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Monoclonal Antibody-Based Blocking Enzyme-Linked Immunosorbent Assay for Specific Detection and Titration of Peste-des-Petits-Ruminants Virus Antibody in Caprine and Ovine Sera

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A blocking enzyme-linked immunosorbent assay (B-ELISA), using two neutralizing monoclonal antibodies (MAbs), was established and compared with the virus neutralization test (VNT) for detecting specific peste-des-petits-ruminants virus (PPRV) antibody in caprine and ovine sera. This technique was developed because VNT, the only available specific serological test for PPRV and the cross-reactive rinderpest virus (RPV), is time-consuming and unaffordable for most laboratories in regions where both peste des petits ruminants and rinderpest occur. The test depends on the blocking of the binding of the MAb to a specific epitope in the presence of positive serum. Test conditions were optimized by using peste-des-petits-ruminants and rinderpest sera that were known to be VNT positive and negative. A blocking format, in which serum is preincubated with a solid-phase PPRV antigen and then incubated with the MAb, yielded levels of sensitivity and specificity superior to those of a competitive format, in which the two reagents are added simultaneously. A threshold value of 45% inhibition, representing the mean for a negative population (n = 277) plus 2.7 standard deviations, was adopted for routine screening. A total of 605 serum samples were screened by B-ELISA and the VNT. The sensitivity and specificity of B-ELISA relative to the VNT were 90.4 and 98.9%, respectively. Of 264 field serum samples tested, 11 (4.2%) could not be assayed by the VNT because of contamination or cytotoxicity; the overall agreement quotient between results of the two tests (n = 253) was 0.91. A high correlation ($r \geq 0.98$) was observed between B-ELISA and the VNT for endpoint titration of sera (n = 57). Because B-ELISA proved to be nearly as sensitive and specific as the VNT while being simpler and more rapid, it would be an adequate substitute for the VNT for assessing herd immune status and for epidemiologic surveillance.

Peste des petits ruminants (PPR) is an acute or subacute viral disease of goats and sheep characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia (25). PPR virus (PPRV) is a paramyxovirus of the *Morbillivirus* genus that includes rinderpest virus (RPV), canine distemper virus (CDV), measles virus (MV), and phocid distemper virus (PDV) (11, 20, 21). RPV is essentially a virus of cattle, but it also causes a disease syndrome in sheep and goats that is clinically indistinguishable from PPRV infection (25).

Various techniques have been applied in the serological diagnosis of PPR in goats and sheep. These include the microplate virus neutralization test (VNT) (22), agar gel precipitation (6, 7), immunoelectroosmophoresis (15), countercurrent immunoelectroosmophoresis (8), and indirect enzyme-linked immunosorbent assay (ELISA) (18, 19). There is a close antigenic relationship between PPRV and RPV (and indeed among all five morbilliviruses) which translates into serological cross-reactivity (11, 12, 17). Serological tests done with polyclonal antibody may therefore be incapable of differentiating between serum antibody to PPRV and antibody to RPV. In recent years, the seroepidemiology of PPR has been further complicated by massive use of the heterologous rinderpest (RP) tissue culture-attenuated vaccine for PPR prophylaxis in several African countries (13).

The VNT is currently the only available test that can distinguish between PPR and RPV serum antibodies (25). Serum raised against either virus may neutralize both viruses but would neutralize the homologous virus at a higher titer than it would the heterologous virus (24, 26). The VNT takes at least 7 days to reach an end point, is laborious, and requires a laboratory with cell culture facilities.

There is a need, therefore, for a rapid, reliable, and simple test that can discriminate PPRV antibody from antibody to the other morbilliviruses, particularly RPV. This paper reports on a blocking ELISA (B-ELISA) using two neutralizing monoclonal antibodies (MAbs), specific for the hemagglutinin protein of PPRV, for rapid detection and endpoint titration of PPRV antibody in sera.

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TABLE 1. Characteristics of MAbs used in B-ELISA

MAb and fluid	ELISA titer[a]		IFAT reaction[b]		VNT titer[c]	
	All PPRV isolates	RPV, CDV, MV, PDV	All PPRV isolates	RPV, CDV, MV, PDV	All PPRV isolates	RPV, CDV, MV, PDV
B_2G_3						
TC sup.[d]	4–6	<1	++	−	5–10	<2
Ascites	7–9	<1	++	−	8–14	<2
B_2G_4						
TC sup.	4–6	<1	++	−	6–12	<2
Ascites	7–9	<1	++	−	10–14	<2

[a] ELISA titers are expressed as $\log_{10}$ of the reciprocal of the highest dilution of antibody that gave an OD reading at least three times the OD for negative controls (hybridoma culture media or normal mouse ascites) by indirect ELISA.

[b] For the indirect fluorescence antibody test (IFAT), hybridoma culture supernatants were used undiluted while ascites fluids were diluted 1:500. ++, strong fluorescence signal; −, negative reaction.

