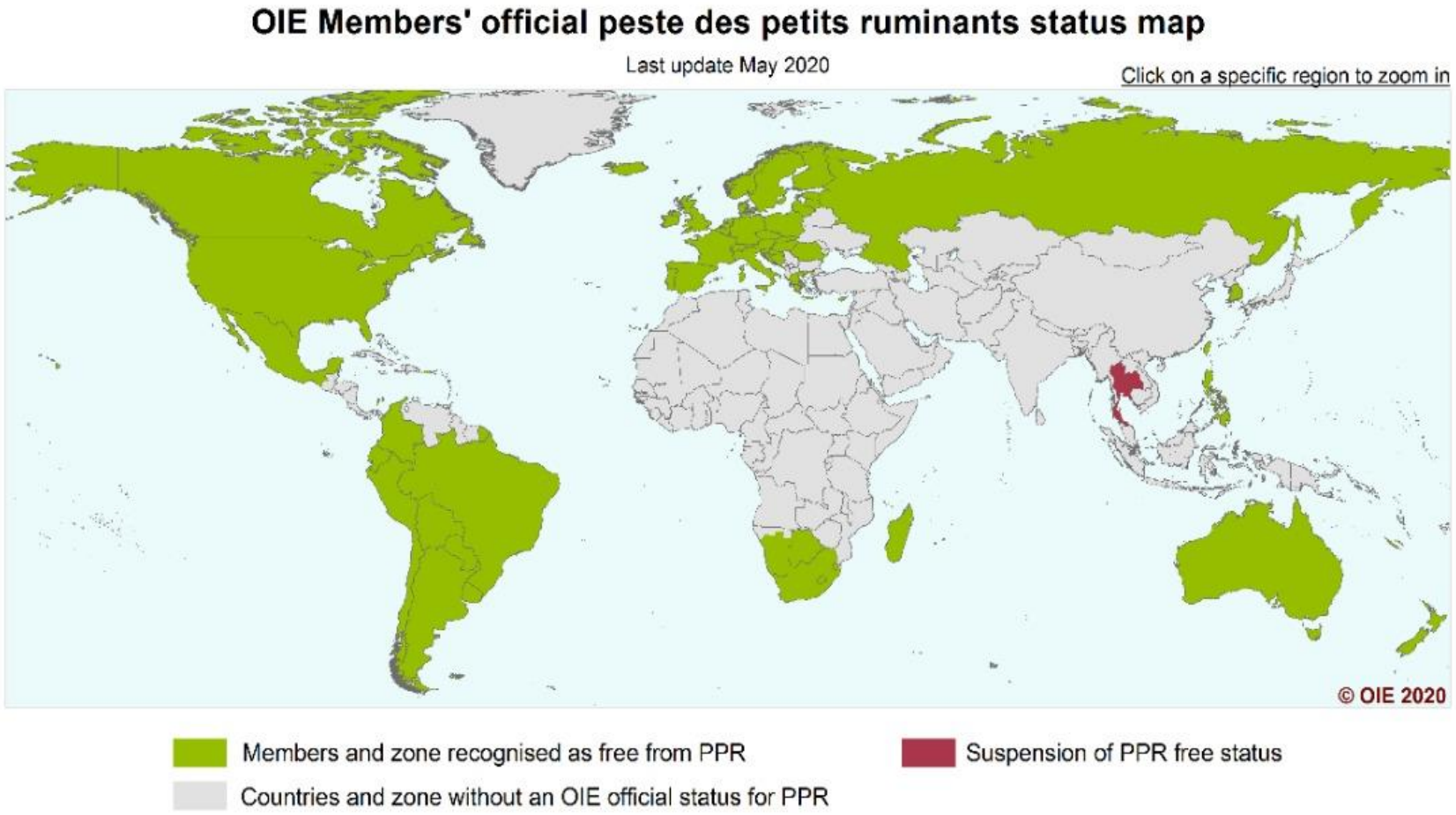

Figure 1. Official peste des petits ruminants status map. World Organization for Animal Health (OIE, 2020): http://www.oie.int/en/animal-health-in-the-world/ppr-portal/distribution/

1.6.2. Transmission of PPR:

Shedding of PPRV occurs in nasal and lacrimal secretions, milk, saliva, urine and faeces (Couacy-Hymann *et al.,* 2007; OIE, 2019). PPRV shedding by infected animals happens during a relatively short period after infection (Banyard *et al.,* 2010). Therefore, close contact with infected animals is necessary for PPR virus transmission. PPRV infection is transmitted mainly by inhalation of aerosols or by contact with secretions or excretions of infected animals. Oral transmission is really via ingestion of contaminated feed and water (OIE, 2019). Animals may acquire the infection by licking or muzzling each other. Virus may transmit indirectly by fomites, in which the virus can survive for up to 72 hours, depending on humidity, temperature, the amount of sunlight and many other factors (Abubakar *et al.,* 2008). Additionally, PPR is generally considered as a seasonal disease, with a peak of infections usually occurring in the dry, cool season in endemic areas of Africa (Abubakar *et al.,* 2009).

1.6.3. Host range:

PPR affects small ruminants and many other animal species but it is not a zoonotic disease. Nanda *et al.* (1996) reported that goats are more susceptible than sheep, but this has not been confirmed in other outbreaks (Singh *et al.,* 2014). Considering different breeds, West African goats were more susceptible than European varieties (Couacy-Hymann *et al.,* 2007). Cattle and pigs can be infected with the virus with detectable sero-conversion, but infected the animals showed no clinical signs and cannot transmit the disease (Nawathe and Taylor, 1979; Anderson and McKay, 1994; Lembo *et al.,* 2013; Schulz *et al.,* 2019).

