بسم الله الرحمن الرحيم

SUDAN UNIVERSITY OF SCIENCE & TECHNOLOGY
COLLEGE OF VETERINARY MEDICINE

اختبار الترسيب المناعي في الأجار للتشخيص المصلي لمرض طاعون المجترات الصغيرة في الخراف الملقحة وغير الملقحة بولاية النيل الابيض ، السودان

AGAR GEL PRECIPITATION TEST (AGPT) FOR SERODIAGNOSIS OF PESTE DES PETITS RUMINANTS (PPR) IN VACCINATED AND NON-VACCINATED SHEEP IN WHITE NILE STATE, SUDAN

by

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A Dissertation Submitted in partial fulfillment of the requirements For the Degree of Bachelor (Honours) of Veterinary Medicine (B.V.M)

Supervisor

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September, 2016

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالي:

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صدق الله العظيم

Dedication

We dedicate our Dissertation to:

Our father's "Whom we hold their names with pride"

Our Mathers "Our angels in life"

Our Sisters and Brothers "With whom we shared the most beautiful moments of our lives

&

Whom were always supported us whole heartedly

Preface

The work described in this dissertation was carried out in the Virology laboratory in the Department of Pathology, Parasitology and Microbiology at the college of Veterinary Medicine, Sudan University of Science & Technology from February to August 2016 under the supervision of Dr. Nussieba Ahmed Osman Elhag.

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

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Table of Contents

List of Figures

List of Abbreviations

AGID:	Agar gel immunodiffusion test
AGPT:	Agar gel precipitation test
C & V:	non-structural proteins
CDV:	Canine distemper virus
C-ELISA:	Competitive ELISA
CIEP:	Counter immunoelectrophoresis
CPE:	Cytopathic effect
DDW:	Deionized distilled water
DMV:	Dolphin distemper virus
ELISA:	Enzyme linked immunosorbent assay
F:	Fusion protein
H:	Haemagglutinin
HA:	Haemagglutination test
HI:	Haemagglutination inhibition test
IC-ELISA:	Immunocapture ELISA
IHC:	Immunohistochemistry
IP:	Immunoperoxidase staining
L:	Large protein/Polymerase
LK:	Lamb kidney
M:	Matrix protein

MV:	Measles virus
N:	Nucleoprotein
OIE:	Office International des Epizootics/ World Organization for Animal Health
P:	Phosphoprotein
PCR:	Polymerase chain reaction
PDV:	Phocine and porpoise distemper virus
PPR:	Peste des petits ruminants
PR:	Rinderpest
RTCV:	Rinderpest tissue culture vaccine
RT-PCR:	Reverse-transcription PCR
rt-PCR:	Real time PCR
SLAM:	Signaling lymphocytic activation molecule
SUD 72/1:	PPRV Sudanese Sinnar strain
SUD 72/2:	PPRV Sudanese Mieliq strain
Vero:	African green monkey kidney
VN:	Virus neutralization

Abstract

In the present study, agar gel precipitation test (AGPT) was performed for the detection of antibodies against peste des petits ruminants (PPR) in vaccinated and non-vaccinated sheep sera from Kosti, White Nile State, Sudan. The results showed that AGPT detected PPRV antibodies in only 2 sera (2%) out of the 100 sera from the vaccinated animals and in only 3 sera (6%) out of the 50 sera from the non-vaccinated sheep sera.

The results indicated that AGPT was not sensitive enough for the detection of PPRV antibodies and proved not suitable as a serological diagnostic tool for PPR.

Keywords:

Peste des petits ruminants (PPR), Antibody, vaccinated and non-vaccinated animals, agar gel precipitation test (AGPT).

الخلاصة

في هذا البحث تم استعمال اختبار الترسيب المناعي في الأجار للكشف عن وجود الأجسام المضادة لفيروس طاعون المجترات الصغيرة في امصال الخراف الملقحة وغير الملقحة ضد المرض في مدينة كوستي بولاية النيل الابيض ، السودان. وأظهرت النتائج أن اختبار الترسيب المناعي في الأجار كشف عن وجود الأجسام المضادة للفيروس في مصل حيوانين فقط (وتمثل نسبة 2٪) من اصل 100 عينة مصل من الحيوانات الملقحة ، كما كشف الاختبار عن وجود الأجسام المضادة للفيروس في امصال 3 حيوانات فقط (وتمثل نسبة 6٪) من اصل 50 عينة مصل من الحيوانات غير الملقحة. وأشارت النتائج إلى أن اختبار الترسيب المناعي في الأجار لم يكن حساساً بما يكفي للكشف عن وجود الأجسام المضادة للفيروس في امصال الخراف الملقحة وغير الملقحة واتضح ان هذا الإختبار غير ملائم كوسيلة للتشخيص المصلي لمرض طاعون المجترات الصغيرة.

الكلمات المفتاحية:

مرض طاعون المجترات الصغيرة - الاجسام المضادة - الحيوانات الملقحة وغير الملقحة - اختبار الترسيب المناعي في الأجار.