[c] VNT titers are expressed as $\log_2$ of the highest dilution of antibody that neutralized approximately 100 50% tissue culture infective doses of virus in two of two wells.

[d] TC sup., hybridoma culture supernatant.

MATERIALS AND METHODS

Viruses. Fifteen PPRV isolates, ten RPV strains, and one representative of each of the three other morbilliviruses were used. The viruses included PPRV (Nigeria 75/1 wild type, Nigeria 75/1 attenuated, Nigeria 75/2, Nigeria 75/3, Ghana 76/1, Ghana 78/1, Senegal 77, Côte d'Ivoire 89, Burkina Faso 89, RCA 89, Sudan 72/1, Sudan 72/2, Egypt 88, Dorcas, and Ibri), RPV (RBOK vaccine, RBT1, RGK/1, Kuwait, Egypt, Sokoto, Nigeria buffalo, India, Pakistan, and Yemen strains), CDV Onderstepoort strain, MV Edmonston attenuated strain, and PDV1-2-6A isolate. PPRV and RPV viruses came from the Foreign Animal Disease Diagnostic Laboratory, Plum Island, N.Y., and the Institut d' Elevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, France, repositories; CDV and MV were provided by Max J. G. Appel of Cornell University, Ithaca, N.Y.; and PDV1 was a gift from Albert D. M. E. Osterhaus of the National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands. With the exception of PPRV Nigeria 75/1 attenuated, RPV RBOK, MV, and CDV, all viruses were low-cell-passage wild-type strains. To establish the specificity of the MAbs, all 28 viruses were used in indirect ELISA, while only the 15 PPRV isolates (RPV RBOK, MV, CDV, and PDV1) were tested by the VNT. The specificity of the test sera was determined against only one representative of each of the five morbilliviruses by the VNT and ELISA.

Preparation of morbillivirus antigens for use in the ELISA. Viruses were grown on African green monkey (Vero R) cells in minimum essential medium supplemented with Earle's salts, 5% fetal bovine serum (FBS), and antibiotics (40 μg of gentamicin and 2.5 μg of amphotericin B per ml). Cells, seeded into 150-cm^2 cell culture flasks, were infected in suspension at a multiplicity of infection of about 0.01 50% tissue culture infective dose per cell and allowed to form monolayers at 37°C. When virus-specific cytopathic effects were observed on 80% or more of the monolayer, cells were scraped into the medium, sonicated, and clarified by low-speed centrifugation, and virus was concentrated from the supernatant by centrifugation at 125,000 × *g* for 1 h. Gradient purification of virus was then done essentially as described for RPV by Underwood and Brown (28) and modified by Bhavani et al. (4). Briefly, concentrated virus was layered onto a 20 to 60% step sucrose gradient and centrifuged at 125,000 × *g* for 1 h. The virus band at the interface of the two sucrose layers was removed, pelleted at 125,000 × *g* for 1 h, and then layered onto a continuous 15 to 40% potassium tartrate gradient. After centrifugation for 4 h, the virus band was collected, diluted 1:15 in sterile phosphate-buffered saline (PBS), and centrifuged at 125,000 × *g* for 1 h. The resulting pellet was resuspended in sterile PBS by sonication and used as the ELISA antigen.

MAbs. Two MAbs, designated B_2G_3 and B_2G_4 and raised in our laboratory against the Nigeria 75/1 wild-type isolate of PPRV, were selected for use in B-ELISA on the basis of their ability to neutralize all our 15 PPRV isolates but none of the other morbilliviruses, their immunoglobulin G (IgG) isotype, and their strong ELISA signal. Both recognize the hemagglutinin protein of PPRV, as determined by radioimmunoprecipitation assay (data not shown). Their reactivity was also evaluated by indirect ELISA, an indirect fluorescence antibody test, and the VNT against all 28 viruses used in this study. Table 1 summarizes their properties.

Test sera. A total of 352 serum samples of laboratory origin and 264 field serum samples were used. The antibody specificity of each sample was established by the VNT by using cross neutralization (i.e., PPRV and at least one other

TABLE 2. Origin by species and characteristics of 352 laboratory serum samples tested by PPRV-specific B-ELISA[a]

Origin	No. of samples with antibody to:			
	No morbillivirus	RPV	PPRV	CDV, MV, PDV
Goat	89	16	64	
Sheep	19		4	
Cattle	11	131		
Rabbit			1	
Wildlife[b]	10			
Dog	1			4
Seal	1			1
Total	131	147	69	5

[a] The VNT titer (see Table 1, footnote *c*) ranges are as follows: negative, ≤3; RPV, 5 to 13; PPRV, 4 to 13; CDV, MV, and PDV, 7 to 11. The B-ELISA percent inhibition values (calculated from mean OD values by using the formula in the text) are as follows: negative, −4.6 to 17.6; RPV, 0.0 to 41.6; PPRV, 45.7 to 97.9; CDV, MV, and PDV, −9.2 to 30.6.

