Full Length Research Paper

Comparison of two competitive ELISAs for the detection of specific peste-des-petits-ruminant antibodies in sheep and cattle populations

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Peste-des-petits-ruminant (PPR) continues to be a major problem of small ruminants in Africa, the Middle East and Asia. The closely related paramyxovirus causing rinderpest (RP) has been largely eradicated by a global vaccination campaign. However, PPR screening of large populations has lacked a sufficiently reliable, fast and cheap screening test. This study compares two commercially available PPR antibodies ELISA kits using serum collected from experimental sheep and cattle populations with four different vaccination histories for RP and PPR. The aim was to estimate the levels of cross-reaction between antibodies to the two diseases for each kit and their test parameters in the different populations. There was considerable variation between kits and between the different vaccination groups. There was a clear problem of cross-reaction in both PPR kits with RP positive sera. However, in areas where RP has been eradicated and vaccination stopped both tests could be useful for screening small ruminants for PPR.

Key words: Peste-des-petis-ruminants, rinderpest, ELISA, sheep, cattle, antibodies.

INTRODUCTION

Peste-des-petits-ruminants (PPR) are a severe viral disease of sheep and particularly goats causing fever, nasal discharge, necrotic lesions on the gums, diarrhea, with high morbidity and mortality. It is endemic in many parts of Africa, the Middle-East and Southern-India (Shaila et al., 1989; Lefevre and Diallo, 1990). In clinical terms, PPR is very similar to bovine rinderpest (RP). Both viruses are antigenically closely related and belong to the morbillivirus genus of the family *Paramyxoviridae* (Gibbs et al., 1979). PPR infects cattle as well as small ruminants but only causes disease in small ruminant species. However, a specific seroconversion to PPR is observed in cattle (Taylor et al., 1979) which cross-pro-

tects cattle against RP which may have helped raise herd immunity levels higher than measured by serological tests (Taylor, 1979; CouacyHymann et al., 1995; Diallo, 2003). Of concern is the possibility that PPRV transmission into an increasingly RP susceptible cattle population might lead to a cattle adapted form of PPR. In small ruminants, disease is caused by both viruses and differential diagnosis relies on laboratory confirmation.

The gold-standard serological test is the comparative viral neutralization test (VNT) where a serum is considered to be PPR positive when the neutralisation titre is at least twofold higher for PPR than for RP (Rossiter et al., 1985). However, this test is time consuming, has a low throughput of samples, needs cell culture facilities and experienced technical support to perform and interpret the results. Recently, with advances in molecular biology new diagnostic tests based on the ELISA system have become available for detecting PPR antibodies (An-

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derson et al., 1991; Libeau et al., 1995). However these tests are not able to differentiate post-vaccination antibodies to post-infection ones.

This study compares two commercial kits for specific PPRV antibody detection based on the competitive ELISA technique. The H-ELISA is produced by the Institute for Animal Health, Pirbright, UK and the N-ELISA is produced by CIRAD/EMVT, Montpellier, France. The agreement between the two tests was assessed in non-vaccinated, RP vaccinated, PPR vaccinated and RP/PPR vaccinated experimental groups of cattle and sheep in Ivory-Coast in 2005.

MATERIALS AND METHODS

Selection and treatment of animals-sheep

Lambs were screened from five villages in the rainforest region of Ivory-Coast selected for their high densities of small ruminants. In each village, two farms were selected based on the flock size and the farm management. Two hundred lambs (6 - 12 months old) were randomly selected (20 from each farm) and were screened for RP and PPR antibodies using the VNT (Rossiter and Jessett, 1982; Taylor, 1984). One hundred and sixty lambs were found to be negative for both (RP-/PPR-). Eighty out of these 160 RP-/PPR-lambs were then vaccinated with 1 ml of 10^3 TCID$_{50}$/ml of attenuated RP vaccine by subcutaneous injection (Plowright and Ferris, 1962). These were then the RP+/PPR- group. A second group of 80 RP-/PPR- lambs were vaccinated with 1 ml of 10^3 TCID$_{50}$/ml homologous PPR vaccine 75/1 (Diallo et al., 1989) by subcutaneous injection to be RP- / PPR+ group. The difference, 40 lambs (200 – 160), were withdrawn from the study at this step by the farmers for sale.

