Serological Techniques for Detection of Antibodies Against Peste des Petits Ruminants Virus

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A thesis submitted to the University of Khartoum in partial fulfillment of the requirements for the degree of Master Science in Microbiology

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November 2009
بسم الله الرحمن الرحيم

قال تعالى: صدق الله العظيم

سورة يوسف (الآية 76)
DEDICATION

This work is dedicated
To

To my beloved Parents
Sisters, brothers and friends
ACKNOWLEDGMENTS

First of all my thanks to Almighty Allah for giving me the strength, and willpower to the accomplishment of this study.

I would like to thank my supervisor Dr. Sulaiman Mohammed El Hassan, Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum for his help and advice.

My gratitude is extended to Dr Mohammed Abdalla Fadol, viral vaccines production complex, Central Veterinary Research Laboratory (CVRL) for his help and advice.

Special thanks to Dr Abd Elgadir Ballal, Head Department of Viral Vaccines Production complex, Central Veterinary Research Laboratory (CVRL), and staff of the department for their unlimited help, and advice.

My gratitude is extended to Professor Mahasin El nur Abdel Rhaman, Head Department of Virology, Central Veterinary Research Laboratory (CVRL) for her help in carrying out the cELISA.

My gratitude is extended to the Director of the Central Veterinary Research Laboratory (CVRL) Soba for his permission to carry out this work in the Central Veterinary Research Laboratory.

Also, I would like to express my deep sense to everyone who helped me to carry out this work.
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ABSTRACT

Peste des petits ruminants (PPR) is an acute disease of sheep and goats. The disease is highly contagious and causes varying degrees of morbidity and mortality. In Sudan, the disease is endemic resulting in heavy economic loss. The objective was to assess the seropositivity of PPR virus antibodies in sheep and goats and to evaluate sensitivity and specificity of agar gel immunodiffusion test (AGID) and serum neutralization test (NT) in relation to competitive immunocapture enzyme–linked immunosorbent assay (cELISA). A total of 400 serum samples collected from sheep and goats were screened for PPR virus antibodies by AGID test and NT. Out of these 400 samples, 266 were screened by cELISA test.

The overall PPR virus antibodies serosurveillance recorded in sheep and goats was 25.75%, 41.75% and 51.87% by AGID test, NT and cELISA test, respectively.

cELISA proved to be a more sensitive test than AGID and NT tests, and this confirms previous studies which proved that cELISA is efficient, sensitive and specific diagnostic technique for detection of PPRV antibodies. When AGID and NT test were evaluated in relation to cELISA, statistical analysis showed NT had higher sensitivity than AGID test but lower specificity in relation to cELISA. This indicates that NT is the second test of choice for detection of PPRV antibodies.

The present investigation confirms previous studies in detection of PPRV antibodies in serum samples of sheep and goats with no vaccination history. This indicates the exposure of sheep and goats to PPR virus and the disease remains prevalent in Sudan.
المستخلص

مرض طاعون المجترات الصغيرة مرض حاد الإصابة في الضأن والماعز شديد الاعتداء ويسبب درجات متغايرة من الإصابة والنفوق. ويعد من الأمراض المستوطنة في السودان ويتسبب في خسائر اقتصادية عالية. وقد أجريت هذه الدراسة لتقديم إيجابية الأجسام المضادة في أملاح من دماء الضأن والماعز لفيروس طاعون المجترات الصغيرة، وتقييم حساسية ونوعية اختبار الإصابة المناعي في الأجور (الهitemap) واختبار التعامل المصلي مقارنه بإختبار الإليرة التنافسية.

فحصت 400 عينة مصل جمعت من الضآن والماعز للكشف عن الأضداد لفيروس طاعون المجترات الصغيرة. أجبراء اختبار الإنتشار المناعي في الهمال والتعامل المصلي. وفحصت 266 عينة من المجموع الكلي بواسطة اختبار الإليرة التنافسية. كان معدل إنتشار الأضداد للفيروس في الضآن والماعز 25.75%، و14.75% بواسطة اختبار الإنتشار المناعي في الهمال والتعامل المصلي والإليرة التنافسية على التوالى.

كشفت الدراسة أن اختبار الإليرة التنافسية أكثر حساسية من اختبار الإنتشار المناعي في الهمال والتعامل المصلي في الكشف عن الأضداد للفيروس مما يتوافق مع الدراسات السابقة التي أثبتت أن تقنية التشخيص بواسطة الإليرة التنافسية أكثر حساسية وفعالية ودقة في الكشف عن الأضداد للفيروس.

أظهر التحليل الإحصائي لنتائج الدراسة أن اختبار التعامل المصلي أكثر حساسية من اختبار الإنتشار المناعي في الهمال ولكنه أقل نوعية مقارنة مع اختبار الإليرة التنافسية مما يشير إلى أن اختبار التعامل المصلي هو الاختبار الثاني للكشف عن الأضداد للفيروس.

أكدت الدراسة البحوث السابقة التي تقصدت عن الأضداد للفيروس في أملاح من الضآن والماعز التي لم يسبق تحصينها ضد المرض مما يشير إلى تعرض هذه الحيوانات لفيروس طاعون المجترات الصغيرة وإنتشار المرض في السودان.
INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious systemic viral disease of goats and sheep. Infected animals present clinical signs similar to those of rinderpest in cattle from which it must be differentiated, and caused by closely related morbillivirus. Unlike rinderpest, however many infections are subclinical.

PPR is known as goat plaque and it is referred to as kata in Nigeria and as pseudo rinderpest (Otte, 1960; Rowland and Bourdin, 1970; Hamdy et al., 1976). The course of the disease may be peracute acute or chronic, however the virus does not persist. PPR is often rendered more severe by secondary bacterial infections, concomitant helminthiasis, which has led to the introduction of descriptive term stomatitis-pneumonitis complex (Fenner et al., 1987). It occurs mainly in West Africa, although outbreaks have also been described elsewhere. The disease is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and ulceration of the mucous membrane and inflammation of gastro-intestinal tract leading to severe diarrhea, clinical signs develop after incubation period of 3-10 days (Gibbs et al., 1979). The disease is highly contagious causing varying degree of morbidity and mortality in susceptible animals (Radostits et al., 2000). Morbidity and mortality rates can be as high as 100 and 90 per cent, respectively (Abu-Elzien et al., 1990). Mortality rates usually lower in endemic areas and mortality can be as low as 20% and serosurveillance is sometimes the only indicator of infection (Roeder and Obi, 1999).

In the basis of mortalities, morbidity, PPR has great economic importance through body wastage, poor feed efficiency, loss meat, milk and offspring (Nawathe, 1984). Although PPR remains the principal killing disease of small ruminants in most Africa, Asia and Middle East
countries as recognized in international survey report published in 2002 (Perry et al., 2002), few economic studies have been made on this disease. The most recent one was published in 1992, it was conducted in Nigeria (Chip, 1993).

PPR is an important disease in its own right, but it has also created problems because of its apparent similarity to rinderpest. The clinical signs of PPR closely resemble those of rinderpest, making differential diagnosis difficult. It should, however, be borne in mind that clinical disease caused by rinderpest in small ruminants is a relatively rare event, even in Asia. PPR is considered to be one of the main constraints to improving productivity of small ruminants in the regions where it is endemic (Ikede, 1983).

In Sudan PPR have been reported in 1971 (El Hag, 1973; El Hag and Taylor, 1984). The rinderpest (RP) tissue culture vaccine used to control the occurrence of the epizootics of the disease based on the principal of antigenic relationship that exists between PPR and RP viruses (El Hag, 1973). The use of rinderpest vaccine against PPR was avoided in Sudan when rinderpest seromonitoring studies were performed. Recently, PPR attenuated virus vaccine for control of PPR disease is produced in Sudan at the CVRL.