[b] From laboratory stocks of sera determined to be free of morbillivirus antibody. Sera originally came from three *Artiodactyla* species (impala, gemsbok, and duiker) from Africa.

morbillivirus). Table 2 presents the origin (by species) of the laboratory sera. They included experimental sera from goats vaccinated with either attenuated PPRV or RPV and challenged with virulent PPRV (76 samples) and from cattle vaccinated with attenuated RPV and challenged with virulent RPV (45 samples). Dog anti-MV and anti-CDV sera were a gift from Max J. G. Appel, and dog and seal anti-PDV sera were kindly provided by Albert D. M. E. Osterhaus. The laboratory sera were collected from various experiments performed at the Foreign Animal Disease Diagnostic Laboratory and the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux between 1988 and 1992. The field serum samples were collected from goats (n = 166) and sheep (n = 98) in Cameroon between 1987 and 1991 during suspected outbreaks of PPR.

Indirect ELISA. Indirect ELISA was used to evaluate the MAbs and to determine the optimal MAb and serum dilutions for use in the blocking assay. Indirect ELISA was performed by a modification of established procedures (3, 9). Immulon-2 96-well flat-bottom microtiter plates (Dynatech Laboratories, Alexandria, Va.) and volumes of 100 μl were used throughout. Antigen was diluted in 0.05 M carbonate buffer, pH 9.6, and coating was carried out at 37°C for 1 h or overnight at 4°C. The optimal dilution of solid-phase antigen, previously determined by checkerboard titration against mouse anti-PPRV polyclonal ascitic fluid in indirect ELISA, was 4 μg/ml. Following four washes in PBS containing 0.05% Tween 20 (PBST), antibody was incubated in wells for 1 h at 37°C. Hybridoma supernatants were used undiluted, while mouse ascitic fluids and sera were diluted in PBST with 2% FBS (PBST-FBS). Plates were again washed, and specific binding of antibody was detected with horseradish peroxidase-conjugated rabbit anti-mouse IgG-IgM-IgA (Sigma Chemical Co., St. Louis, Mo.) diluted in PBST-FBS. The optimum dilution of conjugate, 1:1,500, had been determined by direct ELISA after the plates were coated with serial dilutions of mouse anti-PPRV polyclonal ascitic fluid. Following the addition of conjugate and incubation for 1 h at 37°C, the plates were washed and substrate was added. The substrate-chromogen mixture consisted of 0.01% hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (Sigma Chemical Co.) at 0.1 mg/ml in 0.05 M citrate-phosphate buffer (pH 5.0). Color development was stopped after 25 min by adding 50 μl of 2 M H_2SO_4, and optical density (OD) was read at a 450-nm wavelength.

B-ELISA. B-ELISA depends on the blocking of the binding of MAb to a PPRV-specific epitope in the presence of positive serum (2). Blocking is detected as a reduction in the OD reading obtained with the MAb alone. The amount of decrease in ELISA signal would be proportional to the titer of PPRV-specific antibody in the serum. The test can be done in two ways, namely, simultaneous addition of serum and MAb to antigen-coated wells leading to a competitive ELISA, or addition of MAb after preincubation with the serum (B-ELISA). Both formats were tested in this study.

The wild-type isolate of PPRV Nigeria 75/1, against which the MAbs were raised, was used as the B-ELISA antigen at the previously determined concentration of 4 μg/ml. Immulon-2 96-well flat-bottom microtiter plates (Dynatech Laboratories) were coated with antigen diluted in 0.05 M carbonate buffer (pH 9.6) at 37°C for 1 h or overnight at 4°C. Following four washes in PBST, 50 μl of serum diluted in PBST was added and plates were incubated at 37°C for 1 h. Then, 50 μl of MAb diluted in PBST was added to the serum (i.e., without washing) for a further incubation of 1 h. The plates were again washed, and 100 μl of a 1:1,500 dilution of horseradish peroxidase-conjugated rabbit anti-mouse IgG-IgM-IgA (Sigma Chemical Co.) diluted in PBST-FBS was added to each well. The rest of the assay was done exactly as described for indirect ELISA.

Establishment of optimal MAb and serum dilutions and test format. For initial establishment of test parameters, four weak-positive ($\log_2 VN_{100}$, 5 to 8) PPR serum samples (three goats and one sheep), four strong-positive ($\log_2 VN_{100}$, 10 to 13) RP serum samples (three cattle and one goat), and four negative ($\log_2 VN_{100}$, ≤2) serum samples (two goats, one sheep, and one bovine) were compared ($\log_2 VN_{100}$ is the $\log_2$ of the reciprocal of the highest dilution that completely neutralized virus-specific cytopathic effect). First, the MAbs (hybridoma supernatant or ascitic fluid) were titrated by adding serial twofold dilutions to antigen-coated plates and running an indirect ELISA (Fig. 1a and 2a). Then, three dilutions of MAb were arbitrarily chosen, corresponding to 100, 75 to 80, and 50% of the plateau (saturation) OD on the titration curve, and tested by B-ELISA and competitive ELISA against serial twofold dilutions of the sera (Fig. 1b, 2b, and 3). Finally, to establish the optimal incubation periods for serum and MAb, optimal dilutions of serum and MAb were tested while the relative incubation periods were varied (data not shown). All samples were tested in duplicate. Controls included wells with no serum (0% inhibition) and wells with no serum and no MAb (100% inhibition) (see below), the missing component being replaced with diluent (PBST).