Camels are susceptible to PPR viral infection but their ability to transmit the disease to other susceptible in-contact animals is still to be clarified by experimental infections. Camels showed sero-conversion following PPRV infection (Roger *et al.,* 2001). Camels in Sudan had been infected by PPR and showed clinical expression of the disease (Khalafalla *et al.,* 2010; Kwiatek *et al.,* 2011). Antibodies against PPR were detected in Ethiopian camels (Abraham *et al.,* 2005). Nevertheless, experimental infection of dromedary camels in Dubai, with an Ethiopian PPRV strain originated from goats, did not produce any clinical disease (Wernery, 2011). In Morocco, camels vaccinated with PPRV Nigeria 75/1 vaccine strain showed very poor immunological responses

(Wernery, 2011). There is a need for more research to find out if camels can excrete the virus and to elucidate their role in the epidemiology of PPR.

1.6.4. PPR in the Sudan:

Elhag Ali (1973) firstly reported appearance of a PPR-like disease in sheep and goats in Gedarif in Eastern Sudan in 1971. During 1971-1972 outbreaks of the disease were reported in Sinnar and Mieliq, Central Sudan (Elhag Ali and Taylor, 1984). Based on the clinical signs, the disease was firstly diagnosed as rinderpest (RP) but virus isolation confirmed the causative agent as PPR (Elhag Ali and Taylor, 1984). Detection of PPRV antigen and isolation of viruses were performed from different outbreaks occurred in many areas of the Sudan (Zeidan, 1994, Saeed *et al.,* 2004; Osman, 2005; Osman *et al.,* 2008; Saeed *et al.,* 2010e; Saeed *et al.,* 2017). Since then continuous outbreaks of the disease were reported annually and almost in all areas (Saeed *et al.,* 2004; Osman, 2005; Saeed *et al.,* 2010e; Enan *et al.,* 2013; Saeed *et al.,* 2017). Sero-prevalence studies were performed in different area in the Sudan (Haroun *et al.,* 2002; El Amin and Hassan, 1999; Osman *et al.,* 2009; Saeed *et al.,* 2010e; Saeed *et al.,* 2017; Osman *et al.,* 2018).

At the beginning of the 2000th antibodies against PPRV had been detected in sera of camels in Sudan (Haroun *et al.,* 2002). Interestingly, Khalafalla *et al.* (2010) reported the first severe outbreak of PPR in camels in Kassala, Easten Sudan which was characterized with high morbidity and mortality rates. Many viral diseases affecting the respiratory system of camels such as bovine herpes virus-1 (BHV-1) (Saeed *et al.,* 2009), bovine viral diarrhea virus (BVDV) (Saeed *et al.,* 2010a), respiratory syncytial virus (RSV) (Saeed *et al.,* 2010b), parainfluenza virus 3 (PIV3) (Saeed *et al.,* 2010c), and adenovirus (Saeed *et al.,* 2010d) had been detected in pneumonic camel lungs in the Sudan. A recent study demonstrated a mixed infection of PPRV with many respiratory viruses "Parainfluenza virus 3 (PIV 3), respiratory syncytial virus (RSV), bovine herpes virus-1 (BHV-1), bovine viral diarrhea (BVD), and adenovirus in pneumonic lungs of dromedary camels in Sudan (Saeed *et al.,* 2015). The presence of PPRV antigens in pneumonic lung tissues of camels in slaughterhouses was demonstrated (Saeed *et al.,* 2015; Abdalla, 2019).

Recently, a sero-prevelance study for detecting antibodies against PPRV in cattle sera was performed (Ali *et al.,* 2019).

1.7. Clinical signs:

The disease represents in different forms; peracute, acute and subacute (Losos, 1986). The disease is characterized by presence of high morbidity and mortality rates that can reach 100% among susceptible animals (Dhar *et al.*, 2002). In the per-acute form, infected animals cannot survive more than one week after onset of fever (Diallo, 2003). In the majority of cases, PPR is an acute disease which appears after an incubation period of 3 to 6 days after direct contact or inoculation of the infectious material (Hamdy *et al.*, 1976; Bundza *et al.*, 1988). The disease is characterized by the sudden onset of depression, fever, discharge from the eyes and nose, serous fluid from the mouth, disturbed breathing and cough, foul smelling diarrhea and finally death (Khan *et al.*, 2007; Al-Majali *et al.*, 2008). The disease begins with presence of pyrexia up to 41°C that lasts for 3-5 days; then these animals become depressed, anorexic and develop a dry muzzle. Serous oculo-nasal discharges which progressively turned into mucopurulent and finally may block the nares and encrust the muzzle causing the animal to sneeze, whereas the ocular discharges may cause matting of the eye lids (OIE, 2019). After few days of the onset of fever, the gums become hyperemic, then erosive lesions develop in the oral cavity which may later become necrotic with presence of excessive salivation. In the later stages of the disease, animals have watery bloody diarrhoea and respiratory signs, death may occur within one week of the onset of the disease (OIE, 2019).

1.8. Post-mortem findings:

The post-mortem changes caused by PPRV infection involve the alimentary and respiratory tracts (Losos, 1986; Barker *et al.*, 1993). These lesions are very similar to those present in cattle infected with rinderpest virus, except that prominent crusty scabs along the outer lips and severe interstitial pneumonia were frequently occur in PPR infected animals. Erosive lesions extend from the mouth to the reticulo-rumen junction. Characteristic linear red areas of congestion or haemorrhages called as Zebra stripes were present along the longitudinal mucosal folds of the large intestine and rectum, but they are not a consistent finding. Erosive or haemorrhagic enteritis is a usual finding where the ileo-caecal junction is commonly involved. Lymph nodes are enlarged while the spleen and liver showed necrotic lesions (OIE, 2019).

1.9. Diagnosis of PPR:

1.9.1. Clinical diagnosis of PPR:

The characteristic clinical signs can be used for a tentative diagnosis of PPR. However, laboratory diagnosis using many virological, serological and molecular techniques is necessary for confirmatory diagnosis of PPRV infections (OIE, 2019).