The objectives of this investigation were
1- To continues the study of the PPR disease that done by Intisar (2002), and cover different localities in Sudan by different serological tests.
2- To compare sensitivity and specificity of serological tests, and to select the most reliable test to be applied for monitoring of PPR antibodies.
CHAPTER ONE
LITERATURE REVIEW

1.1. History of the disease

PPR has comparatively a shorter history which only dates back to 1940, when it was first identified as a distinct disease by Francophone workers in Benin and Senegal and described by Cathou in Benin and Gargdennec and Lananne in Ivory Coast of West Africa (1942). PPR existence was associated with West Africa countries, where the disease known as pest des petits ruminant.

Zwart and Rowe (1966) found neutralizing antibodies to rinderpest virus in sera from goats and sheep in northern Nigeria and interpreted this to suggest the presence of PPR in Nigeria(Obi, 1984). Also Whitney et al. (1967) described a disease known as Kata in south western Nigeria. Later Rowland and Bourdin (1970) and Rowland et al. (1971) found that PPR and Kata were clinically and pathologically indistinguishable and then in Nigeria the disease was reported by Isoun and Maun (1972), Nduakas and Ihemalanuss (1973), and Hamdy et al. (1976). After development of specific diagnostic tools in late 1980s onwards, the understanding of the distribution of PPR has grown very quickly (Diallo et al., 1995) and recent data indicates the presence of PPRV in all countries of Africa. In India PPR was first confirmed in sheep suspected of having rinderpest. It is believed since then that many outbreak in India previously attributed to rinderpest were actually PPR (Taylor, 2002).

1.1.1. PPR in Sudan

In Sudan, outbreak of rinderpest involving goats and sheep and cattle in eastern Sudan was investigated in 1971, and the antigen which was isolated from this outbreak produced a positive reaction in immune tests with rinderpest hyperimmune serum. The presence of rinderpest
immune serum in the culture medium inhibited the isolation of an antigen which produced cytopathic effects characterised by the production of syncytia. For these reasons the outbreaks were originally diagnosed as rinderpest but new evidence presented suggests they were probably due to PPRV which was repeatedly isolated by El Hag (1973) and El Hag and Taylor (1984).

1.2 Etiology

The causative agent of this economically important disease of small ruminants is Peste de petits ruminants virus (PPRV). This virus belongs to the genus *Morbillivirus*, under the family *Paramyxoviridae* of the order *Mononegavirales* (Murphy *et al.*, 1999). The virus is closely related to Rinderpest virus (RPV), another member of *Morbillivirus* genus, which causes similar disease in large ruminants (Anderson *et al.*, 1990; Couacy-Hyman *et al.*, 1995). The virus is also serologically related to measles and canine distemper virus and phocid distemper virus (PDV) of sea mammals (seals) (Gibbs *et al.*, 1979).

For many years, PPR, virus was considered a variant of RPV, specifically adapted for goats and sheep that had lost its virulence for cattle. It is now known that the two viruses are distinct though closely related antigenically (Gibbs *et al*; 1979, Appel *et al*; 1981).

1.2.1 Classification of the virus

On the basis of it’s similarity to viruses of rinderpest, canine distemper and measles, the PPR virus has been classified within the genus *morbillivirus* in the family *paramyxoviridae* which included the etiological agents of the most important Veterinary diseases: Canine distemper, rinderpest of cattle and Newcastle disease of chicken. It is subdivided into three genera, *paramyxovirus*, *morbillivirus* and *pneumovirus*. The virus of the genera *paramyxovirus* and *pneumovirus* caused respiratory infections and occasionally generalized infections.
Viruses of the genus *Morbillivirus* usually cause generalized infection: Rinderpest of cattle, peste des ruminants of sheep and goats, and canine distemper as well as measles (Fenner *et al.*, 1987).

### 1.2.2. Virion properties

The ultrastructure of PPR virus has been examined by electron-microscope using negative staining technique. The virus particle was found to be pleomorphic in shape (spherical as well as filamentous forms occur) 130-390 nm in diameter. Virions are enveloped, covered with large peplomers 8-15 nm in length, and contain a herringbone-shaped, helically symmetrical nucleocapsid, 600-800 nm in length and 14-23 nm in diameter (Durojaiye *et al.*, 1985). The genome consists of a single linear molecule of negative-sense, single-stranded RNA 15-16 kb in size. There are 6 to 10 genes separated by conserved noncoding sequences that contain termination, polyadenylation, and initiation signals. Most of gene products are structural proteins found in virions. The peplomers are composed of two glycoproteins a hemagglutinin-neuraminidase protein (HN) and a fusion protein (F). Both proteins play key roles in the pathogenesis of all *paramyxovirus* infections. Hemagglutination involves the agglutination of red blood cells. It relies on the ability of a virus to bind to receptors on red blood cells. Since viruses have multiple attachment proteins per virion, they can bind to more than one red blood cell and so they can serve to link red blood cells into a network. Inactivated virus can still hemagglutinate as long as its attachment proteins are intact. If someone has antibodies to a viral hemagglutinin, the antibodies will bind to the attachment protein and prevent its binding to the red blood cells. The serum of that person will
inhibit the agglutination reaction by the virus to which they have antibodies, but not by other hemagglutinating viruses. This can be used to determine which hemagglutinating virus a person has been exposed to (Hunt, 2008). Hemadsorption during infection, the viral attachment protein will be inserted into the plasma membrane of the infected cell. If the viral attachment protein can bind to red blood cells, the infected cell will bind red blood cells because it has the viral attachment protein on its surface, this is called hemadsorption. In the clinical laboratory, this may enable virally-infected cells to be detected at an early stage in infection, and may allow detection of viruses which do not visibly damage the cell (Hunt, 2008). The genome of attenuated vaccine strain of PPRV (Nigeria 75/1) has entirely been sequenced and the physical map of the genome is the same as that of the other morbilliviruses (Rima et al., 1986; Diallo et al., 1990). Although, there is only one serotype of the virus (Barrett et al., 1993), PPRV isolates on the basis of partial sequence analysis of the fusion (F) protein gene, can be grouped into four distinct lineages. Lineage 1 and 2 are found exclusively in West Africa, whereas lineage 3 has been found in eastern Africa and Arabia. The fourth lineage is confined exclusively in the Middle East, Arabia and Indian subcontinent (Shaila et al., 1996). Except one isolate (TN92/1) from southern India, which belonged to lineage 3, all Indian PPRV isolates identified so far belonged to lineage 4 only (Nanda et al., 1996; Dhar et al., 2002).

1.3. Viral replication

The PPR virus has cytoplasmic replication. The none segmented negative -sense, single- stranded RNA of paramyxovirus carries RNA-dependant RNA polymerase, transcriptase, which transcribes five or more subgenomic positive sense RNAs, each of which serves as amonocistronic mRNA in contrast, transcription in the replication mode (by the same polymerase acting as a replicases) produce a full- length
positive template for the synthesis of new negative sense viral RNA. The envelope of the new generation of the virus acquired during the budding from the plasma membrane. Eclipse period is 4 hours (Frederick et al., 1999).

1.3.1. Adsorption and penetration

The H (N) G protein recognize receptors on cell surface. The F protein facilitates fusion between membranes at physiological pH, so although paramyxoviruses can be taken up by endocytosis, they also often enter the cell by direct fusion with the plasma membrane. Because the F protein functions at physiological pH, this can result in syncytia being formed in paramyxovirus infections (Hunt, 2008).

1.3.2. Transcription, translation and replication of RNA

Viral multiplication occurs in the cytoplasm. The viral RNA polymerase uses the nucleocapsid as a template. The RNA polymerase does not need a fully uncoated nucleocapsid. Viral mRNAs are transcribed; these are capped, methylated and polyadenylated. Since this is a negative-strand RNA virus, RNA polymerase and RNA modification enzymes are packaged in the virion. The viral mRNAs are translated to give viral proteins. There is no distinction between early and late functions in gene expression. Viral RNA replication involves full length plus strand synthesis. This is used as a template for full length minus strand. Both full length strands are coated with nucleocapsid protein as they are made. New full length minus strands may serve as templates for replication, or templates for transcription, or they may be packaged into new virions.

1.3.3. Assembly

Both viral glycoproteins i.e. attachment protein and F (fusion) protein) are translated as transmembrane proteins and transported to the cell plasma membrane. M (matrix) protein enables nucleocapsids to
interact with the regions of the plasma membrane which have the glycoproteins inserted. The virus buds out through membrane (Hunt, 2008).