Eighty ewes (2 - 3 years old) from two state farms in the central region of Ivory Coast was randomly screened following annual vaccination with 1 ml of 10^3 TCID$_{50}$/ml heterologous attenuated RP vaccine by subcutaneous injection. Sixty-five were found to be seropositive using the RP cELISA (Anderson et al., 1991) and were subsequently vaccinated with 1 ml of PPR homologous vaccine 75/1 as above (RP+ / PPR+).

Selection and treatment of animals-cattle

One hundred and sixty young cattle (1 - 2 years old) from villages located 10 - 20 km from the Central Laboratory were screened for RP and PPR antibodies using the VNT. They were divided in two groups of 80 young cattle each. Eighty negative animals (RP-/PPR-) were then vaccinated with 1 ml of attenuated RP vaccine as above (RP+/PPR-). The second group of 80 young cattle was vaccinated with 1 ml of PPR vaccine as above (RP-/PPR+). Finally, 100 cows aged more than 2 years, with a history of 2 - 3 vaccinations (ear-marks) from the Pan African Rinderpest Campaign, were randomly selected from a state farm 30 km from the Central Laboratory. These animals were then vaccinated with 1 ml of PPR vaccine as above (RP+/PPR+). These cattle popu-lations were not mixed with sheep or goat flocks before or after selection for the study.

Serological analysis

Serum samples were collected at the beginning of the study to check the antibodies status of each animal prior to vaccination and

removal of positives except those vaccinated previously with the RP vaccine. Animals were bled 1 to 2 months after vaccination for testing with the two ELISAs of interest. Sera were stored at –20 °C until use. The two cELISA tests were performed by the same operator on the same days for each sample.

During this study a number of animals were lost to follow-up as follows: 10 lambs (80 - 70) from the RP+/PPR- group, 1 lamb (80 - 79) from the RP-/PPR+ group, 3 young cattle (80 - 77) from the RP+/PPR- group and 5 young cattle (80 - 75) from the RP-/PPR+ group.

H-cELISA PPR antibody test kit

The competitive PPR ELISA (cELISA) test was developed at IAH, Pirbright by Anderson et al. (1991). This has been developed as a commercial kit supplied by BDSL (UK). This kit uses whole attenuated PPR virus as the capture antigen to coat the plate and a mouse monoclonal antibody (Mab) directed against the H protein of the PPR virus as the competitive antibody. Briefly, 50 μl of 1/100 diluted PPR antigen in coating buffer (PBS 0.1 M, pH 7.4 - 7.6) was dispensed in every well and incubated at + 37 °C for 1 h on an orbital shaker. After washing 3 times with the washing solution (PBS 0.2 M), 40 μl of the blocking buffer (PBS 0.1 M at pH 7.4 - 7.6 with 0.01% between 20 and 0.3% negative sheep serum supplied with the kit) were dispensed in each well. The two conjugate control wells receive an additional 50 μl of blocking buffer while the monoclonal antibody wells control wells receive only 10 μl. Then 10 μl of testing sera were dispensed in the test wells giving a dilution of 1/5 of test sera. Strong positive, weak positive and negative controls were included in duplicate. Next 50 μl of Mab 1/100 in blocking buffer were dispensed in each well except the conjugate control wells. The plates were incubated for 1 hour on a shaker followed by 3 wash. 50 μl of conjugate (anti-mouse I g of G coupled with radish peroxidase, Dako A/S, Denmark) diluted 1/1000 in blocking buffer was added to each well and incubate for a further 1 hour followed by 3 washes. Finally, 50 μl of substrate/chromogen, OPD/H_2O_2 solution was added to each well and incubate in a dark room for 10 min. The color development was stopped with 50 μl of sulfuric acid 0.1 M. The optical density was measured with a Multiskan MKII plate reader at 492 nm.

The percentage of inhibition (Pi) values was determined with the EDI software v2.3 supplied by the joint division FAO/IAEA, using the formula:

Pi (%) = 100 − [mean of OD of tested serum / median of Mab control OD] * 100

Pi% values greater than or equal to 50% were considered as positive.