1.9.2. Laboratory diagnosis of PPR:

1.9.2.1. Sample required for diagnosis:

The ideal samples for diagnosis of PPR include nasal and lacrimal swabs, whole blood from live animals. These samples are better to be collected during the very early febrile stage of the disease and are used for diagnosis of PPR using antigen detection, virus isolation and identification, polymerase chain reaction (PCR) and haematology (OIE, 2019).

Alternatively, tissues such as lymph nodes, especially the mesenteric and bronchial lymph nodes, lungs, spleen and intestinal mucosa are collected aseptically from dead animals. For the purpose of histopathology, organ samples are collected in 10% neutral buffered formalin (OIE, 2019).

1.9.2.2. PPR virus isolation:

Virus isolation is a gold standard test for PPR diagnosis, primary cell culture such as goat and sheep kidney cells, and cell lines like Vero cells or marmoset B-lymphoblastoid (B95a) cell lines (Sreenivasa *et al.*, 2006; OIE, 2019) can be employed. Vero cell line is usually used for PPR virus isolation from clinical samples and also for infectivity assays (Sreenivasa *et al.*, 2006; Kumar *et al.*, 2013).

1.9.2.3. Antigen detection methods:

In general, agar gel immunodiffution test (AGID) or agar gel precipitation test (AGPT) (Osman *et al.*, 2008; OIE, 2019), counter-immuno electrophoresis (CIEP) (Majiyagbe *et al.*, 1984), haemagglutination (HA) test (Wosu, 1985; 1991), serum or virus neutralisation test (VNT) (OIE, 2019), nucleic acid hybridization (Pandey *et al.*, 1992) and different ELISAs using PPRV-specific monoclonal antibodies (MAbs) (Saliki *et al.*,

1993; Anderson and Mckay, 1994; Libeau *et al.*, 1994; Singh *et al.*, 2004) are used for diagnosis of PPR.

1.9.2.3.1. Agar gel immunodiffution test (AGID):

Agar gel immunodiffusion (AGID) is a very simple, cheap test for detection of PPRV antigen that can be performed in any laboratory although it is not sensitive. It is a group-specific test thus cannot differentiate between PPRV and RPV and other morbilliviruses. The test is not rapid with results being obtained after 24 or 48 hours (Osman *et al.*, 2008; OIE, 2019).

1.9.2.3.2. Enzyme-linked immunosorbent assay (ELISA):

ELISA is a very rapid, simple and sensitive assay for detection of PPRV antigen. The assay based on the use of monoclonal antibodies (MAbs) prepared against PPRV for detection of antibodies and antigens in different ELISAs (Saliki *et al.*, 1993; Anderson and Mckay, 1994; Libeau *et al.*, 1994; 1995). Libeau *et al.* (1994) developed a specific viral antigen detection ELISA that is able to differentiate between PPR and RP. Singh *et al.* (2004) developed MAb-based immuno-capture ELISA and sandwich ELISAs (sELISA) which had been used extensively for detection of PPRV antigen in clinical specimens. The immuno-capture ELISA, using two monoclonal antibodies (MAb) raised against the N protein of PPRV, is very specific and sensitive in rapid identification of PPRV (Libeau *et al.*, 1994). A sandwich ELIS using MAb (4G6) directed against an epitope of the N protein of PPRV is routinely being used for PPR antigenic prevalence in India (Singh *et al.*, 2004; 2006).

1.9.2.3.3. Haemagglutination (HA) test:

Among morbilliviruses, only PPR virus and measles virus, havehaemagglutination properties for red blood cells (Wosu 1985; Wosu 1991; Ramachandran *et al.*, 1993; Ezeibe *et al.*, 2004). Therefore, the haemagglutination (HA) test can differentiate between PPRV and RPV which has no detectable haemagglutinating properties (Huygelen, 1960).

Haemagglutination (HA) test is known as a quick, easy, simple, cheap and reliable confirmatory test for diagnosis of PPR (Wosu, 1985; 1991). The HA test, is useful for

detection of PPRV in samples from live as well as dead animals (Wosu, 1991; Ezeibe *et al.*, 2004; Osman *et al.*, 2008). RBCs from different animal species can be used in the HA test, however, chicken RBCs is found as the most sensitive for detection of PPRV and obtaining the highest HA titers in a short time (Ezeibe *et al.*, 2004; Osman *et al.*, 2008).

1.9.2.4. Serological Diagnosis of PPR:

Serological tests such as agar gel immuno-diffusion test (AGID)/ agar gel immunoprecipitation test (AGPT) (Obi and Patrick, 1984), counter-immuno electrophoresis (CIEP) (Obi and Patrick, 1984) and indirect ELISA, can be used for tentative diagnosis of PPR, however, these tests does not differentiate between PPR and RP viral infections (Obi *et al.*, 1990; Palaniswami *et al.*, 2005). Competitive ELISA and virus neutralization tests are recommended by the OIE for the specific and sensitive serological diagnosis of PPR (OIE, 2019)

1.10. PPR in camels:

Many studies confirmed camels as susceptible to PPR viral infection with outbreaks and seroconversion being reported in Ethiopia (Roger *et al.*, 2000, 2001; Abraham *et al.*, 2005), Sudan (Khalafalla *et al.*, 2010), Iran (Zakian *et al.*, 2016) and Kenya (Omani *et al.*, 2019). PPR in camels in Sudan characterized by colic, yellowish and later bloody diarrhea, difficulty in breathing, sudden death of apparently healthy animals, high mortality rates, abortion of pregnant she camels and other clinical signs of PPR (Khalafalla *et al.*, 2010). Infected camels in Iran developed clinical signs included sudden death, fever, oral erosion, an ecthyma-like lesions, yellowish diarrhea, pneumonia and respiratory distress, enlargement of lymph node, severe dehydration, dermatitis, ulcerative keratitis, and conjunctivitis (Zakian *et al.*, 2016). The necropsy findings of PPR in camels included lung congestion and consolidation, paleness and fragility of the liver, enlarged lymph nodes, congestion and hemorrhage of the small intestines and stomach (Khalafalla *et al.*, 2010). Zakian *et al.* (2016) reported keratoconjunctivitis, congestion and consolidation of the lung, paleness of the liver, and enlargement and edema of lymph nodes in PPRV-infected camels. Camels infected by PPRV in Kenya manifest characteristic clinical signs of the disease included in-

appetence, loss of body condition, and general weakness, diarrhoea, conjunctivitis, and ocular nasal discharges preceding death (Omani *et al.*, 2019).