1.3.4. Role of neuraminidase

In those *paramyxoviruses* which have neuraminidase, the neuraminidase may facilitate release. In these viruses, sialic acid appears to be an important part of the receptor. The neuraminidase removes sialic acid (neuraminic acid) from the cell surface. Thus, since sialic acid will have been largely removed from the cell surface and the progeny virions, neither will have functional receptors, so progeny virions will not stick to each other nor to the cell, they have just budded out from infected cell. They will therefore be able to diffuse away until they meet an uninfected cell. The neuraminidase may also help during infection since, if the virus binds to sialic acid residues in mucus, it would not be able to bind to a receptor on a cell and infect that cell. But if the sialic acid in the mucus is eventually destroyed, the virus will be freed and may then reach a receptor on the cell surface (Hunt, 2008).

1.3.5. Activation of the F protein

The F protein needs to be cleaved before it can function in facilitating fusion when the virus binds to another cell. This is a late event in maturation (Hunt, 2008).

1.4. Physico-chemical properties

PPR virus may survive at 60°C for 60 minutes, and it is stable between pH 7.2–7.9 but is rapidly inactivated at pH values less than 5.6 or greater 9.6 (Lefever, 1982). Possible airborne spread the virus over several hundred meters, mainly at night. High and low humidity aid survival but virus is rapidly destroyed at relative humidity 50–60%. Contaminated pastures would be non-infective after 6–24 hours, depending on sun/shade (FAO, 1999)
The infectivity of enveloped viruses is readily destroyed by lipid solvent such as ether alcohol, chloroform, and detergent like phenol as well as by most disinfectants e.g. Glutaraldehyde is excellent disinfectant, sodium deoxycholate and sodium hydroxide 2% (24 hours), sodium carbonate are very effective against PPR virus. Anderson (1999) stated that the virus might survival long time in chilled and frozen tissue.

1.5. Epidemiology

1.5.1. Host range

PPR is a primarily disease of goats and sheep. Goats are usually more severely affected than sheep and involve goats of all ages (Singh et al., 2000). There have been several reports of PPR occurring in captive wild ungulates from the following families: Gazellinae (Dorcas gazelle), ibex (Capra inbex nubiana, laristan sheep) and gemsbok (Oryx gazelle) (Saliki, 2002). Experimentally, the American white–tailed deer (Odocoileus virginianus) is fully susceptible to PPR. The role of a wild life on the epizootiology of the PPR in Africa should be investigated (Taylor, 1984).

Cattle, buffaloes, camels, and pigs are also susceptible to infection but do not exhibit clinical signs and are unable to transmit the disease to other animal (Taylor, 1984). PPRV was also suspected to be involved in the epizootic disease that affected single humped camels in Ethiopia in 1995–1996 (Roger et al., 2000, Roger et al., 2001). The isolation of virus from an outbreak in Indian buffalo (Bubalus bubalis) has been reported (Govindarajan et al., 1997).

1.5.2 Geographic distribution

PPR has comparatively a shorter history which only dates back to 1940, when it was first described by Gargadennec and Lalane (1942) in Ivory Coast of West Africa. For a long time, its existence was associated with west African countries. After development of specific diagnostic
tools in late 1980s onwards, the understanding of the geographical distribution of PPR has grown very quickly (Diallo et al., 1995) and recent data indicates the activity of PPRV in all countries of Africa lying between Sahara and the Equator. It has been reported in Sudan (El hag and Taylor, 1984), Kenya, Uganda (Wamwayi et al., 1995) and also in Ethiopia (Roeder et al., 2002). The disease has been seen in Arabian Peninsula and the Middle East including Islamic Republic of Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, the United Arab Emirates and Yemen (Dhar, 2002), with extension to Turkey, and Pakistan. In India PPR was first reported in 1987 from Tamil Nadu (Shaila et al., 1989). Outbreaks of PPR are now known to be common in Bangladesh and Nepal (Shaila et al., 1996; Dhar et al., 2002; Taylor et al., 2001). In Africa and Asia the disease is particularly devastating, as these countries often use small ruminants as components of agricultural food production (Ozkul et al., 2002).

PPRV was repeatedly isolated in Sudan by El Hag (1973); El Hag and Taylor (1984); Rasheed (1992); Zeidan (1992); Awad El Karim et al. (1994) and El Amin and Hassan (1998);Intisar (2002); Nussieba (2005); Abu Obieda (2006).

1.5.3. Transmission

For PPR to spread it requires close contact between infected and susceptible animals (Ozkul, 2002). Saliki. (2002) stated that, there are several means of transmission between animals. The disease is transmitted by inhalation of aerosols produced by sneezing and coughing of infected animals living in close contact, or by contamination of the food and water by the main sources of the virus which are ocular, nasal, or oral secretions. Feces, urine, milk and products of abortion contain large amounts of the virus. Fine infective droplets are released into the air from these secretions and excretions. During the rainy season or dry cold
season PPR outbreaks are more frequent. Fomite such as bedding, water and feed trough may also contribute to the onset of an outbreak. In the few animals that do recover, there is no “carrier” state but milk may be infectious 45 days after clinical recovery.

The appearance of clinical PPR may be associated with any of the following: History of recent movement or gathering together of sheep and/or goats of different ages with or without associated changes in housing and feeding; introduction of recently purchased animals; contact in a closed/village flock with sheep and/or goats that had been sent to market but returned unsold; change in weather such as the onset of the rainy season (hot and humid) or dry, cold periods (for example the harmattan season in West Africa); contact with trade or nomadic animals through shared grazing, water and/or housing; a change in husbandry (e.g. towards increased intensification) and trading practices (FAO, 1999).

As in rinderpest (RP), there is no known carrier state. Infected animals may transmit the disease during the incubation period (Lefèvre and Diallo, 1990).

1.6. Clinical features

When PPR occurs in an area for the first time, it is possible that acute high fever with extreme depression and death occur before any other typical signs have been seen. A more typical picture, however, is that of a fast-spreading syndrome in sheep and/or goats characterized by the sudden onset of depression, discharges from eyes, nose and mouth, abnormal breathing with coughing, diarrhoea and deaths. Both goats and sheep are susceptible to infection and may show disease, they are not always affected simultaneously. For example, in Africa PPR is seen most commonly in goats, while in western and south Asia sheep are usually the most noticeable victims. The disease can, however, strike both species with equally devastating consequences (FAO, 1999). Virus appears in
blood, excretions and secretions 1–2 days before clinical signs (FAO, 1999). The clinical disease of PPR in sheep and goats resembles to the rinderpest in cattle. It usually appears in the acute form with an incubation period of 2-10 days. There is a sudden rise in body temperature up to 40-41°C. The temperature usually remains high for about 5-8 days before slowly returning to normal, preceding recovery or dropping below normal before death (Roeder and Obi, 1999; Pawaiya et al., 2004). The animals become depressed, anorexic and develop a dry muzzle. Serous nasal discharge, which becomes progressively mucopurulent, can crust over and occlude nostrils. Purulent ocular discharge with congested conjunctiva can encrust cementing eye lids together. If the death does not ensue, persist for around 14 days. Within 4 days of onset of fever, the gums become hyperaemic, and erosive lesions develop in the oral cavity with excessive salivation but not to point of drooling. These lesions may become necrotic. Inflammation of gastrointestinal tract leading to severe diarrhea, occasional with blood and mucus. Pneumonia, dyspnea, coughing and sneezing in an attempt to clear nose, abdominal breathing also occur, dehydration and emaciation followed by secondary latent infections may be activated and complicate the clinical features (EL Hag and Taylor, 1984). Abortions were noted in pregnant females (EL Hag and Taylor, 1984). Mortality rate can be up to 100% in severe outbreaks (Abu-Elzein, 1990). Mortality is usually low in endemic areas, but when associated with other diseases such as capripox, it can approach 100 per cent (Kitching, 1988). Death usually occurs after 5-10 days (Taylor, 1984; Saliki, 1998).