Comparison of the VNT and B-ELISA for titration of sera. The assay for morbillivirus antibody in sera was done by the microplate virus neutralization method (18, 22) with Vero cells. Test viruses were PPRV Nigeria 75/1 attenuated, used after 1 passage in sheep kidney cells, 1 passage in bovine kidney cells, and 62 passages in Vero cells (5); RPV RBOK vaccine strain (98 passages in bovine kidney cells and 4 passages in Vero cells); CDV Onderstepoort strain (unknown passage history); MV Edmonston (unknown passage history); and PDV1-2-6A (unknown passage history). Serial twofold dilutions of serum samples were tested in duplicate against approximately 100 50% tissue culture infective doses of the appropriate virus(es). The VNT titer was determined after a 3- to 10-day incubation period (depending on the cytopathogenicity of the virus) and expressed as $\log_2 VN_{100}$.

The B-ELISA was evaluated with field sera that were screened at the optimal dilution (1:20) and MAb B_2G_4 ascitic fluid. Assay for PPRV-specific antibody by the VNT and B-ELISA was also done with two sets of goat serum samples. One set (27 samples) came from nine goats that had been vaccinated with RPV and challenged with virulent PPRV Sudan 72/2, and the other (30 samples) came from six goats that had been vaccinated with attenuated PPRV Nigeria 75/1 (5) and challenged with virulent PPRV Sudan 72/2. Serial twofold dilutions were added in duplicate to antigen-coated plates, and B-ELISA was performed as described above. Titers were expressed as $\log_2$ of the reciprocal of the highest dilution of serum samples that gave a positive B-ELISA value.

Quantification of B-ELISA results and statistical analysis. The inhibition of MAb binding in the presence of serum was expressed as percent inhibition (%inh), calculated from mean OD values using the following formula

$$\%\text{inh} = \left[\left(1 - \frac{(OD_{test} - OD_{100})}{(OD_0 - OD_{100})}\right) \times 100\right]$$