Other studies reported presence of PPRV antigens in pneumonic lung tissues of camels in slaughterhouses (Ayelet *et al.*, 2013; Saeed *et al.*, 2015; Abdalla, 2019).

Sero-prevalence studies demonstrated presence of PPRV antibodies among camels population in Egypt (Ismail *et al.,* 1992), Sudan (Haroun *et al.,* 2002; Saeed *et al.,* 2017), Tanzania (Swai *et al.,* 2011), Nigeria (Bello, 2013; El-Yuguda *et al.,* 2013; Woma *et al.,* 2015), Libya (El-Dakhly, 2015), India (Rajneesh and Tanwar, 2011) and Pakistan (Shabbir *et al.,* 2020; Rahman *et al.,* 2020b).

In Germany, an experimental infection study confirmed that camels can be infected by PPRV but are dead-end host for PPRV as there is no evidence of virus shedding or transmission to other susceptible species (Schulz *et al.,* 2019). In Morocco, camels experimental infected with virulent PPRV strain lineage IV did not show any clinical signs and the virus was not detected although animals were seroconvert (Fakri *et al.,* 2019).

Appearance of PPR outbreaks in camels suggested their possible role in the epidemiology of the disease. However, the role of camels and other atypical hosts in the epidemiology of the disease needs more investigations.

Chapter II

Materials and Methods

2.1. Materials:

2.1.1. Study area:

The study was conducted in Khartoum State (Central Sudan) which is located at the coordinates 15º 47 North and 32º 43 East. The samples for this study were collected from Al-Sahafa slaughterhouse during 2019.

2.1.2. Reference PPR viruses:

The reference virus used in the study was PPRV Nigeria 75/1 vaccine strain. Also 20% lung tissue suspension from lungs of camels (Abdalla, 2019), sheep and goats (Alhussein *et al.,* 2020), which were tested positive against PPRV antiserum, were used as the positive control PPRV antigens in the haemagglutination (HA) test, and kept at -20°C.

2.1.3. Chemicals and Reagents:

Chemical Name	**Company**
PBS tablets	Caisson Laboratories, USA
NaCl	Sigma, USA
KCl	Sigma, USA
Na_2HPO_4	Sigma, USA
KH_2PO4	Sigma, USA
Na citrate	Sigma, USA
Citric acid	Sigma, USA
D-glucose	Sigma, USA
Ethanol Absolute	Romil, UK

2.1.4. Antibiotics and Antifungal:

Antibiotic name	**Company**
Benzylpenicillin Sodium	NCPC,China
Streptomycin Sulphate	NCPC,China

Antibiotic name	**Company**
Mycostatin	NCPC,China
Gentamycin	NCPC,China

2.1.5. Apparatus, equipment and instruments:

Name	**Company**
Hood or Laminar Flow Safety Cabinet-Class II	BDSL (Biological & Diagnostic Supplies Ltd.)
Autoclave	SANO clav, Germany
Sanyo OMT Oven	Gallenkamp, UK
Refrigerated Centrifuge	Centurion Scientific Ltd., UK
Jenway pH Meter	Bibby Scientific Ltd., UK
AM69 Microshaker (Cooke Microtiter System)	Denley-Tech Ltd., 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
Plastic ware	-
Mortar and Pestle	-
Scissors	-
Forceps	-
Scalpel and Blades	-
96-well V-bottom micro-well haemagglutination plate	-

2.1.6. Disposables:

Name	**Company**
Sealed Plastic Bags	-

Ice bags or Ice	-
Container for sample transportaion	-
Latex Examination Gloves	Okjo Medical Consumables, Malaysia
Face Mask	-
Yellow Tips (sterile)	Marina Pharma, China
Blue Tips (sterile)	Marina Pharma, China
Trough	-
Syringes	Ava-med Medical Industries, Sudan
Eppendorf tubes (sterile)	Zhejinag Medicines & Health Products, China
Boxes for Eppendorf Tube	-
Tube Rack	-
15 ml Blue cap tubes (Sterile)	Zhejinag Medicines & Health Products, China
Stainless steel tray	-
Absorbent Cotton Wool	Anji Speng Industrial Co. Ltd
Disinfectant/Dettol	-
Aluminum Foil	-
Detergent Micro-90	International Products Corporation, USA

2.1.7. Preparation and sterilization of glassware:

All instrument, glassware and plastic ware used in the virology laboratory were washed by brush using Detergent Micro-90 diluted in water, then the detergent was washed away by rinsing 10 times in tab water followed by 3 times in distilled water, then left to dry under air. The sterilization of instruments, glassware and plastic ware was based on dry heat sterilization using the oven at 180°C or 200°C for 2 hours or wet heat sterilization using the autoclave at 121°C for 30-60 minutes. The choice of the sterilization method depends on the materials it's made from.

Mortar and pestle along with the sharp stainless steel instruments used in dissection (forceps, scalpel and scissors), glassware (measuring cylinders and flasks) were sterilized

by oven at 180ºC-200°C for 2 hours. Disposables and plastic wares such as tips, Eppendorf tubes were sterilized by autoclave at 121ºC for 30 minutes.

2.1.8. Softwares and websites:

www.google.com

www.ncbi.nlm.nih.gov

Microsoft Office Word 2010 software

Microsoft Office excel 2010 software

SPSS version 16.0 was used for data analysis.