1.7. Pathogenesis

PPR virus, like other morbilliviruses, is lymphotropic and epitheliotropic (Scott 1981). Consequently, it induced the most severe lesions in organ systems rich in lymphoid and epithelial tissues. The respiratory
route is likely portal of entry. After the entry of the virus through the respiratory tract system, it localizes first and replicates in pharyngeal and mandibular lymph nodes as well as tonsil. Viremia may develop 2-3 days after infection, and 1-2 days before the first clinical signs appears. Subsequently viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and respiratory system (Scott, 1981).

1.8. Histopathology

PPR virus causes epithelial necrosis of the mucosa of the alimentary and respiratory tracts marked by the presence of eosinophilic intracytoplasmic and intranuclear inclusion bodies. Multinucleated giant cell (syncytia) can be observed in all affected epithelia as well as in the lymph nodes (Brown et al., 1991). In the spleen, tonsil and lymph nodes, the virus causes necrosis of lymphocytes evidenced by pyknotic nuclei and karyorrhexis (Rowland et al., 1971). Brown et al. (1991) using immunohistochemical methods detected viral antigen in cytoplasm and nuclei of trachea, bronchial and bronchio-epithelial cell, type II pneumocytes, syncytial cell and alveolar macrophages.

1.9. The gross lesions

The most characteristic lesions are usually seen in the digestive and respiratory system, but can be seen in the other system. In the digestive system inflammatory and necrotic lesions are seen in mouth and gastrointestinal tract and erosive stomatitis involving the inside of lower lip and adjacent gum. In severe cases, lesion may also be found on hard palate, pharynx, and upper third of esophagus. Rumen, reticulum, and omasum rarely have lesions. Sometimes, there may be erosions on pillars of rumen. The abomasum is a common site of regular outlined erosions and often oozes blood. In small intestine, lesions usually moderate. Extensive necrosis of payer's patch, results in severe ulceration (Saliki,
In the Large intestine, the iliocecval valve was congested together with the folds of the rectal and ceecal mucosa (El Hag and Taylor, 1984). Zebra stripes (discontinuous streaks of congestion) in posterior part of colon and rectum and crests of mucosal folds. In the respiratory system small erosions and petechiae are visible on nasal mucosa, turbinate, Larynx and trachea. Broncho-pneumonia may be present, usually confined to the outer ovental areas and characterized by consolidation and atelectasi. There are pleuritis, which may become exudative and results in hydrothorax (EL Hag and Taylor, 1984).

The spleen may be slightly enlarged and congested. Most of the lymph nodes throughout the body are engorged and edematous (Saliki, 1998; El Hag and Taylor, 1984). Vulvovaginitis lesions similar to the lesions in the oral mucocutaneous junction may be present (Saliki, 1998).

1.10. Diagnosis

1.10.1 Field diagnosis

In the field, a presumptive diagnosis can be made on the basis of the clinical, pathological and by epizootiological findings. Laboratory confirmation is an absolute requirement particularly in areas or countries where PPR has not previously been reported (Taylor et al., 1979; Lefevre et al., 1990).

1.10.2. Laboratory diagnosis

According to Saliki et al (1994), a wide range of laboratory procedures have been described for detecting the virus such as, viral antigen, viral nucleic acid, and as antibodies.

1.10.2.1. Specimens for laboratory diagnosis

For the laboratory diagnosis specimens to be submit included blood in EDTA anticoagulant, clotted blood or serum (if possible paired sera), mesenteric lymph nodes, spleen, lung, tonsils and section of the ileum and large intestine. Swabs of serous nasal and lachrymal discharge may
also be useful. All samples should be shipped fresh (not frozen) on ice within 12 hours after collection (Saliki, 1998; El Hag and Taylor, 1984). The above sample should be collected in the acute phase of the disease, when clinical signs are readily apparent. Ideally, samples should be collected from several animals during an outbreak. Epidemiologic and clinical details should be provided with the samples, and each sample bottle should be marked carefully with an indelible pen. Details of each sample’s origin should be recorded for submission to the laboratory (Taylor et al., 2002).

1.11. Serological test for detection of PPR antigens

1.11.1. Immunocapture enzyme–linked immunosorbent assay (ELISA)

The immunocapture enzyme-linked immunosorbent assay (ELISA), using several anti-N monoclonal antibodies (MAb), allows a rapid differential identification of PPR or rinderpest viruses, and this is of great importance as the two diseases have a similar geographical distribution and may affect the same animal species. The test is very specific and sensitive: it can detect $10^{0.6}\text{TCID}_{50}/\text{well}$ (50% tissue culture infective dose) for the PPR virus. The results are obtained in 2 hours (Libeau et al., 1995).

1.11.2. Counterimmunoelectrophoresis (CIEP)

Counter immunoelectrophoresis (CIEP) is the most rapid test for detecting viral antigen. It is carried out on a horizontal surface using a suitable electrophoresis bath, which consists of two compartments connected through a bridge. The apparatus is connected to a high–voltage source. Agar or agrose (1-2%, [w/v]) dissolved in 0.025 M barbitone acetate buffer is dispensed onto microscope slides in 3 ml volumes and from six to nine pairs of wells are punched in the solidified agar. The electrophoresis bath is filled with 0.1M barbitone acetate buffer. The
pairs of wells in the agar are filled with the reactants. Sera in the anode wells and antigen in the cathode wells. The slide is placed on the connecting bridge and the ends are connected to the buffer in the troughs by wetted porous paper. The apparatus is covered, and a current of 10-12 milliamps per slide is applied for 30-60 minutes. The current is switched off and the slides are viewed by intense light: the presence of 1-3 precipitation lines between pairs of wells is a positive reaction. There should be no reactions between wells containing the negative controls (Durojaiye, 1984; Majiyagbe et al., 1984).

1.11.3. Agar gel immunodiffusion (AGID) test

It is a very simple and inexpensive test that can be performed in any laboratory and even in the field. Standard PPR viral antigen is prepared from mesenteric or bronchial lymph nodes, spleen or lung material and ground up as 1/3 suspensions in buffered saline. These are centrifuged at 500g for 10-20 minutes, and the supernatant fluids are stored in aliquots at −20 oC. They may be retained for 1-3 years. Control antigen is prepared similarly from normal tissue. Standard antiserum is made by hyperimmunising sheep with 5ml of PPR virus with a titer of $10^4$TCID$_{50}$ (50% tissue culture infective dose) per ml given at weekly intervals for 4 weeks. The animals are bled 5-7 days after the last injection. Standard rinderpest hyperimmune antiserum is also effective in detecting PPR antigen and gives results within 1 day but not sensitive to mild forms of PPR. (Durojaiye, 1982).

1.11.4. Virus neutralization test

Prescribed test for international trade, the test is highly sensitive and specific than other serologic test, but can be time consuming. The standard neutralization test is carried out in roller–tube cultures of
primary lamb kidney cell, or Vero cell when primary cells are not available (Taylor, 1979).

1.12. Routine serological tests

Goats and sheep infected with PPR virus develop antibodies that may be demonstrated to support a diagnosis by the antigen–detection tests. Tests that are routinely used are the virus neutralization (NT) test and the competitive ELISA. Other test, such as counter immuno electrophoresis (CIEP), agar gel immune diffusion (AGID), precepitinogen inhibition test, and indirect fluorescent antibody test have remain of little interest compared with the (NT) and ELISA (Durojaiye et al., 1983).

1.13. Molecular diagnosis

1.13.1. Nucleic acid recognition methods

cDNA 32p labeled clones have been used to differentiate PPR and rinderpest but their use in routine diagnosis of PPR is not recommended due to short half-life of the 32P and the need for special equipment to protect the users (Diallo et al., 1988).

1.13.2. Polymerase chain reaction PCR

A PCR technique based on the amplification of the N protein and F protein genes have been developed for the specific diagnosis of PPR. This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction (Forsyth et al., 1995).