where OD_{100} is the OD at 100% competition (no serum and

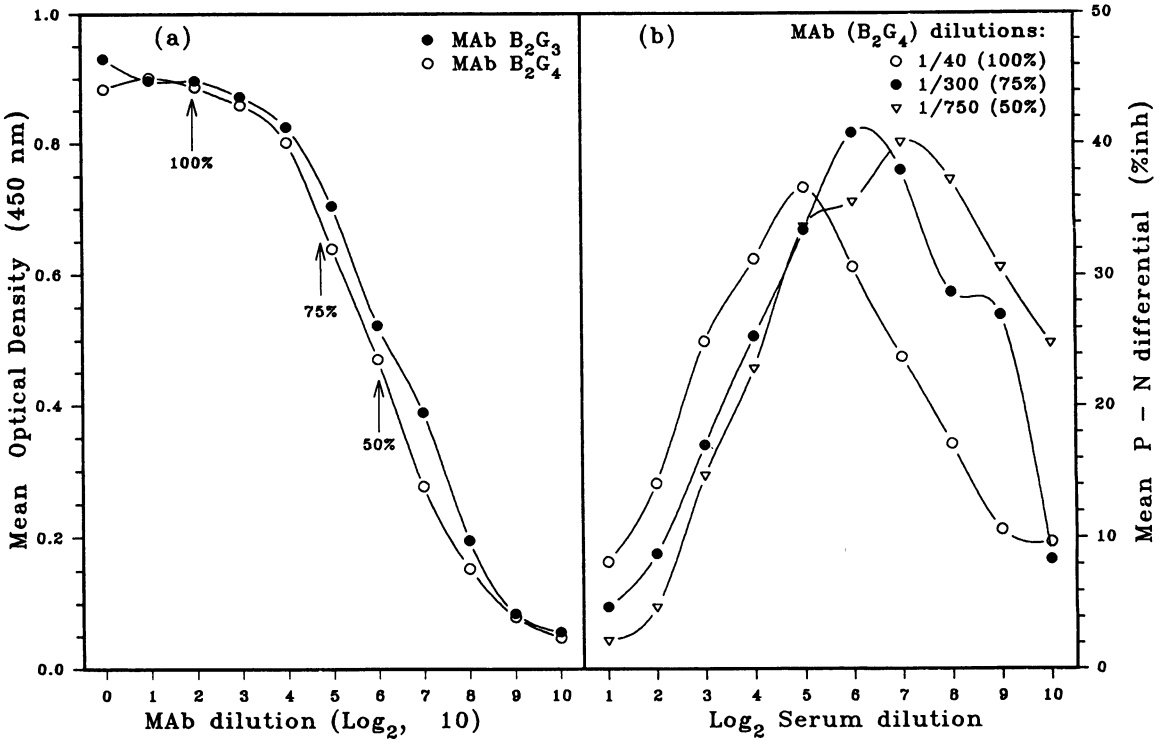

FIG. 1. Establishment of optimal dilutions of hybridoma supernatant and serum. (a) Titration of MAbs by indirect ELISA; (b) titration of sera against three dilutions of MAb. Each datum point is the mean of four values. The P-N differential expresses the difference between the mean percent inhibition (%inh) of four weak PPRV-positive and four strong RPV-positive (i.e., PPRV-negative) serum samples.

no MAb), OD_0 is the OD at 0% competition (MAb alone), and OD_{test} is the OD of the test sample (serum and MAb).

The mean and standard deviation of percent inhibition values from PPRV-negative and -positive serum populations were calculated, and a negative cutoff was established. The correlation between the VNT and B-ELISA was assessed by determining the correlation coefficient (r) by using the Minitab statistical program. The relative specificity and sensitivity of B-ELISA were estimated by using the VNT as a standard test. Agreement between the two tests was also calculated by estimating the agreement quotient (kappa), following established procedures (16).

RESULTS

Optimal dilutions of MAb and serum. The main objective was to differentiate PPRV antibody from RPV antibody. Consequently, the specificity of the test was measured by its ability to discriminate between weak-positive (as defined by the VNT) PPR serum and strong-positive RP serum, at various concentrations. We expressed specificity as the mean difference in percent inhibition values between weak-positive PPR and strong-positive RP sera (or positive-negative [P-N] differential). Figures 1a and 2a present titration curves for the two MAbs (hybridoma supernatants and ascitic fluids, respectively) and the dilutions at which they were further tested. Figures 1b and 2b express the P-N differentials as a function of serum dilution. On the basis of the result in Fig. 1b, we adopted the 75 to 80% saturation dilution (1:300 and 1:20,000 final dilution, for supernatants and ascitic fluids, respectively) as the optimal dilution of the MAb in subsequent testing. At that concentration of the MAb, the optimal dilution for serum was the dilution yielding the peak P-N value. This serum dilution was 1:64 for hybridoma supernatants (Fig. 1b) and 1:20 for ascitic fluid (Fig. 2b). It is remarkable that the two MAbs plateaued at the same dilution with both hybridoma supernatants and ascitic fluids (Fig. 1a and 2a). In subsequent testing, we observed that they gave similar results in every test, and we chose to concentrate on B_2G_4 because of its greater availability.

Test format: competitive ELISA versus B-ELISA and incubation periods. We compared competition and blocking by titrating moderate-PPRV-positive sera (log_2 VN_{100}, 8 to 10) and PPRV-negative sera (Fig. 3). This comparison was extended by evaluating the P-N values for different combinations of the serum and MAb incubation periods, with sera at the optimal dilution. Either serum and MAb were added simultaneously or MAb was added after preincubation with serum, for total incubation periods (serum plus MAb) of 90, 105, 120, and 135 min. Figure 3 clearly indicates that the blocking format yields a sensitivity superior to that of the competitive format, while having the same level of specificity. Furthermore, a 60-min preincubation with the serum and a further 60-min incubation after MAb was added resulted in optimal specificity and sensitivity.

Negative cutoff value. By using 131 serum samples negative for neutralizing activity against any of the morbilliviruses and 147 RPV-positive serum samples (Table 2) at the optimal 1:20 dilution (Fig. 2b), a cutoff value was established for the B-ELISA (Fig. 4). For the two populations combined (Fig. 4a), the cutoff was arbitrarily set at the mean (14.5%) plus 2.7 standard deviations (1 standard deviation = 11.3%), or 45% inhibition. When the RPV-positive population is considered alone (Fig. 4c), the same cutoff point is given by the mean plus 2.6 standard deviations. For the absolute-negative pop-