2.2. Methods:

2.2.1. Sample collection:

During June to December 2019, a total of 78 abnormal lung samples, showing pneumonic lesions at post-mortem, were collected from apparently healthy camels from Al-Sahafa slaughterhouse. Samples were collected aseptically, preserved into sealed plastic bags, kept at -20°C till used. Samples collection was carried out early at 3:00 am in the morning.

Most camels included in the present study were from Darfur breeds originated from Darfur area in northern Darfur State in western Sudan and some were from Butana breed originated from Butana area in eastern Sudan. Camels of different ages were slaughtered at Al-Sahafa slaughterhouse but their ages mostly ranged from 1 to 3 years, however, camels aged up to 7 years old were sometimes slaughtered.

2.2.2. Preparation of antigen samples:

The virus antigen was obtained by preparing a 20% tissue suspension or homogenate from the lung samples in PBS (pH 7.2-7.4). The procedure was performed under cold aseptic conditions inside the laminar flow safety cabinet-class II. Tissues were placed into sterile mortar using a sterile forceps, tissues were then cut into small pieces using a sterile scissors and forceps, small tissues were ground and minced using pestle, around 7 ml of cold PBS solution (pH 7.2-7.4) supplemented with antibiotics and antifungal was added. The minced preparation were then transferred into 15 ml sterile blue cap tubes and centrifuged at 4°C at 3000 rounds per minutes (rpm) for 10 minutes. Then supernatants were aspirated and

distributed into Eppendorf tubes, stored at -20°C till used. The homogenate was used as an antigen source for PPRV.

2.2.3. Collection of blood:

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

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

For preparation of red blood cells (RBCs) suspension, the collected chicken blood was centrifuged at 1500 rpm for 10 minutes at 25°C, then the plasma and buffy coat was aspirated and discarded, the remaining RBCs were washed after addition of 10 ml of PBS (pH 6.8) by centrifugation at 1500 rpm for 10 minutes, supernatant PBS was discarded and washing was repeated 3 times. A 1% RBCs suspension was prepared by addition of 1 ml of washed RBCs to 99 ml of PBS (pH 6.8), the suspension was used as indicator in the HA test.

2.2.4. Detection of PPRV antigen:

2.2.4.1. Haemagglutination (HA) test:

Tissue homogenate of lung samples were thawed at room temperature then stored at 4°C while performing the test. The procedure of the Haemagglutination (HA) test was performed as described recently by Abdalla (2019). The HA test was performed into 96-well V-bottom haemagglutination plate. Firstly, 50 µl of the diluent Phosphate Buffer Saline (PBS, pH 6.8) was distributed into all wells of the micro-well haemagglutination plate. Secondly, 50 µl of the positive control PPRV and antigen tested samples were added to row A, 50 µl of the PPRV was dispensed into well A1 of column 1 whereas the antigen samples Ag1 to Ag10 were dispensed each into wells A2 to A11 of columns 2-11, respectively (Figure 2). Column 12 served as RBCs control (negative control) and receives only PBS and RBCs. Afterwards, a 2-fold serial dilution was prepared for the virus control and Ag samples by mixing well the contents of wells A1-A11 and transferring 50 µl to row B, for the serial dilution we continued by mixing and transferring 50 µl to the next row till

row H (Figure 2) then 50 µl volumes were discarded away. Finally, 50 µl of 1% RBCs suspension was added to column 12 (RBCs control) then 50 µl of 1% RBCs suspension were dispensed in all wells of the plate, shaking was performed for mixing the contents in the wells of the plate, the plate was incubated for 17-18 minutes in a room temperature (15-20°C). When the RBCs settled down at the bottom of the wells in the RBCs control wells (column 12) and the haemagglutination sheets formed in the wells of the virus control and tested antigens, the results of the HA test was recorded by the naked eye.

2.2.4.1.1. Calculation and interpretation of the HA results:

The haemagglutination test is basically used for identification and quantification of PPRV antigen in a suspected tissue homogentae. The end-point dilution for the haemagglutinating virus was determined as the last well that showed complete haemagglutination of RBCs, the end-point dilution is equal to one haemagglutination (HA) unit (1 HAU) which is the minimum amount of the virus that will cause complete agglutination of the RBCs. The haemagglutination titre (HA titre) of the virus in the sample was determined as the reciprocal of the end-point dilution and expressed as haemagglutination units (HAU).

	Control (Column 1)	Ag Samples (Columns 2-11)									Control (Column 12)	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Ag dilution**
A	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:2**
B	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:4**
C	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:8**
D	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:16**
E	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:32**
F	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:64**
G	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:128**
H	PPRV	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	RBCs	**1:256**

Figure 2. Layout of the haemagglutination (HA) plate.

Notes:

PPRV = Positive control antigen (Column 1)

Ag1-10 = Antigen samples 1-10 (Columns 2-11)

RBCs = Red blood cells control (Column 12)

Chapter III

Results

3.1. Clinical signs and lesion in slaughtered camels:

At ante-mortem, no apparent clinical signs were observed in camels.

At post-mortem, many types of pneumonia were observed and lesions noted included generally abnormal appearance of lung, the colour of the lung tissue is turned to dark-red or purple with some areas firm at touching, these lungs were shrink to a smaller size compared with the normal ones.

3.2. Haemagglutination (HA) test for detection of PPRV antigen:

The prevalence of PPR among camel populations was investigated in Al Sahafa slaughterhouse, Khartoum State. Seventy-eight pneumonic lung tissue samples from camels were examined for the presence of PPRV antigen by the haemagglutination (HA) test. PPRV has the ability to bind with red blood cells (RBCs) and cause haemagglutination, so the HA test is used to detect PPRV antigen in 20% tissue suspension.

Positive HA results were indicated by presence of a diffused sheet or film representing the agglutinating RBCs caused by PPRV. In contrast, negative HA results appeared as a sharp red button composed of non-agglutinated sediment RBCs formed due to absence of the haemagglutinating virus (Figure 3).