1.14. Virus isolation

PPR virus may be isolated in primary lamb kidney or in African green monkey kidney (vero) cell culture. Monolayer cultures are inoculated with suspect material and examined daily for cytopathic effect (CPE). The CPE produced by PPR virus can develop within 5 days and consists of cell rounding and aggregation culminating in syncytia formation in lamb kidney cells. In vero cells sometimes difficult to see
the syncytia. Syncytia are recognized by a circular arrangement of nuclei giving a clock face appearance (Lefevre and Diallo, 1990; Durojaiye et al., 1983).

1.15. Differential diagnosis

Rinderpest with clinical sign is rare in goats and sheep in Africa. In India, these species are quite often involved in RP outbreaks. Clinically, RP and PPR are similar, but the former should be the prime suspect if disease involves both cattle and small ruminants. Confirmation requires virus isolation and cross-neutralization test. In addition to rinderpest, other condition that should be considered in differential diagnosis included pasteurellosis, contagious caprine pleurapneumonia, bluetongue, heart water, contagious ecthyma (contagious pustular dermatitis), foot and mouth disease, Nairobi sheep disease, coccidiosis, gastrointestinal helminthes infestations and plant or mineral poisoning (OIE, 2002).

1.16. Control

1.16.1. Treatment

There is no treatment for PPR. However, mortality rates may be decreased by the use of drugs that control the bacterial and parasitic complication. Specifically, oxytetracycline and chlortetracycline are recommended to prevent secondary pulmonary infection (OIE, 2000).

1.16.2. Vaccination

In the past, the rinderpest vaccine has been used to protect goats for at least 12 months against PPR. However, this practice is being phased out to avoid confusion during retrospective serologic studies. A homologous attenuated PPR vaccine is now available and gives strong immunity. There are also genetically engineered recombinant vaccines undergoing limited field trial (OIE, 2002).
1.16.3. Control and eradication

Methods applied for rinderpest eradication may be appropriate for PPR and eradication is recommended when PPR appears in new area. These should include quarantine, combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations. Until recently, the most practical vaccination against PPR made use of tissue culture rinderpest vaccine. Recently, a homologous PPR vaccine has been developed and the vaccine seed is available through the Pan African Veterinary Vaccine Centre (PANVAC). This vaccine of choice is becoming increasingly available. The vaccines can protect small ruminants against PPR for at least three years (FAO, 1999). Slaughter and proper disposal of carcasses and contact fomite, decontamination of facilities and equipment and restrictions on importation of sheep and goats from affected areas (Taylor, 1984; Diallo et al., 1988; lefevre, 1990). Awa et al. (2000) recommended that control program of vaccinating of small ruminants against PPR accompanied by strategic of antihelmintic treatment, should be considered.

1.17. Public health

There are no known health risks to humans working with PPR virus as no report of human infection with the virus exists (OIE, 2002).
CHAPTER TWO
MATERIALS AND METHODS

2.1. Preparation and sterilization of glassware

Glassware: beakers, flasks, pipettes, cylinders, centrifuge tubes were boiled in water with a detergent for 20 minutes then rinsed in running water five times to remove the detergent completely. They were then immersed overnight in distilled water (DW), left to dry and then sterilized in hot air oven at 180 °C for 2 hours.

2.2. Sera

2.2.1. Serum samples

A total of 400 serum samples were collected randomly from sheep and goats in different localities in Sudan in November 2007 as shown in Table 1. Animals were bled from the jugular vein, 5 ml of blood were collected from each animal and kept overnight at room temperature. Then serum was separated by centrifugation at 1500 rpm for 10 min. The separated sera were stored at −20 °C till examined. Samples from Blue Nile state were collected kindly by (Dr. Ibrahim, Daoud).

2.2.2. Hyperimmune serum (Positive control)

Standard antiserum was raised in rabbits as described by Obi et al. (1990). PPR virus vaccine was injected subcutaneously 2 ml in the flank region. Subsequent three 2 ml injections were given intramuscularly at 2 weeks interval. The blood was collected 7 days after last injection and the serum was separated and stored at −20 °C till used.

2.2.3. New born calf serum (sigma) (Negative control)

New borne calf serum kindly supplied by Department of Viral Vaccines Production (CVRL) was used as a negative control.
2.3. Cell culture.
African Green Monkey Kidney Cell (Vero cell), kindly supplied by Department of Viral Vaccines Production, CVRL, Soba was used.

2.3.1. Cell culture preparation
Confluent monolayer cell culture of Vero cell was used. The growth medium was removed and the cell briefly washed with sterile PD. Three ml of trypsin — versene solutions were added and the bottle incubated at 37 °C until cells flew freely when the bottle was tilted. Few drops of calf serum were added to stop the action of trypsin and versene and the suspension was centrifuged at 1000 rpm for 5 minutes. The supernatant was poured out and the cell pellet was resuspended in 10 ml of growth medium and mixed well by pipeting. The suspension was diluted in growth medium and distributed in tissue culture flasks or tubes and then incubated at 37 °C.

2.4. PPR Virus
PPR virus used in this study was obtained from the Central Veterinary Research lab, Soba, Sudan. It is a PPR virus vaccine strain [PPR 75/1] originally isolated in Nigeria in 1975 and attenuated in vero cell culture (Diallo et al, 1988).

2.5. Serological tests
2.5.1. Agar gel immunodiffusion test
2.5.1.1. Preparation of agar
The test was carried out as described in the Manual of Standards, Diagnostic Tests and Vaccines (OIE, 2002) as follows: Agarose (1%) in normal saline containing 0.125 g sodium azide as anticontaminent agent was dispensed into Petri dishes as 6ml /5cm dish.
Table 1: Serum samples collected from sheep and goats from different localities in Sudan for serological detection of PPRV antibodies.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Serum samples collected from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Goats</td>
</tr>
<tr>
<td>Khartoum state</td>
<td>67</td>
</tr>
<tr>
<td>El Gezera state</td>
<td>_</td>
</tr>
<tr>
<td>Blue Nile state</td>
<td>_</td>
</tr>
<tr>
<td>River Nile state</td>
<td>_</td>
</tr>
<tr>
<td>El Ghadaref state</td>
<td>_</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
</tr>
</tbody>
</table>

=_ No sample collected
2.5.1.2. Preparation of PPR virus antigen

PPR virus antigen was prepared as described by Taylor and Abequnde (1979). The PPRV (NIG.75/1) was grown in vero cell. At maximum cytopathic effect (CPE), the cell and the supernatant medium were harvested and clarified at 2000 rpm for 10 min. The cell deposit was then washed with cold PBS, recentrifuged at the above speed and the supernatant was discarded. The cell deposit was resuspended in 1/250 original volume in cold PBS and then subjected to three alternate cycles of freezing and thawing, followed by centrifugation at 2000 rpm for 10 min. The supernatant was then collected and stored at –20°C and was used as a positive PPRV antigen.

2.5.1.3. Test procedure

Wells were punched in the agar following a hexagonal pattern with central well. The wells were 5 mm in diameter and 5 mm apart. The central well was filled with positive control Ag and three peripheral well with positive antiserum and one well with negative antiserum and the remaining peripheral wells were filled with test sera a total of 400 serum samples collected from sheep and goats (2.1.1) were examined. Lines when developed between the serum and positive antigen within 18–24 hours were intensified by washing the agar with 5% glacial acetic acid for 5 minutes. Positive serum reactions showed lines of the same identity as the positive control serum. This procedure was also carried out with all apparently negative tests before recording a negative result. Another arrangement was done by filled the central well with control Ag and peripheral well with test sera and one well with hyperimmune serum fig (1).
2.5.2. Serum Neutralization test (SNT)

A total of 400 serum samples collected from sheep and goats (2.1.1) were tested. Before testing the sera were inactivated at 56 °C for 30 min.

2.5.2.1. Test procedure

The test was conducted as described by Taylor (1979). One ml of inactivated serum was mixed with equal volume of stock virus suspension containing approximately $10^3$ TCID$_{50}$/ml. Then the serum–virus mixture was held at 37 °C for 2 h. The serum–virus mixture (0.2) ml was inoculated into 3 wells of tissue culture plate of 24 wells, followed immediately by the addition of 1.0 ml of growth medium containing $10^5$ of freshly suspended cell into each well. Then the plate was incubated at 37°C and were examined for cytopathic effect (CPE) on days 5, 8, 11 and finally on day 13. When the test was terminated complete protection of (CPE) even one out of the 3 wells per serum sample was interpreted as an evidence of neutralizing antibodies.