The N-cELISA PPR antibody test

The second kit for the detection of PPR antibodies used the recombinant N-protein of PPR virus as the capture antigen and a Mab against the N-protein as the competitive antibody (Libeau et al., 1995). This kit is directly supplied by CIRAD-EMVT (Montpellier, France). Briefly, this test was run is a similar way to the H-cELISA except that the recombinant N-protein was used at a 1/3000 dilution in the coating buffer (PBS 0.01 M, pH 7.4 - 7.6); the blocking buffer consisted of PBS 0.01 M at pH 7.4 - 7.6 with 0.05% tween 20 and 0.5% negative sheep serum. The washing buffer was PBS 0.002 M at pH 7.2 -7.6 with 0.05% between 20. Test and control sera were diluted 1/10 in blocking buffer. Optical density (OD) readings were converted to percentage inhibition (Pi%) values using the same formula as above and the cut-off was set at 50% Pi value as above.

Table 1. Serological results of H-cELISA and N-cELISA antibodies detection of naïve and vaccinated sheep.

H-cELISA PPR			Pos.	Neg.	Total	% Agreement	Kappa	Yules Y
N-cELISA PPR	RP-/PPR-Sera	Pos.	0	2	2			-
		Neg.	0	78	78	0.975	0	
		Total	0	80	80			
	RP+/PPR-Sera	Po	10	6	16			0.461
		Neg.	10	44	54	0.771	0.404	
		Total	20	50	70			
	RP-/PPR+Sera	Pos.	64	1	65			0.933
		Neg.	1	13	14	0.975	0.913	
		Total	65	14	79			
	RP+/PPR+Sera	Pos.	40	4	44			0.603
		Neg.	8	13	21	0.815	0.560	
		Total	48	17	65			Yules Y

Pos: positive sera; Neg: negative

Table 2. Serological results of H-cELISA and N-cELISA antibodies detection of naïve and vaccinated cattle.

H-cELISA PPR			Pos.	Neg.	Total	% Agreement	Kappa	Yules Y
N-cELISA PPR	RP-/PPR-Sera	Pos.	0	0	0	1	-	-
		Neg.	0	80	80			
		Total	0	80	80			
	RP+/PPR-Sera	Pos.	28	9	37	0.649	0.304	0.322
		Neg.	18	22	40			
		Total	46	31	77			
	RP-/PPR+Sera	Pos.	37	13	50	0.640	0.182	0.199
		Neg.	14	11	25			
		Total	51	24	75			
	RP+/PPR+Sera	Pos.	45	15	60	0.520	-0.081	-0.113
		Neg.	33	7	40			
		Total	78	22	100			

Pos : positive sera ;   Neg : negative

Statistical analysis

The agreement between the 2 tests were calculated for each vaccination group and species and this was then compared with the Cohen's kappa statistic (Martin et al., 1987) and Yules Y statistic (Spitznagel and Helzer, 1985). The Cohen's kappa statistic, which corrects the agreement for chance, and Yules 'Y' statistic, which is less dependent than kappa on the prevalence of the condition, was estimated as follows:

$$Kappa = \frac{(\%\ Observed\ agreement) - (\%\ Expected\ agreement\ by\ chance)}{100\% - (\%\ Expected\ agreement\ by\ chance)}$$

Yules Y statistic was calculated using the formula:

$$Y = \frac{\sqrt{(ad)} - \sqrt{(bc)}}{\sqrt{(ad)} + \sqrt{(bc)}}$$

where a, b, c, d are the cells of the standard 2 x 2 table.

Table 3. Diagnostic sensitivity and specificity of the two cELISA kits on naive and vaccinated sheep and cattle.

	Rinderpest negative sera				Rinderpest positive sera			
	SHEEP							
	H-cELISA		N-cELISA		H-cELISA		N-cELISA	
	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+
Test +	0	65	0	65	0	65	0	65
Test -	80	14	80	14	80	14	80	14
Se (%)	82.3%		82.3%		82.3%		82.3%	
Sp (%)	100%		100%		100%		100%	
	CATTLE							
	H-cELISA		N-cELISA		H-cELISA		N-cELISA	
	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+	RP- / PPR-	RP- / PPR+
Test +	0	51	0	51	0	51	0	51
Test -	80	24	80	24	80	24	80	24
Se (%)	68%		68%		68%		68%	
Sp (%)	100%		100%		100%		100%	

Se: sensitivity;   Sp: specificity.

The recommended cut-off values are <0.4 poor agreement, 0.4 - 0.75 fair to good agreement, and >0.75 very good agreement (Sargeant and Martin, 1998). The Yule's Y statistic generally gives higher estimates of agreement compared to the kappa statistic.