Introduction

Peste des petits ruminants (PPR) is an acute or subacute fatal viral disease of goats and sheep. PPR is characterized by fever, necrotic stomatitis, conjunctivitis, gastroenteritis, diarrhea, pneumonia and sometimes death. The causative agent peste des petits ruminants (PPR) virus is classified in the genus *Morbillivirus* in the family *Paramyxoviridae* in the order Mononegavirales. PPRV is closely related to Rinderpest (RP) virus and the two viruses cause similar clinical diseases in susceptible animals (Gibbs *et al.,* 1979; Murphy *et al.,* 1999). Goats and sheep appear to be equally susceptible to the virus, but goats exhibit more severe clinical disease. Several wild small ruminant species are also susceptible to PPRV infection.

PPR was firstly described in East Africa in Cote d'Ivoire (the Ivory Coast) in 1942 and subsequently in other parts of West Africa then expands to the East. Currently the disease is endemic in most of Africa, most of Asia, the Middle East and Turkey. PPR was firstly reported in the Sudan in 1971 (Elhag Ali, 1973; Lefevre and Diallo, 1990; OIE, 2013).

Objectives of the Study

The objectives of the present study are to:

1. Detect antibodies against PPRV in sera from vaccinated and non-vaccinated sheep from Kosti, White Nile State, Sudan.
2. Test the suitability of the agar gel precipitation test (AGPT) for detection of antibodies against PPRV in serum samples.

Chapter 1

Literature Review

1.1. Peste des petits ruminants (PPR):

Peste des petits ruminants (PPR) is a contagious and fatal viral disease of domestic and wild small ruminant. PPR is one of the notifiable diseases to the World Organization for Animal Health "OIE" (OIE, 2013). It has an economic importance due to its high rates of morbidity and mortality among susceptible animals which may reach 100% and 50-80%, respectively (Losos, 1986; Kitching, 1988). The disease is characterized by fever, ocular and nasal discharges, stomatitis, gastroenteritis, diarrhea, conjunctivitis and pneumonia (Lefevre and Diallo, 1990; OIE, 2013).

1.2. Causative Agent:

Peste des petits ruminants (PPR) is caused by peste des petits ruminants virus (PPRV) which belongs to the *Morbillivirus* virus genus of the *Paramyxoviridae* family of the *Paramyxovirinae* subfamily of the Order *Mononegavirales* (Gibbs *et al.,* 1979). PPR virus is closely related to Rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV), dolphin distemper virus (DMV), phocine and porpoise distemper virus (PDV) (Gibbs *et al.,* 1979; Murphy *et al.,* 1999).

1.3. Virus Structure:

It is an RNA enveloped virus. The RNA genome of PPRV is single-stranded negative sense and is 15,948 Kb in size. PPRV and other members of the Morbillivirus genus have six structural proteins namely: the nucleoprotein (N), the phsophoprotein (p), the matrix protein (M), the fusion protein (F), the haemagglutinin (H) and the large protein or polymerase (L) in addition to two non-structural proteins (C and V) (Figure 1) (Bailey *et al.,* 2005; Chard *et al.,* 2008).