2.5.3. Competitive ELISA

2.5.3.1. Principle of the test

It is an assay to determine the presence of anti–PPRV antibody in serum. It is based on the competition between the anti-PPRV monoclonal antibody and the antibodies in the serum sample binding to the PPRV antigen. The presence of antibodies to PPRV in the serum sample will block reactivity of the monoclonal antibody resulting in reduction in expected color following the addition of conjugate and substrate/chromogen solution. As this is a solid phase assay, wash step are required between each step to ensure removal of unbounded reagents.
Competitive ELISA kit used was that of Biological Diagnostic Suppliers LTD (BDSL), Flow Laboratories and Institute for Animal Health. For use in seromonitoring and surveillance of cattle, sheep and goats for PARC, WAREC and SAREC. Pirbright, Surrey, England. Product and the technique described sheet fact accompanied the kit was followed.

2.5.3.2. Antigen

PPRV antigen: One ml of sterile distilled water supplied with the kit, was added to the freeze-dried contents of the vial and mixed till completely dissolved and stored at –20°C till used.

2.5.3.3. Control sera

Strong positive (++)c, week positive (+c), negative (-c): one ml of sterile distilled water supplied with the kit, was added to the freeze-dried contents of the each vial and mixed well until dissolved and stored at –20°C till used.

2.5.3.4. Monoclonal antibody (MAb)

One ml of sterile distilled water supplied with the kit, was added to the freeze-dried contents of the vial and mixed till completely dissolved and stored at –20°C till used.

2.5.3.5. Anti species conjugate

One vial contents of monoclonal (Mouse anti–PPR monoclonal antibody) was dissolved in 1ml of sterile distilled water supplied with the kit.

2.5.4. Reagents

2.5.4.1. Coating buffer (phosphate buffer saline (PBS))

Phosphate buffered saline (PBS, pH 7.4): was prepared by dissolving content of one liter PBS pouch in 100 ml of fresh glass distilled water to make 10X PBS. The stock was diluted 1 in 10 depending on requirements and stored at 4°C.
2.5.4.2. Washing buffer (WB)

Washing buffer was prepared by mixing 200 ml of PBS with 800 ml of DDW, and pH was adjusted to 7.4 ± 0.2.

2.5.4.3. Blocking buffer (BB)

Blocking buffer containing 0.1% Tween-20 and 0.2% negative serum. It was prepared fresh every time when needed.

2.5.4.4. Chromogen-substrate solution

One tablet (30 mg) of chromogen; Orth-phenylenediamine (OPD) was dissolved in 75.0 ml fresh glass distilled water and stored at –20°C till used. H₂O₂ solution (3%) was added just before use at the rate of 4.0 µl H₂O₂ per ml of OPD solution.

2.5.5. Test procedure

The test was carried out according to the manufacture instructions as follows:

Fifty µl of an antigen diluted (1\100) with PBS were dispensed to all wells of an ELISA micro plate. The plate was covered and placed on a shaker for one hour, then incubated overnight at +4°C.

After 3 wash cycles with washing buffer, 40 µl of blocking buffer were dispensed to all 96 wells of the plate.

According to the plate layout (Figure, 5), 10 µl from test and control sera (++)c, +c, -c) and monoclonal sera were added to appropriate wells.

Ten µl of blocking buffer were added to the monoclonal antibody control wells and 60 µl of blocking buffer to the conjugate control wells.

Fifty µl of MAb (1\100 BB) were added to all wells except the conjugate control wells. Then the plate was covered and incubated at 37°C on a shaker for one hour.
Then the plate washed vigorously 3 times with washing buffer and dried. Then 50µl of conjugate (1/1000 BB) were dispensed to all wells of the plate, then covered and incubated at 37°C on shaker for one hour. The plate was washed 3 times with washing buffer and dried. Fifty µl of substrate\chromogen were dispensed to all wells. Other clean micro titer plate was used for blanking. Fifty µl substrate\chromogen solutions were added to the row of blanking plate and incubated for 10 min in the dark. The reaction was stopped by addition of 50µl stop solution (H₂SO₄) to all wells of the test plate and the row of the blanking. Plates were read in a micro plate reader( BDSL) Immunoscan. MS serial No RS-232C at 492 nm filter. The reader was connected to a computer loaded with ELISA Data Interchange (EDI) software, which was used to automate the reading and calculation of percentage of inhibition (PI) values. The optical density (OD) values were converted to percentage inhibition by using the following formula: PI=100-(absorbance of the test wells/absorbance of the MAb control wells) X100. Sera showing PI greater than 50% were considered positive.

2.6. Data analysis

2.6.1. Principle of parameters

<table>
<thead>
<tr>
<th>Condition Present</th>
<th>Condition Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test positive</td>
<td>True Positive</td>
</tr>
<tr>
<td>Test negative</td>
<td>False Negative</td>
</tr>
</tbody>
</table>

The sensitivity (Sn) is defined as the probability that the test is positive when given to a group of patients who have the disease. It is calculated
by the formula $Sn = \frac{TP}{(TP+FN)} \times 100$. It is expressed as percentage (Munro, 2005).

The specificity (Sp) of a screening test is defined as the probability that the test will be negative among patients who do not have the disease. It is calculated by the formula $Sp = \frac{TN}{(TN+FP)} \times 100$ (Munro, 2005).

The positive predictive value (PPV) of a test is the probability that a patient who tested positive for the disease actually have the disease. It is calculated by the formula $PPV = \frac{TP}{(TP+FP)} \times 100$ (Munro, 2005).

The negative predictive value (NPV) of a test is the probability that a patient who tested negative for the disease will not have the disease. It is calculated by the formula $NPV = \frac{TN}{(TN+FN)} \times 100$ (Munro, 2005).

The efficiency (EFF) of a test is the probability that the test result and the diagnosis agree. It is calculated by the formula $EFF = \frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100$ (Munro, 2005).

**2.6.2. Statistical analysis**

The sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and the efficiency (EFF) of the AGID and NT test were determined in relation to ELISA as described by Munro (2005) and SPSS software (Statistical Package for Social Sciences) version 11.5, was used for statistical analysis of data.
Figure 1: The different arrangements of Ag and hyperimmune serum and serum samples in agar gel immunodiffusion test

HS: Hyperimmune serum

Ag: Antigen

S: Serum sample
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cc</td>
<td>++c</td>
<td>++c</td>
<td>+c</td>
<td>+c</td>
<td>cm</td>
<td>Cm</td>
<td>C-</td>
</tr>
<tr>
<td>2</td>
<td>Cc</td>
<td>++c</td>
<td>++c</td>
<td>+c</td>
<td>+c</td>
<td>cm</td>
<td>cm</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
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<tr>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

**Figure 2: ELISA Plate layout**

Cc: conjugate control, ++C: strong positive control, +C weak positive control, Cm: monoclonal antibody control, C- : negative control.
CHAPTER THREE
RESULTS

Four hundred serum samples collected from sheep and goats in different localities in Sudan as shown in Table 1 were examined by different serological method for screening PPR virus antibodies.

3.1. Examination of the sera by agar gel immunodiffusion (AGID) test

Out of 400 sheep and goats serum samples tested by AGID test, 103 were found positive (25.8%) for PPR antibodies. The detection of the PPRV antibodies in different localities by AGID test is shown in Table 2.

3.2. Examination of the sera by neutralization test (SNT)

Out of 400 sheep and goats serum samples tested by SNT, 167 were found positive (41.8%) for PPR antibodies. The detection of the PPRV antibodies in different localities by SNT is shown in Table 2.

3.3. Examination of the sera by cELISA test

Out of 266 sheep and goats serum samples tested by cELISA test, 138 samples were found positive (51.9%) for PPR antibodies. The detection of the PPRV antibodies in different localities by cELISA test is shown in Table 3.