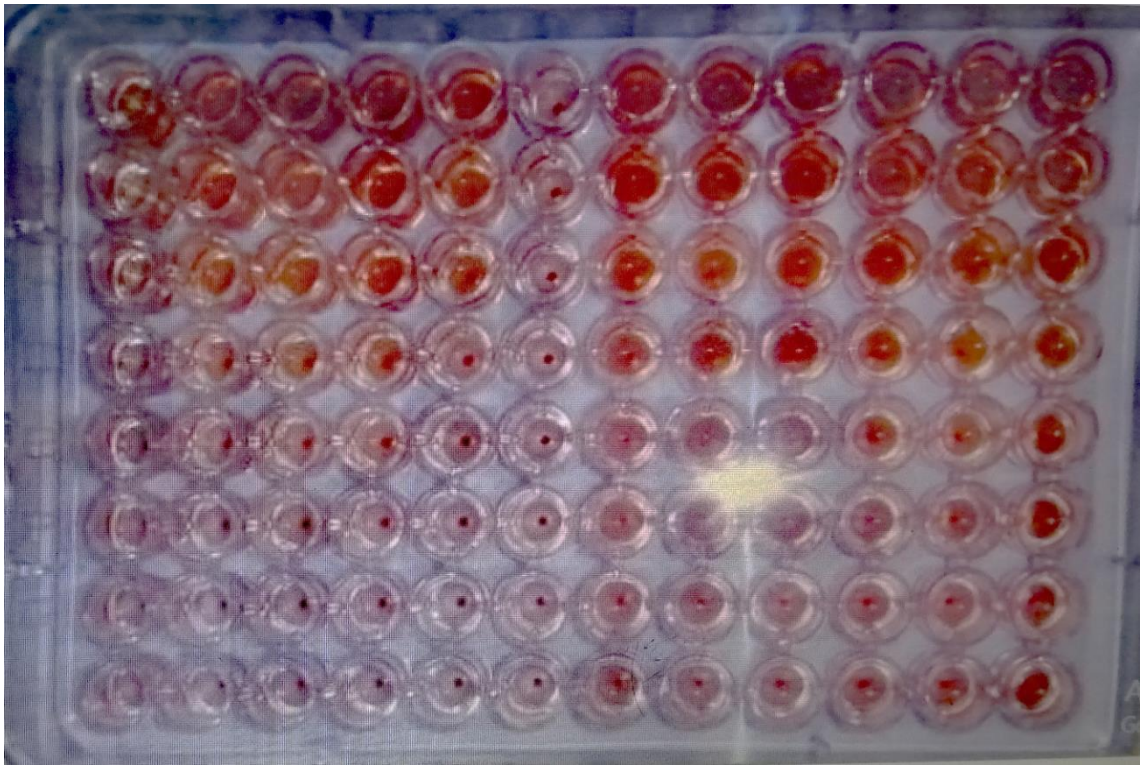

Figure 3. Haemagglutination test for detection of PPRV antigen. HA plate showing positive HA result as a diffused sheet or film and negative HA result as a sharp red button of sediment RBCs. Samples are in Vertical position, column 1: PPRV Control, columns 2-5: antigen samples), column 6: RBCs control, columns 7-12: antigen samples.)

3.3. Prevalence of PPRV in pneumonic camel lung samples by haemagglutination (HA) test:

Out of the 78 pneumonic lung camel samples screened using the haemagglutination (HA) test for the presence of PPRV antigen, 76 (97.4%) samples were found positive whereas only 2 (2.6%) samples were found negative (Table 1, Figure 4). The results demonstrated the highest overall PPRV antigenic prevalence of 97.4%.

3.4. Haemagglutination (HA) test detected PPRV antigen at different haemagglutination titres:

When camel lung tissue samples were tested using the haemagglutination test, results revealed heamagglutination titres ranged from 2 to 32 HAU with mean titre of 10.0 (Table 1). Moreover, 29 (37.2%) samples had titre of 8 HAU and 27 (34.6 %) samples had the HA titre of 16 HAU. These samples constitute the majority and nearly more than half of the samples. The remaining samples include, only 2 (2.6%) samples had the highest HA titre of 32 HAU, 10 (12.8%) samples found with titre of 4 HAU, and 8 (10.2%) samples found with titre of 2 HAU (Table 1; Figure 4).

Table 1. Detection of PPRV antigen in pneumonic camel lung samples by haemagglutination (HA) test. Number of positive and negative camel lung samples for PPRV antigen, end-point dilutions and number of samples at different HA titres expressed as HAU were shown.

End-point	HA Titre (HAU)	+ve (%)	-ve (%)
0	0	0 (0%)	2 (2.6%)
1/2	2	8 (10.2%)	0 (0%)
1/4	4	10 (12.8%)	0 (0%)
1/8	8	29 (37.2%)	0 (0%)
1/16	16	27 (34.6%)	0 (0%)
1/32	32	2 (2.6%)	0 (0%)
1/64	64	0 (0%)	0 (0%)
1/128	128	0 (0%)	0 (0%)
1/256	256	0 (0%)	0 (0%)
Total		76 (97.4%)	2 (2.6%)