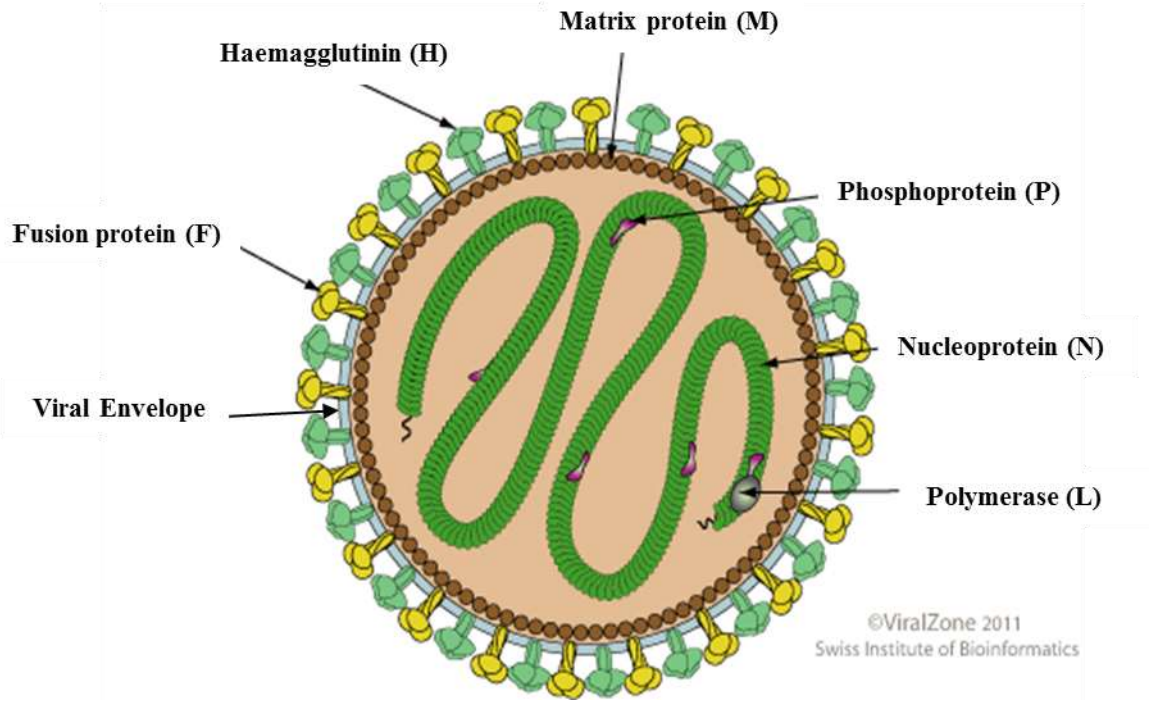

Figure 1. Schematic diagram of a PPR viral particle showing the viral structural proteins: the nucleoprotein (N), the phosphprotein (P), the large protein/polymerase (L) the matrix (M) protein, the haemagglutinin (H), the fusion (F) protein and the viral envelope. (ViralZone, SIB Swiss Institute of Bioinformatics, 2011).

1.4. History of the disease:

PPR was firstly described in Cote d'Ivore (Ivory Coast) in West Africa in 1940 (Gargadennec and Lalanne, 1942). After that the disease starts to appear in other countries in East Africa then distributed further towards middle and east Africa. According to the accompanied clinical signs, the disease was recognized by different names or synonyms, especially in west Africa, including Kata (Whitney *et al.,* 1967), stomatitis pneumoentritis complex, (Nduaka and Ihemelandu, 1973), goat plaque, pest of small ruminants, Rinderpest-like disease or syndrome pseudorinderpest of small ruminants (Johnson and Ritchie, 1968; Durtnell and Eid, 1973), contagious pustular stomatitis and contagious vulvovaginitis (Wosu, 1992).

1.5. Epidemiology:

PPRV has a single serotype, although four genetically distinct lineages of PPR strains were identified; lineage 1 and 2 viruses in West Africa, lineage 3 in East Africa, Arabian and Southern India and lineage 4 in the Middle East and Asia subcontinent. Recently, the incursion of lineage IV was reported in some countries in North and Middle Africa. PPRV lineages reflect the epidemiology of the disease by describing the origin of the virus causing a new outbreak (Shaila *et al.,* 1996; Dhar *et al.,* 2002; Kwiatek *et al.,* 2010).

1.5.1. Geographic Distribution:

Starting from 2003 and coincides with the beginning of the control strategy for the global eradication of rinderpest (RP), PPR emerged in countries which was previously known as free from PPR including north and middle African countries and some Asian countries. PPR is now endemic in most of African countries except few countries in South Africa, most of Asia, the Arabian Peninsula, the Middle East and in Turkey (Figure 2) (Nanda *et al.,* 1996; Shaila *et al.,* 1996; Banyard *et al.,* 2010; OIE, 2013).