3.4. Comparison between cELISA, SNT and AGID tests

The detection of PPRV antibodies in a total of 266 serum samples examined by AGID, SNT and cELISA were compared. cELISA test detected the highest percentage (51.9%) of seropositive while SNT and AGID test detected 41.8% and 25.8% seropositive respectively. The percentage of seropositive sample detected by the three serological tests in each locality is shown in Table 4 and Figure 3. ELISA test detected higher seropositive samples (51.9%) when compared to AGID test (25.8%) and SNT (41.8%). The AGID test agrees with ELISA in detection of 62 seropositive and 123 seronegative samples (Table, 5),
while SNT agrees with ELISA in detection of 95 seropositive and 115 seronegative samples (Table, 6).
The statically analysis showed SNT had higher sensitivity than AGID (68.8% Vs 44.9%), but lower specificity than AGID (89.8% Vs 96.1%) in relation to ELISA (Table, 7).
Table 2: Detection of PPRV antibodies in 400 serum samples collected from sheep and goats in different localities by AGID test and virus neutralization test (SNT).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Samples examined</th>
<th>AGID Positive</th>
<th>SNT Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khartoum state</td>
<td>67 sheep</td>
<td>15 (22.4%)</td>
<td>25 (37.3%)</td>
</tr>
<tr>
<td></td>
<td>50 goats</td>
<td>21 (42%)</td>
<td>34 (68%)</td>
</tr>
<tr>
<td>El Gezera state</td>
<td>49 sheep</td>
<td>12 (24.5%)</td>
<td>24 (49%)</td>
</tr>
<tr>
<td>Blue Nile state</td>
<td>114 sheep</td>
<td>29 (25.4%)</td>
<td>43 (37.8%)</td>
</tr>
<tr>
<td>River Nile state</td>
<td>38 sheep</td>
<td>2 (5.3%)</td>
<td>5 (13.2%)</td>
</tr>
<tr>
<td>El Ghadaref state</td>
<td>82 sheep</td>
<td>24 (29.3%)</td>
<td>36 (43.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>103 (25.8%)</td>
<td>167 (41.8%)</td>
</tr>
</tbody>
</table>
Table 3: Detection of PPRV antibodies in 266 serum samples collected from sheep and goats in different localities by cELISA test.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Goats</th>
<th></th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples Examined</td>
<td>Samples positive (%)</td>
<td>Samples Examined</td>
</tr>
<tr>
<td>Khartoum state</td>
<td>36</td>
<td>22 (61.2%)</td>
<td>47</td>
</tr>
<tr>
<td>El Gezera state</td>
<td>__</td>
<td>__</td>
<td>30</td>
</tr>
<tr>
<td>Blue Nile state</td>
<td>__</td>
<td>__</td>
<td>75</td>
</tr>
<tr>
<td>River Nile state</td>
<td>__</td>
<td>__</td>
<td>22</td>
</tr>
<tr>
<td>El Ghadaref state</td>
<td>__</td>
<td>__</td>
<td>56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>36</td>
<td>22 (61.1%)</td>
<td>230</td>
</tr>
</tbody>
</table>

− = No sample collected and examined
Table 4: PPRV seropositive serum samples collected from goats and sheep in different localities in Sudan detected by AGID, SNT and cELISA tests (n=266).

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of Sample</th>
<th>AGID Positive</th>
<th>SNT Positive</th>
<th>cELISA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Khartoum state</td>
<td>47 sheep</td>
<td>12 (25.5%)</td>
<td>32 (68.0%)</td>
<td>40 (85.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 (27.8%)</td>
<td>12 (33.3%)</td>
<td>22 (61.1%)</td>
</tr>
<tr>
<td></td>
<td>36 goats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El Gezera state</td>
<td>30 sheep</td>
<td>6 (20%)</td>
<td>10 (33.3%)</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>Blue Nile state</td>
<td>75 sheep</td>
<td>22 (29.3%)</td>
<td>32 (42.6%)</td>
<td>41 (54.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (13.6%)</td>
<td>5 (22.7%)</td>
<td>8 (36.4%)</td>
</tr>
<tr>
<td>River Nile state</td>
<td>22 sheep</td>
<td>3 (13.6%)</td>
<td>5 (22.7%)</td>
<td>8 (36.4%)</td>
</tr>
<tr>
<td>El Ghadaref state</td>
<td>56 sheep</td>
<td>14 (25%)</td>
<td>17 (30.4%)</td>
<td>16 (28.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>67 (25.2%)</td>
<td>108 (40.6%)</td>
<td>138 (51.9%)</td>
</tr>
</tbody>
</table>
Table 5: PPRV antibodies detection by agar gel-immunodiffusion test in relation to cELISA test (n=266).

<table>
<thead>
<tr>
<th>AGID</th>
<th>cELISA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Positive</td>
<td>62</td>
<td>5</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>Negative</td>
<td>76</td>
<td>123</td>
<td></td>
<td>199</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>128</td>
<td></td>
<td>266</td>
</tr>
</tbody>
</table>
Table 6: PPRV antibodies detection by neutralization test in relation to cELISA test (n=266)

<table>
<thead>
<tr>
<th>SNT</th>
<th>cELISA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>95</td>
<td>13</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>43</td>
<td>115</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>128</td>
<td>266</td>
<td></td>
</tr>
</tbody>
</table>
Table 7: The Sensitivity, specificity, positive predictive value, negative predictive value and efficiency of AGID and SNT tests in relation to cELISA test (n=266).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AGID</td>
<td>SNT</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>44.9%</td>
<td>68.8%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.1%</td>
<td>89.8%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>92.5%</td>
<td>88.0%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>69.6%</td>
<td>72.8%</td>
</tr>
<tr>
<td>Efficiency</td>
<td>69.6%</td>
<td>79.0%</td>
</tr>
</tbody>
</table>
Figure 3: Frequency of seropositive and seronegative PPRV antibodies detected by AGID test, SNT and cELISA test in serum samples of sheep and goats (n=266).
DISCUSSION

Peste des petits ruminants (PPR) is an acute viral disease. The disease mainly affects sheep and goats, however, goats are affected more often and more severely than sheep (Singh et al., 2000). The disease is highly contagious and causes varying degree of morbidity and mortality in susceptible population (Radostits et al., 2000).

In Sudan, PPR was first reported by El Hag et al. (1984). Within a decade, PPR became endemic in the country, imposing severe threat to small ruminants resulting in heavy economic loss to the farmers as well as small ruminant industry.

The present study assessed the seropositivity to PPR virus antibodies in sheep and goats. A total of 400 serum samples from sheep and goats were collected from different localities in Sudan and were screened for PPR specific antibodies by SNT, AGID and cELISA. The overall PPRV antibodies seroprevalence recorded in sheep and goats was 41.8%, 25.8%, and 51.9% by SNT, AGID and cELISA respectively.

Three methods may be used to diagnose and monitor the distribution and prevalence of PPR: Case recording of PPR outbreaks, serological detection of PPR specific antibodies and detection of the virus. Although, case recording of PPR outbreaks could give some clues in the areas where the disease is endemic, serological tests for monitoring the antibodies against PPRV must be both highly specific and sensitive to provide accurate results in field studies. Further, a diagnostic test to be used for serological surveys must be rapid and economic. However, laboratory diagnosis is essential for confirmation by detection of virus.

Conventional serological tests such as AGID and Counter immunoelectrophoresis (CIEP) have been used in the past for diagnosis of rinderpest but they often fail in specific diagnosis of PPR due to cross reaction between PPRV and RPV. Thus, monoclonal antibody based
cELISA developed by Anderson and McKay (1994) became the most popular test for diagnosis of PPR, as it was highly sensitive and specific. Although, the assay was highly specific and sensitive, mere detection of antibodies could not confirm the presence of virus at the locations where specific vaccination against the PPRV was practiced (Saliki et al., 1994).

AGID test is known to be reliable for detection of specific antibodies in species where there is no available commercial conjugate to their antibodies (Taylor, 1990; OIE, 2000).