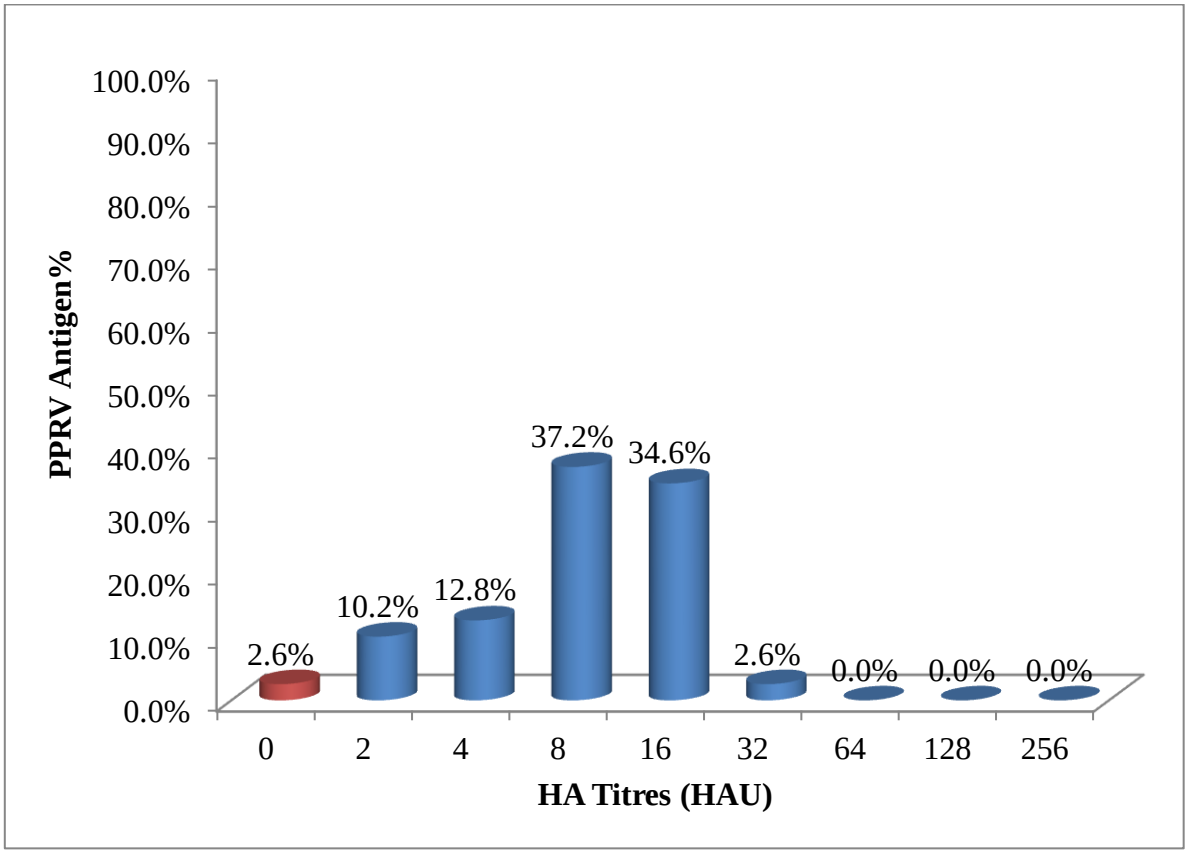

Figure 4. PPRV antigen detected in pneumonic camel lung samples at different haemagglutination titres by haemagglutination (HA) test. Positive results are in dark blue color and negative results are in red color.

Chapter IV
Discussion

Camels can be infected with PPRV exhibiting clinical signs of the diseases as had been reported in Ethiopia (Roger *et al.,* 2000; 2001), Sudan (Khalafalla *et al.,* 2010), Kenya (Omani *et al.,* 2019), Iran (Zakian *et al.,* 2016) and Pakistan (Rahman *et al.,* 2020a). In many studies, PPRV antibodies had also been detected in dromedary camels from Libya (El-Dakhly, 2015), Nigeria (Woma *et al.,* 2015) and Pakistan (Shabbir *et al.,* 2020; Rahman *et al.,* 2020b). Other studies reported presence of PPRV antigens in lung tissues of camels slaughtered in Abattoir in Ethiopia (Ayelet *et al.,* 2013) and Sudan (Saeed *et al.,* 2015; Abdalla, 2019).

This study aimed to investigate the presence of PPRV antigen and to determine the antigenic prevalence of PPRV among camels slaughtered at Al-Sahafa slaughterhouse, Khartoum State, Sudan. In the present study, seventy-eight lung samples, showing pneumonic lesions at post-mortem, were collected from apparently healthy camels slaughtered at Al-Sahafa slaughterhouse in Khartoum State during 2019. Most camels included in the present study were from Darfur breeds and originated from Darfur area in Northern Darfur State in western Sudan and some were from Butana breed that originated from Butana area in eastern Sudan. Young camels aged 1 to 3 years old were mostly slaughtered although camels aged up to 7 years old, at the end of its productivity, were also slaughtered at Al-Sahafa slaughterhouse. During the first PPR outbreak in Sudan in 2004, infection of camels had been reported from all age groups, both sexes and different breeds however, adult animals were more affected compared to calves and young camels (Khalafalla *et al.,* 2010). Of note, one recent study examined camels regardless of its sex, age and breed, however, most of these slaughtered and examined animals were females (Abdalla, 2019).

At ante-mortem, no apparent clinical signs were observed in camels. However, at post-mortem, generally many types of pneumonia were observed and lesions noted included generally abnormal appearance of infected lungs, the colour of the lung tissue is turned to dark-red or purple with some areas firm at touching. Obviously, these lung tissues were shrink to a smaller size compared with the normal ones. Recently, Abdalla (2019) investigated PPR infection in lungs of apparently healthy camels in central Sudan. At ante-mortem, generally some clinical signs including nasal discharges, an increased respiratory rate associated with severe emaciation were observed in camels. Abdalla

(2019) observed post-mortem lesions included lung congestion and fragility, also changes in color and thickness of the tissue and presence of some abscesses were also observed in the pneumonic lungs of examined camels. The previous report by Khalafalla *et al.* (2010) described presence of lung congestion and consolidation, paleness and fragility of liver, enlarged lymph nodes, congestion and hemorrhage of small intestine and stomach as the main post-mortem findings observed among PPRV-infected camels.