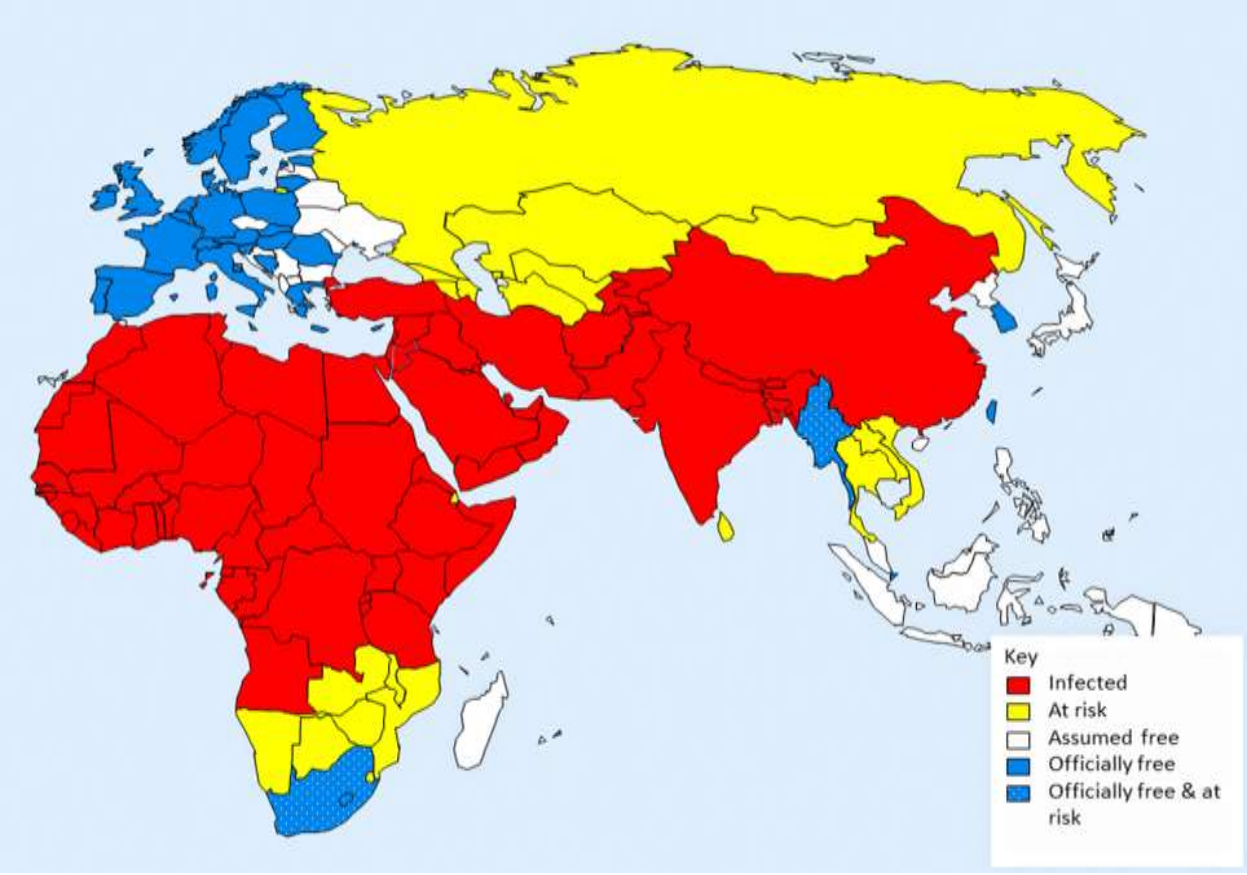

Figure 2. Worldwide distribution of peste des petits ruminants (Jones *et al.,* 2016).

1.5.2. Host Range:

Sheep and goats are the primary hosts for PPRV. Outbreaks of a fatal respiratory disease were reported in camels in Ethiopia and in the Sudan (Roger *et al.,* 2000; Roger *et al.,* 2001; Khalafalla *et al.,* 2010). Cattle, buffalo and pigs develop subclinical disease without excreting the virus, and thus are not considered to be important in the epidemiology of the disease (Nawathe and Taylor, 1979; Anderson and McKay, 1994; Govindarajan *et al.,* 1997). Many reports described infection of wild small ruminants by PPRV, such as white-tailed deer, gazelle, ibex, Bharal, although their role in the epidemiology of the disease needs more studies (Furley *et al.,* 1987; Abu Elzein *et al.,* 2004; Kinne *et al.,* 2010; Bao *et al.,* 2011).

1.6. Transmission:

Transmission of PPR generally requires close contact with infected animals. PPRV is transmitted mainly by aerosols between animals living in close contact. The virus is secreted in all body secretions and excretions (lacrimal, nasal, saliva, feces) of infected animals, thus it can be transmitted to close in-contact susceptible animals (Lefevre and Diallo, 1990; OIE, 2013). PPRV is excreted for several days before the onset of significant clinical signs, such that the spread of the virus is enhanced with the co-mixing of animals. The virus is temperature

sensitive and is easily inactivated in a dry environment (Rossiter and Taylor, 1994).

1.7. Clinical sings:

The incubation period is normally 4-6 days, but in some cases may range between 3 and 10 days. The disease “PPR” has two different clinical forms namely the acute or the subacute form (OIE, 2013).

The clinical disease is acute, with pyrexia up to 41°C that can last for 3-5 days; the animals become depressed, anorexic and develop a dry muzzle. Serous oculonasal discharges become progressively mucopurulent which may block the nares and encrust the muzzle causing the animal to short and sneeze, whereas the ocular discharges may mat the eye lids together. Within 4 days of the onset of fever, the gums become hyperaemic, and erosive lesions develop in the oral cavity which may become necrotic with excessive salivation. Watery bloody diarrhoea is common in the later stages of the disease. Pneumonia, coughing, pleural rales and abdominal breathing also occur and death within one week of the onset of illness (OIE, 2013).

The morbidity rate among susceptible animals can reach 90-100% whereas the mortality rate can reach 50-80% in more severe cases. However, morbidity and mortality may be much lower in milder outbreaks (OIE, 2013).

The subacute form of PPR is more common in sheep but can occur in goats and is manifested by low grade of clinical sings and lesions. Most affected animals recover within two weeks (Lefevre and Diallo, 1990; OIE, 2013).

1.8. Postmortem (PM) lesions:

Animals affected by PPR showed similar postmortem lesions to those observed in cattle affected with Rinderpest, except that the presence of prominent crusty scabs on the outer lips and severe interstitial pneumonia mostly occur with PPR. Erosive lesions may extend from the mouth to the reticulo-rumen junction. Characteristic linear red areas of congestion or haemorrhage may occur along the longitudinal mucosal folds of the large intestine and rectum (zebra stripes), but they are not a consistent finding. Erosive or haemorrhagic enteritis is usually present and the ileo-caecal junction is commonly involved. Peyer's patches may be necrotic. Lymph nodes are enlarged, and the spleen and liver may show necrotic lesions (Lefevre and Diallo, 1990; OIE, 2013).

