



Cell Bound Immunoassay: A Simple Method for Detection and Titration of Antibodies to Peste des Petits Ruminants Virus in Small Ruminant Sera

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ABSTRACT

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants, it may have morbidity of 80-90% and mortality between 50 and 80 %. A total of two hundred serum samples were collected from 120 sheep and 80 goats respectively during winter 2009 from Kalubia governorate, Egypt. Sera were tested for peste des petits ruminants virus (PPRV)-specific antibodies by the serum neutralization test (SNT), and a recently developed cell bound immunoassay (CBIA). The SNT was used as the reference for the estimation of the sensitivity and specificity of the CBIA. Out of that 200 examined sera, 53 (26.5%) and 66 (33%) were found positive for PPRV antibodies by SNT and CBIA respectively. On the other hand, 17 out of 120 sheep sera (14.2%) and 36 of 80 goat sera (45%) were positive, contained PPRV-specific neutralizing antibodies. Concerning the use of CBIA for detection of antibodies to PPRV, 24 out of 120 (20%) and 42 out of 80 (52.5%) of sheep and goat sera were positive, respectively. The CBIA gave higher antibody titers to PPRV in comparison to SNT. There was good percentage of agreement (97%) between the SNT and CBIA for detection of antibody to PPRV, and it is suggested that the 2 methods are interchangeable. The sensitivity and specificity of CBIA to the SNT for detection and titration of antibodies against PPRV were 100% and 91.1 %, respectively. In conclusion, antibody seroprevalence in goats and sheep confirmed natural presentation and transmission of PPRV among ovine under field condition. In addition, the establishment of such a cell bound immunoassay for detection and titration of antibodies against PPRV has many advantages. Firstly, the method is simple, cheap, sensitive and specific for the laboratory conditions in Egypt. Secondly, the cytotoxicity and bacterial contamination of sera play no role in this technique. Thirdly, it is a rapid method for detection and titration of antibodies against PPRV. Fourthly, the microtiter plate containing infected monolayer can be preserved till being used. Key Words: PPRV, sheep, goat, CBIA, SNT.

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1.INTRODUCTION:

Peste des Petits Ruminants (PPR) is an economically important viral disease of sheep

and goats, first described by Gargadennec and Lalane (1942) from Ivory Coast in West Africa.

The disease is highly contagious causing varying degree of morbidity and mortality in susceptible animals (Radostits et al., 2000). Peste des Petits Ruminants Virus (PPRV), is a genus Morbillivirus, under the family Paramyxoviridae of order Mononegavirales (Murphy et al., 1999). Like all members of the family, PPRV is an enveloped pleomorphic particle of size between 150 and 390 nm (Durojaiye et al., 1985) containing nonsegmented single stranded RNA genome of negative polarity. The disease has been recorded in several parts of the world included Egypt (Abd El-Rahim et al. 2010). In a serological survey of antibodies against PPRV in small ruminants; serum neutralization test (SNT), Agar gel precipitation test (AGPT) and counter immuno electrophoresis (CIEP) (Durojaiye and Taylor 1984) indirect precipitation test and neutralization test (Durojaiye 1987) and ELISA (El-Allawy et al. 1993) have been used for diagnosis of seroprevalence of PPR.

Serum neutralization test used as a standard test against which other serological testes were evaluated concerning sensitivity, specificity and agreement (Brown et al., 1991). However, Detection of antibodies to PPRV by neutralization test has many disadvantages as it is time consuming, bacterial contamination and toxic effect of sera on tissue culture (Burleson et al. 1997). Moreover, it is laborious and difficult especially when sample size is large. The presence of Mycoplasma, non-cytopathogenic Bovine Viral Diarrhea virus in bovine fetus sera or other inhibitors may interfere with the growth of indicator virus used in neutralization test and result in false-positive or negative neutralizing antibody assays (Potgieter, 1996)

Cell bound immunoassay (CBIA) is a modified form of cell ELISA. It has been used for detection and titration of antibodies to

canine distemper virus in dogs (Zaghawa, 1993), bovine herpes virus type 1 (Zaghawa, 1997) and Bovine viral diarrhoea virus (BVDV) (Sharawi et al. 2005) in bovine sera and infectious bursal diseases virus in chicken's sera (Khaliel, 1999).

The aim of the present study was to a) establish a simple, cheap, sensitive and specific method for detection and titration of anti-PPRV antibodies in sheep and goat sera and b) determine sensitivity and specificity of cell bound immunoassay (CBIA) in comparison to SNT.

2. MATERIAL AND METHODS:

2.1. Materials

2.1.1. Blood serum samples:

A total of two hundred serum samples were collected from 120 sheep and 80 goats during summer 2009 from Kalubia governorate, Egypt. The animals previously showed signs of suspected PPRV infection and did not received PPR vaccine. Blood samples were collected in sterile test tubes and sera were separated by centrifugation at 4000 rpm for 15 minutes. The clear serum was aspirated, frozen in deep freeze (-20°C) until used and heat inactivated at 56°C for 30 minutes when being tested.