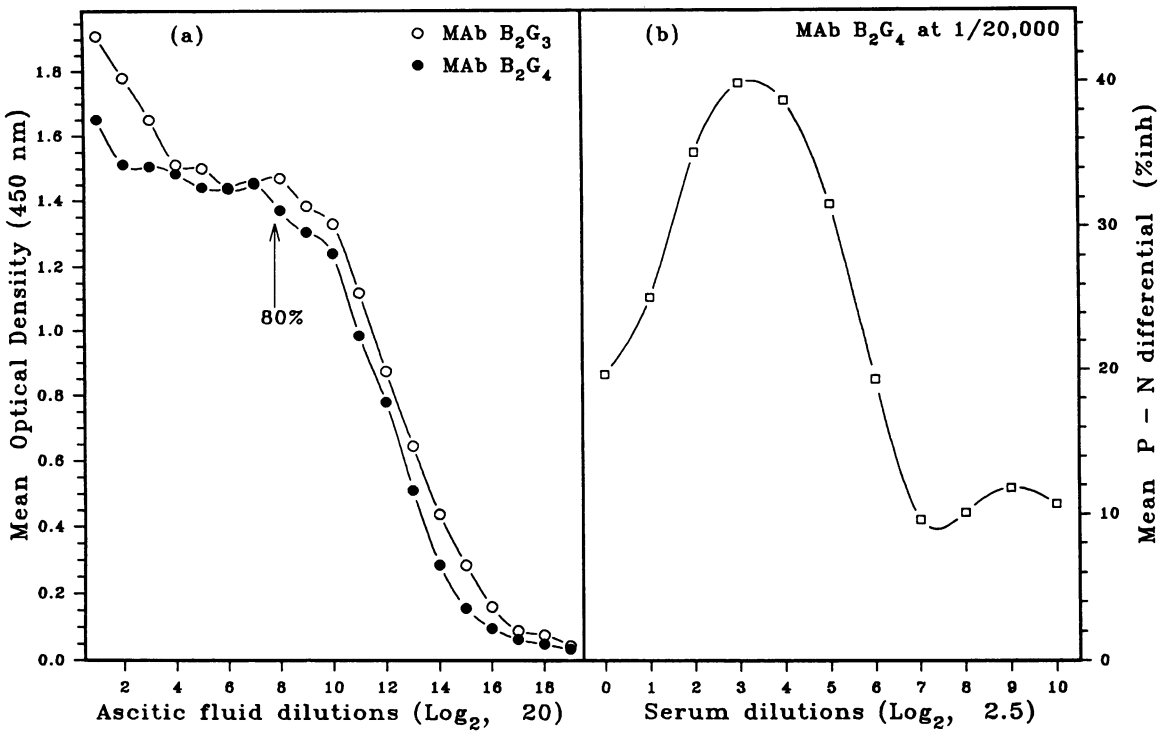

FIG. 2. Establishment of optimal dilutions of ascites and serum. (a) Titration of MAbs by indirect ELISA; (b) titration of sera against a unique dilution of MAb by B-ELISA. Mean and P-N differential are as described for Fig. 1.

ulation (Fig. 4b), the mean plus 2.7 standard deviations gave a cutoff value of 18.7% inhibition.

Comparison of the VNT and B-ELISA. Figure 5 presents mean endpoint titers for the two sets of serum samples, as determined by the VNT and B-ELISA. The overall correlation coefficients between results of the two tests were 0.991 (n = 27) and 0.982 (n = 30) for the two sets of samples.

When 264 field serum samples were titrated by the VNT, an endpoint could not be determined for 11 (4.2%) that were either cytotoxic or contaminated. A total of 39 serum samples (14.8%) were positive by both the VNT and B-ELISA, 6 (2.3%) were negative by B-ELISA but positive by the VNT, and 208 (78.8%) were negative by both tests. Table 3 presents the calculation of agreement quotient based on the 253 samples that could be used in both tests. By combining all the samples, the specificity and sensitivity of B-ELISA relative to the VNT were estimated as shown in Table 4.

DISCUSSION

PPR currently occurs in most African countries located between the Sahara and the equator (13, 25), the Arabian peninsula (1, 10, 27), the Middle East (13), and India (23). It exists in all countries where rinderpest occurs. Most laboratories in those areas cannot afford cell culture facilities that are indispensable for performing the VNT. Because of the immense economic importance of the two diseases, there is need for more affordable tools for differential diagnosis.

Our MAb-based blocking ELISA proved to be a rapid, sensitive, and specific method for detection of PPR antibody. The test offers the additional advantage, over indirect ELISA, of permitting screening of sera from different species with only one anti-immunoglobulin-horseradish peroxidase conjugate. Compared with the VNT, B-ELISA offers