1.9. Immunity:

Infected animals that recover from disease develop a lifelong protective immunity and no carrier state has been identified (Hamdy *et al.,* 1976).

1.10. Diagnosis of PPR:

A tentative diagnosis of PPR can be made depending on disease history, epidemiology, high morbidity and mortality rates, characteristic clinical signs and postmortem lesions. A provisional diagnosis can be made by laboratory confirmation of PPR and differential diagnosis with other diseases which have similar clinical signs (OIE, 2013).

1.10.1. Samples for diagnosis of PPR:

The ideal samples for diagnosis of PPR from live animals include swabs from the conjunctival discharges and from the nasal and buccal mucosa. Whole blood is also collected in anticoagulant, during the very early stage of the disease, for virus isolation, polymerase chain reaction (PCR) and haematology. Samples from dead animals should be collected aseptically from lymph nodes, especially the mesentric and bronchial nodes, lungs, spleen and intestinal mucosa. For histopathology samples of organs were collected in 10% neutral buffered formalin (OIE, 2013).

1.10.2. Laboratory diagnosis of PPRV

For confirming diagnosis of PPRV, virus isolation in cell culture can be performed using primary lamb or goat kidney (LK), African green monkey kidney (Vero), CV1 cells expressing sheep/goat SLAM, Vero Dog-SLAM cell cultures have been used for isolation and propagation of

PPRV. Virus isolation or growth in cell culture can be confirmed by observing the cytopathic effect (cpe) in the days following infection of a monolayer culture with suspected material. The cpe is characterized by cell rounding, syncytia formation and intracytoplasmic and intranuclear inclusion bodies (Hamdy *et al.,* 1976; Taylor and Abegunde, 1979; Durojaiye *et al.,* 1983; Adomi *et al.,* 2011).

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

Detection of antibodies to PPRV by using competitive ELISAs (C-ELISA) (Libeau and Lefevre, 1990; Saliki *et al.,* 1993; Anderson and McKay, 1994; Libeau *et al.,* 1992; Libeau *et al.,* 1995; Singh *et al.,* 2004; Choi *et al.,* 2005; OIE, 2013) and virus neutralization (VN) tests (Rossiter *et al.,* 1985; OIE, 2013) which are recommended by the OIE for serological diagnosis of PPR (OIE, 2013).

Molecular techniques for detection of PPRV in reference laboratories is based on standard reverse-transcription-PCR (RT-PCR) assay followed by sequencing of new PPRV isolates, quantitative RT-PCR and realtime-PCR

(rt-PCR) (Couacy-Hymann *et al.,* 2002; Forsyth and Barrett, 1995; Bao *et al.,* 2008; Kwiatek *et al.,* 2010).

1.11. Control of PPR:

Control of PPR outbreaks relies on control of animal movement, quarantine, slaughter, proper disposal of carcasses and contact fomites, decontamination of facilities and equipment, restriction on the import of sheep and goats from affected areas combined with animal vaccination (OIE, 2013).

1.11.1. Vaccination

Vaccination is the most effective way to control PPR in endemic areas. Due to the cross-relationship between PPRV and RPV, attenuated Plowright Rinderpest tissue culture vaccine (RTCV) was used for many years to control PPR (Roeder and Obi, 1999), however, after the eradication of Rinderpest, its usage was restricted by the OIE. TCRP vaccine provides protection for at least one year and probably for economic life of the vaccinated animal (Taylor and Abegunde, 1979).

A live attenuated vaccine PPR was developed by serial attenuation of PPRV Nigeria 75/1 strain, belongs to lineage I, in Vero cells. The vaccine is effective for the control of PPR and can provide protection against PPR in small ruminant for at least 3 years (Diallo *et al.,* 1989; Diallo *et*

al., 2007; Sen *et al.,* 2010). Subsequently, three attenuated PPR vaccines were developed in India by using PPRV Sungri 96, Arasur 87 and Coimbatore 97 Indian isolate of lineage IV and currently are under use in the field (Saravanan *et al.,* 2010; Sen *et al.,* 2010).

1.12. PPR in the Sudan:

In the Sudan, peste des petits ruminants (PPR) was known since the beginning of the 1970th where outbreaks of a Rinderpest-like disease in sheep and goats were reported in three areas in Elgadarif "a town in Eastern Sudan" in 1971 (Elhag Ali, 1973). Following that the disease was reported in Sinnar during the years 1971 to 1972 and in Mieliq in 1972. At that time the disease was wrongly diagnosed as Rinderpest (RP) and 10 years later it is confirmed to be PPR (Elhag Ali and Taylor, 1984). PPRV was firstly isolated by Elhag Ali and the isolates were named PPRV SUD 72/1 (Sinnar strain) and SUD 72/2 (Mieliq strain) (Elhag Ali and Taylor, 1984). Subsequently, outbreaks of the disease were reported during the years 1989 and 1990 in Elhilalia in Gezira State, the virus isolated was named PPR VHL (El Hassan *et al.,* 1994).