RESULTS AND DISCUSSION

The comparison of the H-cELISA and N-cELISA kits for PPR antibody detection is given in Table 1 for sheep and Table 2 for cattle. The percentage agreement varied with the different vaccination combinations with almost perfect agreement for RP-/PPR- sheep and cattle. Agreement was also still very good for RP-/PPR + sheep but very poor for both RP + /PPR- and RP+/PPR + sheep and cattle. The RP + /PPR- in particular showed that between 23%(16/70) - 29%(20/70) and 48%(37/77) − 60%(46/77) of samples respectively in sheep and cattle species, were coming up falsely positive for PPR depending on which kit was used. This strongly suggests that there is a significant problem of cross-reaction with RP antibodies in both the test kits. In cattle the agreement in the RP-/PPR+ group was also very poor.

The test sensitivities and specificities are given in Table 3. These results suggest that the tests have reasonable PPR specificity in the absence of RP antibodies though not particularly high sensitivities in either sheep or cattle. However, in populations previously vaccinated against RPV test specificities drop dramatically in both sheep and cattle but are particularly poor in cattle.

Although PPR is a major epidemic disease of small ruminants, it has until recently received relatively little attention compared to the other epidemic diseases of cattle such as rinderpest, foot-and-mouth disease (FMD), contagious bovine pleuropneumonia (CBPP), and try-panosomiasis, etc. As of 2006, rinderpest is on the point

of being eradicated globally by wide scale regional vaccination. In Africa this was first under the Pan African Rinderpest Campaign (PARC) and since 2000, has been operated by the Pan African Control of Epizootics (PACE). Control of PPR has until recently relied on vaccination using the heterologous rinderpest vaccination which cross protects in small ruminants against PPR. With the development of a PPR homologous vaccine (Diallo et al., 1989), a new alternative is available to protect small ruminant species against PPR. It is now forbidden to use rinderpest vaccine to control PPR to avoid confusion during serological surveillance. With increased interest in control of PPR there is now a need to have a cheap and rapid testing system for PPR surveillance programmes. In the past the VNT was used to measure post-infection and post-vaccination antibody titres, however, this test is time consuming and requires virus containment and cell culture facilities. In addition, it can not distinguish between RP and PPR positive sera. In contrast the comparative VNT is able to make this differential distinction between specific antibodies where the homologous positive serum gives a neutralisation titre at least two-fold higher than the heterologous serum one (Rossiter et al., 1985). The advantage of this comparative VNT is balanced by the disadvantages of a viral neutralisation test. The development of PPR competitive ELISA techniques offers the potential to overcome some of the problems of differentiating RP and PPR positive animals (Anderson et al., 1991; Anderson and Mckay, 1994; Libeau et al., 1994).

This study has compared two commercially available PPR competitive ELISA kits. The major differences between the two kits are the use of the whole, attenuated PPR virus particle as antigen (H-cELISA) compared to a recombinant nucleoprotein (N-cELISA) and the antibody

used which are an anti-H monoclonal antibody in the H-cELISA and an anti-Np monoclonal antibody in the N-cELISA. The main advantage of the N-cELISA kit is that it can be used anywhere, even in PPR free zones because it does not use whole viral particles. This study has shown that both kits could be used to test sheep serum samples in a PPR survey in small ruminant species but the low sensitivity means it would be better interpreted at a herd /flock level than as a diagnostic test in individuals. The performance of the tests declined markedly when used in groups of RP vaccinated animals due to apparent cross-reaction with RP antibodies. This is disappointing as by contrast the H-cELISA kit for rinderpest antibody detection works well in both bovine and small ruminants (Anderson and Mackay, 1991). PPR, being a major disease, has to be controlled and therefore requires a specific and sensitive serological test like RP where a specific ELISA test is in use during the present eradication programme.

The study was rather limited in scale and unfortunately had to use repeat measures on one group in order to get a RP-/PPR- and RP+/PPR- groups. This independence was not accounted for in the analysis. In addition, for the RP+/PPR+ groups older animals had to be used as these had been vaccinated repeatedly for RP. It is possible that older animals exposed over a longer period to a range of antigens might have more non specific reactions.

In conclusion, the H-cELISA and the N-cELISA kits for PPR performed similarly well in sheep without prior exposure to RP and could make a useful screening tool for flocks. Neither test performed well in either cattle or sheep that had previously been vaccinated against RP with particularly poor specificity in RP vaccinated cattle.

ACKNOWLEDGEMENTS

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