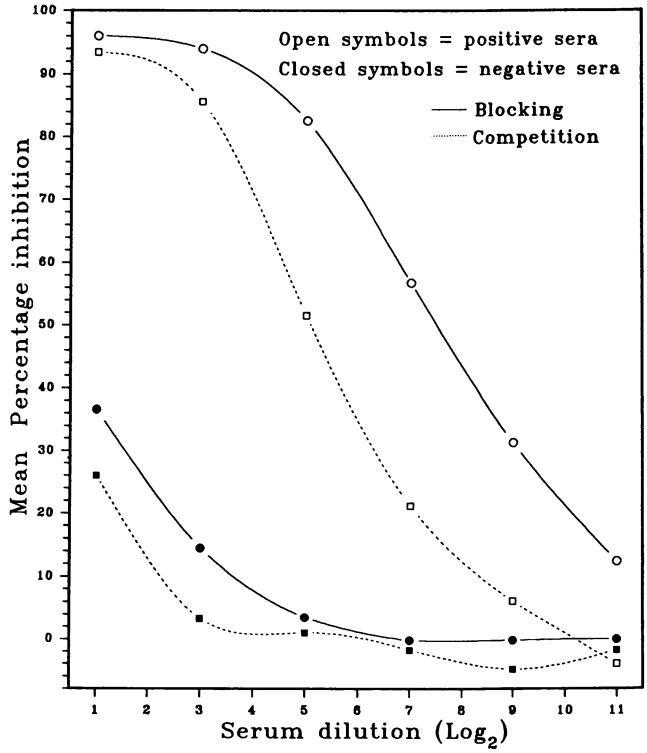

FIG. 3. Comparison of B-ELISA with C-ELISA with MAb B_2G_4. Each datum point is the mean of four values; moderate positive (log_2 VN_{100}, 8 to 10) PPR sera, ○ and □; RPV-positive sera (i.e., PPRV negative), ● and ■.

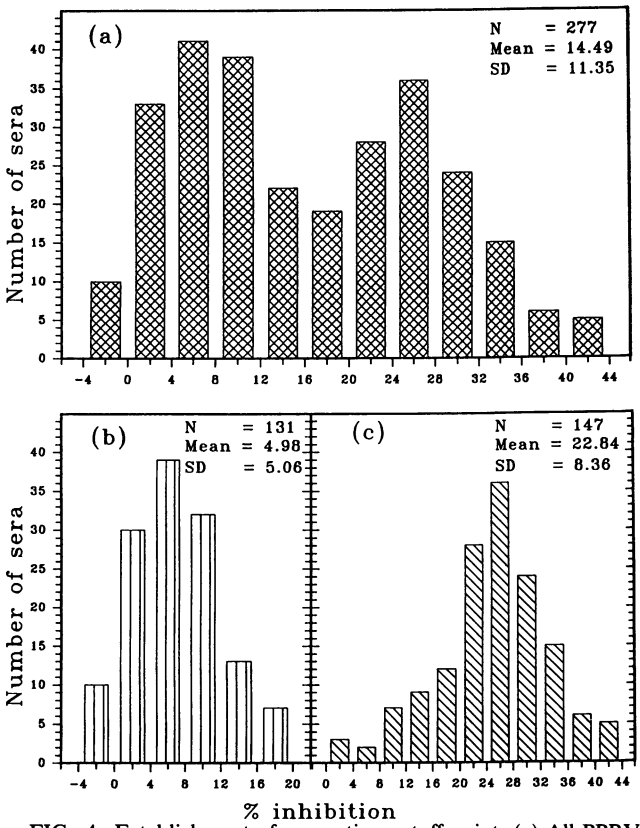

FIG. 4. Establishment of a negative cutoff point. (a) All PPRV-negative sera; (b) absolute-negative sera, i.e., no neutralizing activity against any morbillivirus; (c) RPV-positive sera. SD, standard deviation.

TABLE 3. Agreement between VNT and B-ELISA for detection of antibody in 253 field serum samples

VNT result	No. of B-ELISA serum samples[a]			Apparent prevalence[b]
	Positive	Negative	Total	
Positive	39	6	45	0.178
Negative	0	208	208	0.822
Total	39	214	253	

[a] Observed proportional agreement, (39 + 208)/253 = 0.976; chance proportional agreement, (0.154 × 0.178) + (0.846 × 0.822) = 0.723; observed minus chance agreement, (0.976 − 0.723) = 0.253; maximum possible agreement beyond chance level, (1 − 0.723) = 0.277; agreement quotient (kappa), 0.253/0.277 = 0.91.

[b] Apparent prevalence of B-ELISA sera: positive, 0.154; negative, 0.846.

high levels of sensitivity (≥90%) and specificity (≥98%) while decreasing the run time from 7 days to less than 7 h. Furthermore, unlike the VNT, B-ELISA may be less affected by the quality of sera; 11 of 264 (4.2%) field serum samples could not be titrated by the VNT because of cytotoxicity or contamination but were tested by B-ELISA. Contaminants may affect the outcome of serology in two ways: degradation of antibody and alteration of pH to a level that hinders antibody binding. In practice, the relatively high dilution at which serum is screened (1:20 or above) should nullify the second factor.