Several studies on PPR in the Sudan was carried out including virus isolation (Rasheed, 1992; Zeidan, 1994; Saeed *et al.,* 2004; Osman *et al.,* 2008); virus characterization using agar gel precipitation test (AGPT); haemagglutination test (HA); reverse passive haemagglutination test

(HA); immunocapture ELISA (IC-ELISA) and reverse transcription polymerase chain reaction (RT-PCR) (Saeed *et al.,* 2004; Osman *et al.,* 2008; Ali *et al.,* 2014; Ishag *et al.,* 2015). Molecular characterization studies confirmed the presence of PPRV lineage III and recently the emergence of lineage IV genotype (Kwiatek *et al.,* 2011). Serological surveillance using agar gel precipitation test (AGPT); counter-immunoelectrophoresis (CIEP); and competitive ELISA (C-ELISA) confirmed the presence of the disease in different regions of the country such as Gezira, White Nile, Blue Nile, Khartoum, River Nile, Northern (Elshamalia), Red Sea, Kordofan and Darfur states (Rasheed, 1992; Zeidan, 1994; El Amin and Hassan, 1999; Haroun *et al.,* 2002; Osman *et al.,* 2009; Saeed *et al.,* 2010; Ali *et al.,* 2014).

A peracute fatal respiratory disease of camels in Eastern Sudan and the first report for PPR infection in camels in the Sudan was reported by Khalafalla *et al.* (2010).

Chapter 2

Materials and Methods

2.1. Area of the Study:

White Nile State is one of the major animal producing states in the Sudan. The animal populations in the State were estimated at 5-6 million heads of cattle, 1 million head of equines, 6-7 million head of sheep and goat.

2.2. Collection of Serum Samples:

2.2.1. Equipments:

For collection of blood samples from sheep and for the preparation of the sera the following equipments were used:

1. Syringes.
2. Vacutainer tubes and needles.
3. Eppendorf tubes.
4. Centrifuge (Thermo-electron centrifuge "ACL", USA).

2.2.2. Preparation of the sera:

Blood samples were collected from sheep from Kosti in the White Nile state, Sudan. A total of 100 sera were collected from a herd of sheep which was vaccinated more than a year ago with the PPRV Nigerian

75/1 vaccine strain used routinely in the field. Additionally, a total of 50 sera were collected from non-vaccinated sheep herd.

Blood samples were collected in vacutainers or syringes and kept at room temperature for 1 hour then stored in the refrigerator at 4°C overnight. For preparation of sera, on the following day, the blood was centrifuged at 3000 rpm for 5 minutes and the sera were collected in Eppendorf tubes and stored at -20°C.

2.3. PPR Virus Antigen:

2.3.1. Equipments:

1. 3 vials of PPRV lyophilized vaccine strain.
2. Syringes.
3. Sterile Deionized distilled water.

2.3.2. Reference PPR Virus:

The reference vaccine strain PPRV Nigeria 75/1 (Diallo *et al.,* 1989) was obtained from the Viral Vaccine Production Department at the Central Veterinary Research Laboratory (CVL), Soba, Khartoum, Sudan. The PPRV vaccine was used as a positive PPRV antigen (+ve Ag) in the agar gel precipitation/immunodiffusion test (AGPT/ AGID).

2.3.3. Preparation of the PPRV Antigen:

The PPRV antigen, which was supplied as a lyophilized powder, was prepared by dissolving one vial of the lyophilized PPRV vaccine powder in 500 µl of sterile deionized distilled water.

2.4. PPRV Positive (Control) serum:

Serum against PPRV was kindly obtained from the Friedrich-Loeffler-Institute, Germany and was used as a positive control serum in the agar gel precipitation/immunodiffusion test (AGPT/ AGID).

2.5. Agar Gel Precipitation/Immunodiffusion Test (AGPT/ AGID):

2.5.1. Principles of the Test:

The passive diffusion of soluble antigens and\or antibodies toward each other leading to their precipitation in a gel matrix and formation of white lines of precipitations called precipitinogen lines at the site of the antigen and antibody interaction (Figure 3).

2.5.2. Equipments:

1. Ultrapure Agarose (NEEO "ROTH", Germany).
2. Sodium Azide/ Sodium Nitrate (NaN3) used as a bacteriostatic agent.
3. Water distiller.