2.1.2. VERO cell:

It was kindly obtained from the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The monolayer cell cultures were grown in Egel's minimum essential medium (EMEM) supplemented with 5 % newborn calf serum which has been treated with gamma radiation. It was used in neutralization test (NT) and cell bound immunoassay (CBIA).

2.1.3. PPRV strain:

The PPRV field isolate (Sharawi and Abd El-Rahim 2010) was propagated in VERO cell line

with a titer of 3.3 log₁₀ TCID₅₀/ml after the third passage. The virus was used for SNT and CBIA.

2.1.4. Ant-ovine and caprine immunoglobuline peroxidase conjugate:

both anti-ovine and caprine immunoglobulin conjugated with peroxidase were commercially available by Sigma. The conjugate was used at a dilution of 1: 400 for the cell bound immunoassay.

2.1.5. O-phenylene diamine dihydrochloride (OPD):

It was used as a substrate in cell bound immunoassay (Sigma product no. P8806) with added 30% hydrogen peroxide (4 ul/ml) for being used.

2.2. Methods:

2.2.1. Serum neutralization test (SNT) (Burlison et al. 1997):

A beta micro-neutralization procedure was used for detection and titration of antibodies against PPRV in examined sera.

2.2.2. Cell bound immunoassay (Zaghawa, 1993):

a. Preparation of infected monolayer cell culture in microtiter plates:

VERO cell cultures were grown in microtiter tissue culture plates (Nunc®) at a seeding concentration of 600 cells per well. Cells were inoculated simultaneously with PPRV (200 median tissue culture infectious dose per well). One row of the plate was left without virus inoculation (negative control). The plates were incubated in a CO₂ incubator at 37°C for 48 hours (the incubation period that gives the best virus concentration before the appearance of cytopathic effect). The plates were washed with phosphate-buffered saline (PBS)-tween and fixed by heat at 80 °C for 2 hours. 0.1ml of PBS

was added to each well of the plates and the plates were sealed and kept in a refrigerator (4 °C) till being used.

b. Test proper:

Serial two-fold dilutions of each serum were prepared from the basic 1: 10 dilutions. 0.05 ml of the diluted serum sample was dispensed into wells of microtiter plates containing the infected monolayer cell culture. The plates were incubated either at room temperature for one hour or at 37°C for 30 minutes. The plates were washed 3 times (5 minutes each) with PBS-tween and rinsed finally with distilled water. 0.05 ml of anti-ovine immunoglobuline peroxidase conjugate (diluted 1: 400) was added to each well. Incubation and washing were followed as mentioned above. 0.05 ml of freshly prepared substrate solution (OPD) containing 4% H₂O₂ was added to each well. After 15 - 30 minutes, the substrate solution was discarded and replaced with PBS-tween. The plates were examined under an inverted microscopy where positive wells showed reddish to dark brown cells, while negative cells remained unstained. Antibody titers were expressed as the reciprocal of the highest serum dilution giving positive reaction. The ND₅₀ was calculated according to Reed and Meunch (1938).

2.2.3. Statistical analysis:

It was carried out using Epi-Info computer program designed by Dean et al. (1994) and produced by World Health Organization (WHO). The percentage of agreement, sensitivity and specificity were calculated according to Knapp and Miller (1991).

3. RESULTS:

The results present in Table (1) indicated the prevalence of antibodies to PPRV in ovine sera using the serum neutralization test and the bound immunoassay (CBIA). 53 (26.5%) of

200 examined sera were found positive for PPRV antibodies by SNT, while 66 (33%) were positive for PPRV antibodies by CBIA. It also shows that 36 (45%) and 42 (52.5%) out of the 80 tested goat sera, were found positive for PPR virus antibodies in SNT and CBIA respectively while 17 (14.2%) and 24 (20%) out of the 120 tested sheep sera, were found positive for PPRV antibodies in SNT and CBIA respectively. Table (2) shows that high

frequencies and titers of antibody titer to PPRV in ovine sera were obtained by CBIA comparing to SNT. Table (3) show the percentage of agreement between cell bound immunoassay and serum neutralization test for detection of antibodies to PPRV in ovine sera was 96.9 %. The sensitivity between CBIA and SNT was 100%. While the specificity between CBIA and SNT was 95.5 % (Table 4).

Table (1): Detection of antibodies to PPRV in ovine sera using serum neutralization test (SNT) and cell bound immunoassay (CBIA).

Animal species	No. of examined samples	SNT			CBIA		
		Positive	Negative	*%	Positive	Negative	%
Goats	80	36	44	45	42	38	52.5
Sheep	120	17	103	14.2	24	96	20
Total	200	53	147	26.5	66	134	33

*% was calculated according to number of examined samples of each species.

Table (2): Frequency distribution of antibody titer to PPRV in ovine sera using SNT and CBIA.