In the present study AGID test detected PPR virus antibodies in 103 samples out of 400 sera sample (25.8%) collected from different localities in Sudan (Table, 1), in this study. Durojaiye and Taylor (1984) described AGIDT to monitor the appearance of antibody in experimentally infected sheep and goats. Virus neutralization test of serum samples collected from sheep and goats could detect the presence PPRV antibody (Saliki et al., 1993; Anderson et al., 1991). Out of 400 samples screened from different localities in Sudan (Table, 1),SNT detected PPRV antibodies in 167 samples (41.8%). This is in agreement with the observations of Taylor. (1979); Libeau et al. (1995) and Singh et al. (2004).They were able to detect PPRV antibodies by VNT in field and laboratory serum samples. Durojaiye and Taylor found, of the 137 convalescent field serum samples, 80 were positive to AGID test and 78 to the neutralization test, 71 were positive to both the tests. However, in this investigation NT detected higher prevalence of PPRV (41.8%) than AGID test (25.8%).

In this investigation study significant PPRV antibodies were detected in serum samples of sheep and goats screened by cELISA. Of the 266 serum samples, 138 samples were seropositive to PPR (51.9%). Libeau et al, (1995) and Singh et al, (2004) reported 24 out of 271field serum samples and 691 out of 1700 field and laboratory serum samples were positive.
cELISA test is described as diagnostic technique, which is simple, rapid, specific and sensitive, test for screening of antibodies to various morbilliviruses (Saliki et al., 1993; Libeau et al., 1995). This test is having several advantages over NT, as it does not require cell culture facility and strict sterility of serum samples. Rapid diagnosis and screening of large number of sera sample is therefore, possible using the cELISA. Morbillivirus cELISA detected anti-RPV, and anti-PPRV antibodies, in all the reference RPV and PPRV antisera containing VN titers 1:8, suggesting that the assay could simultaneously detect antibodies against RPV and PPRV (Choi et al., 2004). The test used monoclonal antibody to neutralize epitope of hemagglutinin protein (H-protein) of the virus, revealed presence of PPR virus specific antibodies of PPR without showing cross-reaction with antibodies of RP (Kulkarni et al., 1996; Choi et al., 2004).

Efficacy of cELISA compared very well with SNT, having high relative specificity (98.4%) and sensitivity (92.4%). The sensitivity of c-ELISA for PPR sero-surveillance was more (95.4%), if the target population was non-vaccinated, and it had been employed for detection of PPR circulating antibodies (Anderson et al., 2000).

It was opined that the cELISA developed could easily replace SNT for sero-surveillance, seromonitoring, diagnosis from paired sera samples and end-point titration of PPR virus antibodies (Singh et al., 2004). In India epidemiological scenario, suggested that cELISA kit could prove to be an important tool for sero-monitoring and serosurveillance of PPRV antibodies (Sreenivasa et al., 2002). In previous study it was observed that the test can detect low levels of antibodies in newborn sera containing maternal antibodies. Thus the test may be useful tool for standardization and accurate determination of immune status (Libeau et al., 1995).
Also, the present study revealed cELISA is more sensitive test when compared to AGID test and SNT as cELISA detected more PPRV antibodies positive samples (51.9%) than AGID test (25.8%) and SNT (41.8%).

Since previous studies proved cELISA is efficient diagnostic technique which is specific and sensitive for detection of PPRV antibodies and also this study revealed cELISA is more sensitive than the other two tests, it was decided to examine the sensitivity and specificity of AGID test and SNT in relation to cELISA.

The statistical analysis showed NT had higher sensitivity than AGID test (68.8%Vs 44.9%), but lower specificity (89.8%Vs 96.1%), in relation to cELISA. This indicates NT is second test of choice if facilities are equally available.

In Sudan, prevalence of PPR disease was screened by cELISA (Intisar, 2002). She detected PPRV antibodies in 75.7% serum samples collected from nonvaccinated sheep and goats. This study confirms the previous findings (Intisar, 2002) as it detected PPRV antibodies (51.9%) in serum samples of sheep and goats with no vaccination history. This indicates the exposure of sheep and goats to this virus and the disease remains prevalent in the country.

Conclusions and Recommendation
Conclusions

From the finding of this study, it can be concluded that:

– PPRV antibodies were detected in serum samples collected from sheep and goats in non vaccinated sheep and goats by AGID (25.8%), SNT (41.8%) and cELISA (51.9%).

– The detection of PPRV antibodies indicates the exposure of the sheep and goats to PPRV and the disease remains prevalent in the Sudan.

– Competitive ELISA test detected the highest PPR seropositive.

– The statistical analysis revealed the SNT is second test of choice.

Recommendation
From the results and discussion it can be recommended that:

1. Serosurveillance of PPR in small ruminants in all states of Sudan.

2. Serosurveillance of PPR in wild ruminants and other domestic ruminants to investigate the possible role of the wild life and domestic ruminants in the cycle of PPR.

3. Strategies for the control of PPR need to account for the dynamics of sheep and goats population.

4. Mass vaccination in endemic area, and along the routes of animal movement.

REFERENCES


Fenner, Frank; Peter, A; Bachmann, E; Gibbs, Paul J; Frederick, A; Murphy; Studdert, Michael J.; White, David O. (1987). Veterinary Virology, 2:30-38.


Appendices

Appendix I

Buffers

1. Physiological saline (Normal Saline) 0.85%

NaCl 8.5g
DDW 1000 ml

The solution was autoclaved at 121 °C for 30 minutes and kept at +4 °C until used. The pH was adjusted to 7.2.

2. Phosphate buffered saline (PBS)

Solution A

Na Cl 16 g
K Cl 0.4 g
Na$_2$HPO$_4$ (unhydrous) 2.3 g
K HPO$_4$ 0.4 g
DDW completed to 1500 ml

Solution B

Mg Cl$_2$.6H$_2$O (hydrous) 0.426 g
DDW 200 ml

Solution C

CaCl$_2$ (hydrous) 0.426 g
DDW completed to 200 ml

Solution A, B and C were autoclaved at 121°C for 30 minutes and left to cool. Solution A was added to Solution B then Solution C was added and completed to two employed in medium preparation. Liters with sterile DDW. Antibiotics were added at the same concentration.

3. Phosphate diluents (PD)

Na Cl 16.0 g
KCl 0.4 g
Na$_2$ HPO$_4$ (unhydrous) 2.3 g  
KHPO$_4$ 0.4 g  
DDW completed to 2000 ml  
The solution was sterilized by autoclaving at 121°C for 30 minutes.

Appendix II

Cell dispersing solutions

1. Trypsin (7.5%)  
Trypsin powder 37.5 g  
PD completed to 500 ml  
The solution was sterilized by filtration.

2. Veresene (5%)  
Veresene 25 g  
PD completed to 500 ml

3. Trypsin versene solution  
Trypsin(7.5%) 6 ml  
Versene (5%) 4 ml  
PD 90 ml  
Trypsin and versene were sterilized by filtration. PD was sterilized by autoclaving at 121°C for 15 minutes.
Appendix III

Tissue culture media

1. Growth media

GMEM medium to prepare 1x GMEM medium

- GMEM 5x 200 ml
- Yeast extracts 1% 25 ml
- Lactalbumen hydrolysate (x5) 25 ml
- Sodium bicarbonate 7.5 ml
- Fetal calf serum 10% ml

The complete medium was sterilized by filtration, supplemented with antibiotic (100 iu/ml Penicillin, 50µg/ml Streptomycin, 10µg/ml Gentamycin) and Mycostatin at a concentration of 50 iu/ml and then completed to 1 litter with DDW.

2. Maintenance medium

Same as growth medium with 5% fetal calf serum.

Media additives

1. Lactalbumin hydrolysate (5%)

- Lactalbumin hydrolysate 25g
- DDW 500 ml

The solution was sterilized by autoclaving at 121°C for 30 minutes.

2. Sodium bicarbonate 7.5% (NaHCO₃)

- NaHCO₃ 7.5 g
- DDW completed to 100ml

The solution was sterilized by autoclaving at 121°C for 30 minutes.