4. Deionizer.
5. Deionized Distilled Water (DDW).
6. Sensitive Balance (Adam Scale and Balances, USA).
7. Heavy duty aluminum foil.
8. Eppendorf pipette tips.
9. Single Channel Micropipette 0-50 µl "Assipette".
10. Petri dish plastic diameter: 90 mm.
11. Schott Glass Bottle 500 ml.
12. Template Gel Cutter.
13. Water Bath.
14. Incubator CO_2 Incubator (Model MCO 175, Japan).
15. Autoclave used for sterilization of tools.
16. Serum samples/sera (Antibodies).
17. PPR virus vaccine (Antigen).
18. PPRV Positive serum.

0.5.3. Procedure:

The procedure for performing the agar gel precipitation test was as described previously by White (1958), Anderson *et al.* (1996) and Osman *et al.* (2008). Briefly, 1 grams of agarose were dissolved in 100 ml of sterile deionized distilled water (DDW) in Schott glass bottle then 0.13 g of Sodium Azide were added to the mixture and mixed well. The

orifice/opening of the bottle was covered by aluminum foil and placed in a water bath or boiling water for around one hour until the agarose is completely dissolved. After that, 17 ml of the agar was poured into 90 mm petri dishes and allowed to solidify at room temperature (Figure 4). When the agarose become solid, a pattern consists of a rosette of six peripheral wells around a central well (Figure 5A) were prepared into the agarose by the aid of a template gel cutter. Each well is 4 mm in diameter and 3 mm apart. The agar plugs were removed from the wells by a syringe needle (Figure 5B).

By the aid of a single channel micropipette, 20-25 µl of the antigen were taken and placed into the central well. Afterwards a similar amount was taken from the sera and placed into one of the peripheral wells around the antigen well. The positive PPRV serum was placed in one of the peripheral wells in one rosette in each petri dish. All antigen and sera were placed into the wells following the same described manner (Figure 6).

Agar plates were stored in a humid chamber -in order not to get dry- and were checked at 24, 48 and 27 hours for the appearance of the precipitation lines. After 72 hours plates were stored in a refrigerator at 4°C.

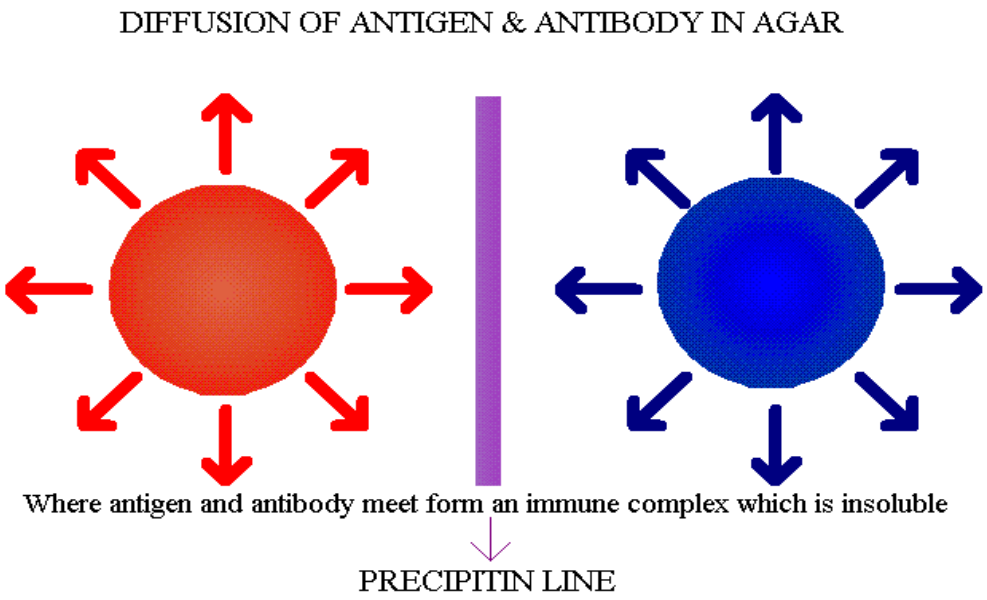

Figure 3. Principles of the agar gel immunodiffusion agar gel precipitation/immunodiffusion test (AGPT/ AGID).

Figure 4. Preparation of the agarose gel for the agar gel precipitation/immunodiffusion test (AGPT/ AGID).