The haemagglutination (HA) test had been described previously as useful, rapid and cheap for detecting the presence and also for quantification of PPRV antigen in pneumonic camel lung tissue samples (Ezeibe *et al.,* 2004; Osman, 2005; Abdalla, 2019; Alhussain *et al.,* 2020). It is known that most paramyxoviruses and particularly PPRV cause haemagglutination by binding with red blood cells (RBCs) of different animal species via its haemagglutinin surface glycoprotein (Wosu, 1985; 1991; Ramachandran *et al.,* 1993; Eziebe *et al.,* 2004; Seth and Shaila, 2001) leading to formation of diffused sheets of agglutinating RBCs. Recently, the HA test was proved as a specific and valuable diagnostic tool for diagnosis of PPR, detection and quantification of PPRV antigen from different animal species as had been confirmed in many studies (Manoharan *et al.,* 2005; Abdalla, 2019; Rahman *et al.,* 2020b; Alhussain *et al.,* 2020). Moreover, it has been demonstrated that the HA test is useful in detecting PPRV antigen in lung samples from slaughtered camels Abdalla (2019), sheep and goats Alhussain *et al.,* 2020, showing pneumonic post-mortem lesions in central Sudan in studies performed recently. The recent study of Rahman *et al.,* (2020b) proved that the HA test is as sensitive an immuno-capture enzyme-linked immunosorbent assay (IC-ELISA) in detecting prevalence of PPRV antigen thus can be employed as an alternative test for diagnosis of PPR in developing countries.

In the present study, haemagglutination (HA) test detected the presence of PPRV antigen in 76 (97.4%) of the 78 examined lung samples. Moreover, the highest overall PPRV antigenic prevalence of 97.4% had been demonstrated among camels slaughtered at Al-Sahafa slaughterhouse, Khartoum State, Sudan. Recently, a relatively similar antigenic prevalence (98%, 98/100) had been demonstrated among camels slaughtered at Tambul slaughterhouse, Gezira State, central Sudan, using the HA test Abdalla (2019). Both studies declared presence of PPRV antigen among slaughtered camels in the Sudan and showed a very highest antigenic prevalence suggesting exposure of these animals to PPRV. In a previous study, a lowest prevalence of 45.1% (214/474) had been

detected in pneumonic lungs of slaughtered camels in the Sudan whereas the highest antigenic prevalence of 59.9% had been detected in Tambool area, central Sudan (Saeed *et al.*, 2015). In this study, heamagglutination titres ranged between 2 and 32 (HAU) with an average HA titre of 10.0 had been detected. A remarkably higher HA titres ranged between 4 and 256 (HAU) with an average HA titre of 14.4 HAU had been detected by Abdalla (2019).

In previous studies, detection of PPRV antigen, infectious virus or RNA had been demonstrated in tissue samples of dromedary camels in the Sudan (Kwiatek *et al.*, 2011; Saeed *et al.*, 2017; Abdalla, 2019). Moreover, in the Sudan many viral diseases affecting the respiratory system of camels had been detected in pneumonic camel lungs such as bovine herpes virus-1 (BHV-1) (Saeed *et al.*, 2009), bovine viral diarrhea virus (BVDV) (Saeed *et al.*, 2010a), respiratory syncytial virus (RSV) (Saeed *et al.*, 2010b), parainfluenza virus 3 (PIV3) (Saeed *et al.*, 2010c), and adenovirus (Saeed *et al.*, 2010d). Also one study demonstrated a mixed infection of PPRV with many respiratory viruses "Parainfluenza virus 3 (PIV 3), respiratory syncytial virus (RSV), bovine herpes virus-1 (BHV-1), bovine viral diarrhea (BVD), and adenovirus in pneumonic lungs of dromedary camels in Sudan (Saeed *et al.*, 2015). Due to the association of PPRV infection in camels with other viral diseases, PPR had recently been recognized as an emerging disease of camels (Khalafalla *et al.*, 2010; Zakian *et al.*, 2016, Omani *et al.*, 2019; Rahman *et al.*, 2020a).

It is obvious that camels can be infected by PPRV but there is no evidence if the infection occurred via contact with infected small ruminants "sheep and goats" or via contact with the infected camels itself, so the source of infection remains unclear. Many studies claimed that no viral shedding from infected camels had been reported thus transmission to susceptible in-contact animals can occur (Schulz *et al.*, 2019; Omani *et al.*, 2019). Experimentally infected dromedaries camels with the wild-type PPRV developed no clinical signs, no viremia, and shed no or low RNA loads in swab samples and there was no clear evidence on onward transmission to in-contact dromedary camels and goats concluded camels as dead-end hosts for PPRV (Schulz *et al.*, 2019). More future studies should be directed to figure out if presence and/or absence of PPR viral shedding in secretions and excretions of infected camels that could be a source of infection to other in-contact camels and/or other susceptible animal species. Understanding the role camels may play in the viral transmission and/or the epidemiology of PPR is an important issue for PPR global eradication program.

Conclusion and Recommendations

Conclusion:

The high prevalence achieved in the present study confirmed that PPR is widely distributed in camels slaughtered at Al-Sahafa slaughterhouse located in Khartoum State, Sudan. Diagnosis of PPR in camels cannot be relies on due to presence of subclinical form of the disease or presence of respiratory signs which are common and requires differential diagnosis from other diseases. Results of the study stated that the Haemagglutination (HA) test is useful, simple, rapid and cheap for detecting the presence and also for quantification of PPRV antigen in pneumonic camel lung tissue samples. Thus the HA test can be employed, as an alternative for an immuno-capture enzyme-linked immunosorbent assay (IC-ELISA), for diagnosis of PPR in developing countries.

Recommendations:

1- PPR is widely distributed in the Sudan, therefore, it is recommended to use the PPRV vaccine to vaccinate camels in all parts of the country.
2- There is a need for more studies to increase knowledge and understanding the role camels may play in the viral transmission and/or the epidemiology of PPR.
3- It is important to control animal movement from endemic areas.
4- Early reporting and notification of PPR outbreaks to the World Organization of Animal Health (OIE) is the key to early control and rapid elimination.
5- Awareness of the camel owner's about the economic problem caused by the disease.

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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.