Antibody titer	SNT		CBIA	
	Frequency	%	Frequency	%
10	5	9.4	0	0.0
20	6	11.3	0	0.0
40	11	20.8	0	0.0
80	17	32.1	8	12.1
160	5	9.4	12	18.2
320	7	13.2	18	27.3
640	2	3.8	25	37.9
1280	0	0.0	3	4.5
Total	53		66	

Table (3): Percentage of agreement between cell bound immunoassay and serum neutralization test for detection of antibodies to PPRV in ovine sera.

No. of examined sera	SNT	CBIA	Results	
			Agreement	Disagreement
36	Positive	Positive	36	0
158	Negative	Negative	96	0
6	Negative	Positive	0	6
200			194	6

Total agreement

Percentage of agreement = ----- X 100

Total No. of examined samples 194

Percentage of agreement = ----- X 100 = 97 %.

200

Table (4): Results of sensitivity and specificity of CBIA in comparison to SNT for detection of antibodies to BVDV in bovine sera.

CBIA results	No.	SNT results		
		Positive	Negative	Total
Positive	66	53 (A)	13 (B)	66
Negative	0	0 (C)	134 (D)	134
Total		53 (A+C)	147 (B+D)	200

A 53

Sensitivity = ----- X 100 = ----- X 100 = 100%.

A + C 53

D 134

Specificity = ----- X 100 = ----- X 100 = 91.1 %.

B + D 147

4. DISCUSSION:

Great efforts are being made in Egypt to improve sheep and goat production. To that end, disease control and eradication have always been priority considerations. The objective of this study was to develop a rapid,

simple, cheap, sensitive and specific assay for detection and titration of PPRV antibodies in sheep and goat sera. To accomplish this aim, we first focused on SNT assay where 53 out of 200 (26.5%) serum samples from the recovered

and apparently healthy goats and sheep were found positive for PPRV neutralizing antibodies. Likewise, thirty six of 80 (45%) examined goats and 17 out of 120 sheep sera (14.2%) had anti-PPR neutralizing antibodies were detected. Previous history and results of SNT confirmed the outbreak to be PPR. PPRV antibodies could be attributed to the fact that usually the antibody titers are low and undetectable at the beginning of the infection and increase to reach high level in recovered and clinically healthy animals in which previous infection were more likely to be occurred specially in endemic area (Sharawi et al. 2010). It is known that PPR primarily affects goats, but sheep may also be affected (Ali, 2004) Although Obi et al. (1983) found 52.3 and 47.2 per cent prevalence of PPRV neutralizing antibodies in sheep and goats, respectively. However, further investigations are needed in this regard. The binding of antibodies to specific antigens is based on complementarity between the antibody combining sites and the antigenic determinants, similarly to enzyme – substrate reaction (Botus and Oncescu 2006). The immunoenzymatic assays are based on an enzymatic marker fixed on a reagent which participates to antigen-antibody interaction on an adsorbant, in a solid phase, emphasizing this interaction. Thus, CBIA (cell bounded Immunosorbent Assay) – indirect variant – is a technique consisting in an antigen-antibody reaction, the formed complex being evidenced by an enzymatic reaction, with an anti-species enzyme labeled conjugate and a chromogen.

Concerning the use of CBIA for detection of antibodies to PPRV, 66 (33%) out of this 200 serum samples were positive, where 42 (52.5%) out of the 80 tested goat sera and 24 (20%) out of the 120 tested sheep sera were found positive for PPRV antibodies in CBIA respectively. The results presented in our work

indicated that CBIA gave higher antibody titers to PPRV in comparison to SNT while the percentage of agreement between CBIA and SNT for detection of antibody to PPRV was 97%. The sensitivity and specificity of CBIA in comparison to SNT for detection and titration of antibodies against PPRV were 100 % and 91.1%, respectively. In a comparative study, El-Allawy et al. (1993) found ELISA more sensitive than SNT and AGPT for detection of specific antibodies against PPR virus.

Regarding the sensitivity and specificity of our CBIA technique for detecting anti-PPRV antibodies in the sera is in agreement with results of Zaghawa, (1993 & 1997), when they compared CBIA with serum neutralization test in detecting anti-canine distemper virus and anti-infectious bovine rhinotracheitis virus. Also, is in agreement with the result of other workers who compared CBIA with serum neutralization test in detecting anti-infectious bursal disease (Khaliel, 1999) and anti-bovine viral diarrhea (Sharawi et al. 2005).

It is concluded that, the establishment of such a cell bound immunoassay for detection and titration of antibodies against PPRV has the following advantages:

1. The method is simple, cheap, sensitive and specific for the laboratory conditions in Egypt and does not need ELISA reader, so it can be used in field study.
2. The cytotoxicity and bacterial contamination of sera play no role in this technique.
3. It is a rapid method for detection and titration of antibodies against PPRV.
4. The microtiter plate containing PPRV-infected tissue culture monolayer can be preserved till being used.

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