The superiority of blocking over competition may be due to the fact that MAb has a higher affinity for the epitope than serum antibodies do. When the two are added simultaneously, only high-affinity serum antibody would compete successfully against the MAb. Indeed, when we ran the test in a competitive format, early-primary-response sera (≤14 days postinoculation) which would contain mainly low-affinity antibody tested negative while secondary-response

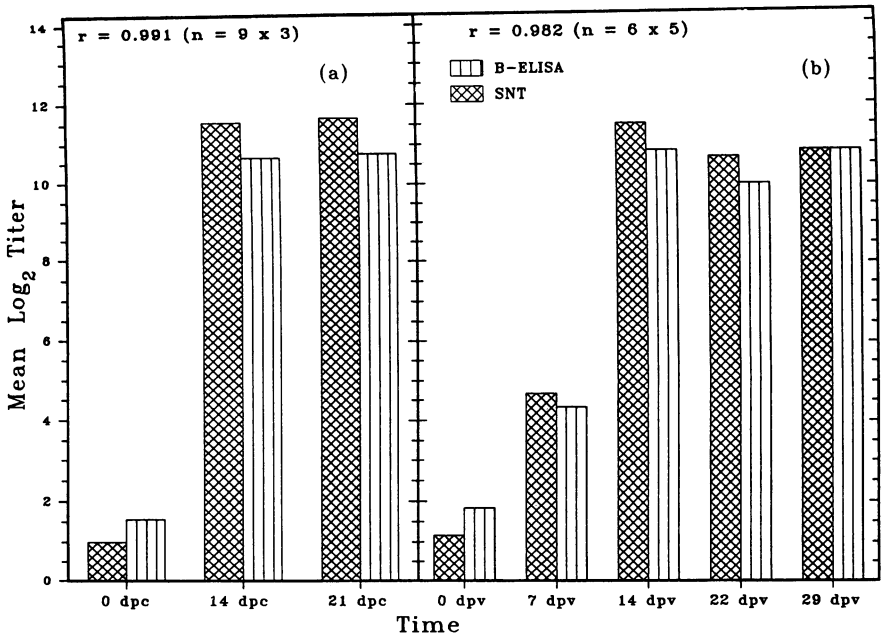

FIG. 5. Correlation between endpoint titers determined by the VNT and B-ELISA. For titers of <2, a value of 1 was arbitrarily used in computing the mean titer. Titers of <3 $\log_2$ were considered negative in both tests. dpc, days postchallenge; dpv, days postvaccination. (a) Sera from nine goats vaccinated with attenuated RPV and challenged with virulent PPRV. Each datum point is the mean of 9 values. (b) Sera from six goats vaccinated with attenuated PPRV and challenged with virulent PPRV. Each datum point is the mean of 6 values.

TABLE 4. Relative sensitivity and specificity[a]

VNT result	No. of B-ELISA serum samples		
	Positive	Negative	Total
Positive	103	11	114
Negative	5	486	491
Total	108	497	605

[a] Relative specificity = 486 of 491, or 98.9%; relative sensitivity = 103 of 114, or 90.4%.

sera, presumably having high affinity, showed a level of sensitivity comparable to that of B-ELISA (data not shown).

When B-ELISA was used in a 1-dilution screening format, a few false negatives (6 of 253, or about 2.4%) were observed. Four of the six had VNT titers of 1:16, while the other two had a titer of 1:256. All those samples became positive when tested at a dilution of 1:10 or lower. To increase sensitivity, it is therefore recommended that a titration be performed whenever possible. From the 605 serum samples tested, the percentage of false positives was remarkably low, especially when one considers that the sera included 152 samples having high VNT titers of antibodies against serologically cross-reactive RPV and the other morbilliviruses (Table 2). It is also noteworthy that the population of RP sera distinguished itself from absolute-negative sera by showing significantly higher mean percent inhibition values ($P < 0.01$), as determined by Student's t-test analysis. This could be due to steric hindrance caused by binding of nonspecific antibody to the numerous morbillivirus cross-reactive epitopes present on the antigen. Unfortunately, our B-ELISA cannot be used to detect RPV antibody because of the overlap between RP-positive and absolute-negative populations (Fig. 4a).

We standardized the B-ELISA to a microneutralization assay for two reasons: (i) the VNT is presently the only available test for differentiation of the PPRV and RPV antibodies, and (ii) neutralizing antibodies are considered the best predictor of host immune status. The B-ELISA results agreed very well with those of the VNT (Table 3), and there was a very high positive correlation between the two test results (Fig. 5). A similar, though slightly lower, correlation coefficient between the VNT and a competitive ELISA for specific detection of RPV antibody with nucleoprotein-specific MAbs was observed by Libeau et al. (14). The higher correlation obtained in our test may be explained in part by our use of a more sensitive test format (Fig. 3) and the fact that our MAbs recognize a neutralizing epitope.

In Africa today, there is an ongoing multinational RP eradication program (the Pan-African Rinderpest Campaign). Because small-ruminant populations, while being fully susceptible to RP, are extremely difficult to vaccinate in a proportion sufficient for eradication in the African context, there is fear that they could represent a refuge for RPV after its eradication in cattle. There is therefore a need for rapid tests to differentiate RP from the clinically similar PPR in small ruminants. The B-ELISA would be very useful in that regard. Its ability to discriminate between RPV and PPRV antibodies would also prove useful in distinguishing sera from animals vaccinated against PPR with tissue culture attenuated RP vaccine from animals that have recovered from natural PPRV infection. Because B-ELISA is rapid, sensitive, and specific, it would be a suitable replacement for the VNT for epidemiological surveillance.

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