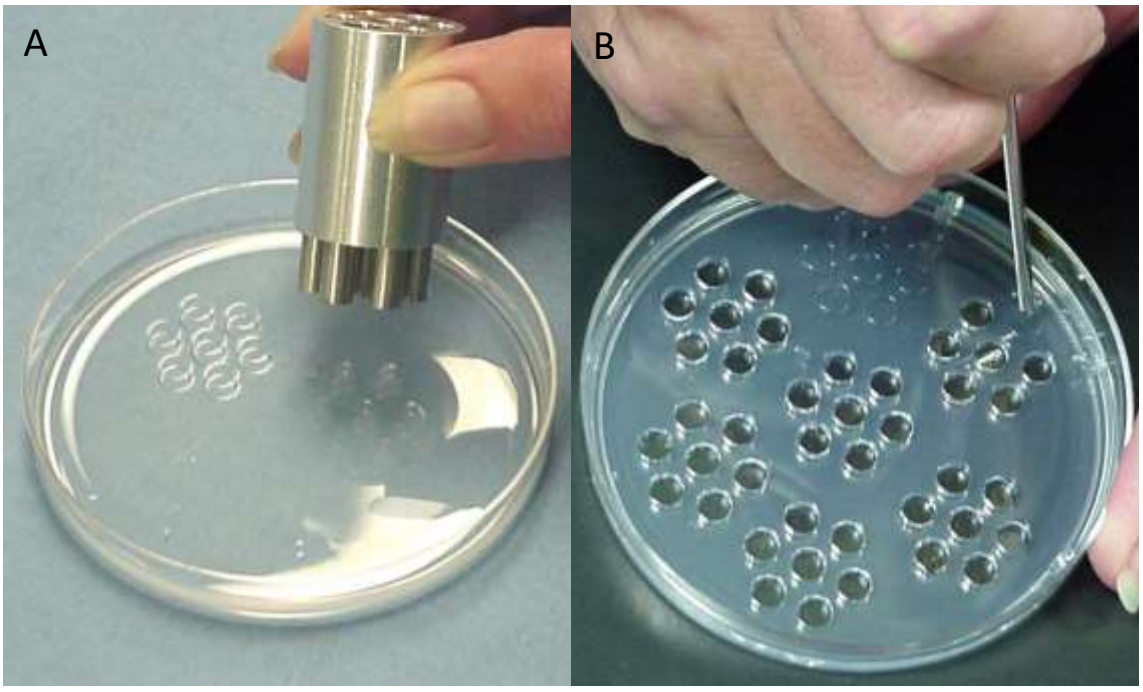

Figure 5. Agar gel precipitation/immunodiffusion test (AGPT/ AGID):
A) Preparation of the rosettes into the agarose by the aid of a template gel cutter. Each rosette consists of six peripheral wells around a central well, each well is 4 mm in diameter and 3 mm apart.
B) The agar plugs were removed from the wells by a syringe needle.

Figure 6. Agar gel precipitation/immunodiffusion test (AGPT/ AGID): Addition of antigen and antibody samples.

Chapter 3

Results

A total of 100 sera samples were collected from a herd of sheep vaccinated with PPR attenuated cell culture vaccine. Additionally, 50 sera samples were collected from a non-vaccinated sheep herd.

All sera were tested for the presence of PPRV antibodies by Agar gel precipitation test (AGPT) against PPRV positive antigen.

When employing AGPT for detection of PPRV antibodies, only 2 sera (2%) out of the 100 sera from the vaccinated sheep were positive whereas 98 sera (98%) samples were negative for the presence of PPR antibodies or precipitinogen.

On the other hand, only 3 sera (6%) out of the 50 sera from the non-vaccinated sheep were positive and 47 sera (94%) sera were negative for the presence of PPR antibodies.

Positive samples produced one white clear line of precipitation at the middle of the antigen (PPRV) and the antibody (sheep sera) wells on the AGPT plate. The bands of the precipitation were seen after 24-48 hours of the onset of the test.

Chapter 4

Discussion

The present study was conducted firstly to detect antibodies against PPRV in sera from vaccinated and non-vaccinated sheep from Kosti, White Nile State, Sudan. Secondly to test the suitability of the agar gel precipitation test (AGPT) for the serological diagnosis of PPR.

A total of 100 sera from a herd of sheep vaccinated with PPRV Nigeria 75/1 vaccine strain beside 50 sera from a herd of non-vaccinated sheep were tested for the presence of PPRV antibodies by agar gel precipitation test (AGPT) against PPRV positive antigen. Of the tested sera, only 2 sera (2%) from the vaccinated animals were positive whereas 3 sera (6%) were positive for the presence of PPR antibodies.

These results indicated that agar gel precipitation test can only detect antibodies early in infection and cannot detect antibodies in later stages of infection or longer periods post vaccination. The result does not agree with the findings of Durojaiye (1982) which employ agar gel precipitation test for detection of antibodies against PPR in the sera of the affected goats.

These findings indicated that agar gel precipitation test appears unsuitable as a serological diagnostic tool for PPR although it is proved to be a useful test for detection of PPRV antigen (Osman *et al.,* 2009).

Conclusion and Recommendations

Conclusion:

In the present study agar gel precipitation test (AGPT) was applied for detection for antibodies against PPR in sheep sera. It is concluded that AGPT is not sensitive enough to detect PPRV antibodies and thus appears unsuitable as a serological diagnostic tool for PPR.

Recommendations:

1. Agar gel precipitation test (AGPT) can only be used for detection of PPRV antigen.
2. Other cheap and simple serological tests such as counter immunoelectrophoresis (CIEP) can be used as an alternative to AGPT.
3. If available, the most sensitive competitive ELISA (C-ELISA) or virus neutralization (VN) tests can also be used as alternatives for detection for PPR antibodies.

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