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The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes

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SUMMARY

Monoclonal antibody-based competitive ELISA (C-ELISA) have been used for the specific measurement of antibodies to both rinderpest and peste des petits ruminants (PPR) viruses in cattle, sheep and goats. Examination of serum samples from sheep and goats in Gambia, before and after vaccination with rinderpest vaccine, suggested that antibodies to PPR virus could prevent an immune response to the rinderpest vaccine. Cattle sera from Nigeria and Ghana showed a high prevalence of antibody against PPR virus which may explain the difficulty experienced in some countries in achieving high post-vaccination immunity levels against rinderpest. Because antibodies against PPR virus are both cross-neutralizing and cross-protective against rinderpest virus further vaccination in the presence of antibodies against PPR virus may be a waste of national resources. This paper presents serological evidence for the transmission of PPR virus from sheep and goats to cattle and highlights the need to include PPR serology in the sero-monitoring programme to give a better indication of national herd immunity.

INTRODUCTION

The Pan African Rinderpest Campaign (PARC) has been in operation for 6 years and has had a major impact on the incidence of rinderpest throughout Africa. In the last year only Sudan, Ethiopia, Uganda and Kenya have reported disease. This has been accomplished through mass vaccination of the cattle population, the efficiency of which has been evaluated through post-vaccination sero-monitoring. The computer model of Rossiter and James [1] suggests that immunity in at least 85% of the cattle population must be attained to prevent a resurgence of the disease. Although some countries have achieved this level of immunity, others have found this extremely difficult to accomplish, despite repeated vaccination. This has been assumed to be due to either poor maintenance of the cold-chain for preserving the viability of the vaccine virus, or failure to vaccinate a high enough percentage of the animals.

Peste des petits ruminants (PPR) virus is closely-related to rinderpest virus and predominantly affects sheep and goats. Although PPR virus has been shown

experimentally to infect cattle, the disease is subclinical and transmission between small ruminants and cattle in the field has been considered unlikely [2].

In the past differential serodiagnosis of rinderpest and PPR could only be accomplished by cross-neutralization tests. The recently-developed monoclonal antibody-based competitive ELISAs (C-ELISA) for the detection of antibodies against rinderpest and PPR virus have allowed rapid, simple, differential serodiagnosis of the two diseases [3]. This has allowed serological surveys on the prevalence of antibodies against the two viruses in sheep, goats and cattle to give a better understanding of the interaction of these two infections under field conditions.

MATERIALS AND METHODS

Rinderpest virus

The tissue culture attenuated RBOK vaccine strain was used throughout. Virus was propagated in the Madin Derby cell line in Eagle's medium supplemented with 5% bovine serum. Culture medium was changed to serum-free Eagle's medium after 3 days and cultures were harvested when there was 90% cytopathic effect.

PPR virus

The Nigerian strain 75-1 was propagated in Vero cells using the protocol described above.

Preparation of ELISA antigen

Cells from infected cultures were pelleted at 1000 g, resuspended in phosphate buffered saline (PBS) pH 7·6, and sonicated at an amplitude of 30 μm for 1 min. Cell debris was again pelleted by low-speed centrifugation, the supernatant removed, and the cells resuspended in PBS. This cycle of sonication and clarification was repeated six times and the clarified supernatant from each cycle tested by indirect ELISA for reactivity. The supernatant fractions with a suitable titre were pooled and freeze-dried.

Monoclonal antibodies

The C-ELISA for the detection of antibodies against rinderpest virus utilized a rinderpest-specific monoclonal antibody (Mab), designated C1, against the virus haemagglutinin. The assay for antibodies against PPR virus utilized a PPR-specific monoclonal antibody, designated C77, against PPR virus haemagglutinin.

C-ELISA

Nunc Maxisorb 96-well microtitre plates were used for all assays. Volumes of 50 μl were used throughout the test. Antigen was adsorbed to the plate using PBS, all other reagents were added in blocking buffer (PBS supplemented with 0·1% [v/v] Tween-20 and 0·3% [v/v] normal bovine serum). All incubation steps were for 1 h at 37 °C on an orbital shaker. Plates were washed three times after each incubation step by flooding the plates with PBS then emptying. Mouse immunoglobulin was detected using rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase (HRPO). Hydrogen peroxide/orthophenylene diamine (OPD) was used as the substrate/chromogen.

Following antigen adsorption, test sera were added at a dilution of 1/5 in blocking buffer followed immediately by the addition of the specific monoclonal antibody at a dilution previously established by titration. Controls were included with known strong positive, weak positive, and negative bovine sera and a monoclonal antibody (0% competition) control. The last contained antigen, Mab and enzyme conjugate (no test serum) and was used in subsequent calculations as the 0% competition optical density (OD) value. Following incubation at 37 °C for 1 h on an orbital shaker, plates were washed and anti-mouse HRPO conjugate added. After a final incubation for 1 h at 37 °C, substrate/chromogen was added and the colour allowed to develop for 10 min. Plates were read on a Titertek Multiskan ELISA reader at an absorbance of 492 nm and the OD values converted to percentage inhibition (PI) values using the following formula:

$$PI = 100 - ([OD \text{ in test well}/OD \text{ in } 0\% \text{ control well}] \times 100).$$

Percentage inhibition values greater than 50% were considered as positive.

Antiserum

Serum samples collected in Gambia from 56 sheep and 53 goats before and after rinderpest vaccination were collected and kindly supplied by members of the 1988/89 Royal (Dick) School of Veterinary Studies Expedition to the Gambia.

Serum samples collected in Nigeria from 75 sheep and goats and 303 cattle were kindly supplied by Dr D. Shamaki, Vom Institute, Nigeria.

Serum samples from 80 cattle in Ghana were kindly supplied by Dr G. Opoku-Pare, Animal Health Division, Ministry for Agriculture, Accra, Ghana.

RESULTS

Antibody response in vaccinated animals

Serum samples from Gambian sheep and goats were collected before, and 21 days after, vaccination with rinderpest vaccine. They were tested for antibodies against rinderpest virus by C-ELISA and the results are summarized in Table 1. In most Districts, the antibody prevalence following rinderpest vaccination was between 60–70%. The highest prevalence of antibody was found in Fulada W5, where all of 12 sheep and goats were seropositive for rinderpest. However, animals from other Districts, showed a lower antibody prevalence following vaccination, the lowest being Niamina E with only 3/13 (23%) sero-converting following vaccination.

All sera were retested by PPR C-ELISA and all animals which had failed to seroconvert following rinderpest vaccination were found to have antibodies against PPR before vaccination. This is exemplified by the results for Niamina E District (Table 2) where only 3/13 animals were rinderpest seropositive following rinderpest vaccination, the remaining 10 animals were PPR seropositive before vaccination.

Serosurvey of cattle, sheep and goats

The results from the examination of sera from cattle, sheep and goats from Nigeria are shown in Table 3. Twenty-nine percent (11/38) of the cattle from the government farm were seropositive for rinderpest virus alone, 55% (21/38) were

Table 1. *Antibody status of Gambian sheep and goat sera before and after rinderpest vaccination, as detected by rinderpest C-ELISA*

District	Rinderpest competitive ELISA positive	
	Pre-vaccination	Post-vaccination
Niamina E	0/13	3/13
Fuladu W1	1/10	6/10
Fuladu W2	0/10	9/10
Fuladu W3	0/10	6/10
Fuladu W4	0/9	5/9
Fuladu W5	1/12	12/12
Kunting 1	0/10	6/10
Kunting 2	1/10	7/10
Kunting 3	1/10	7/10
Kunting 4	0/10	6/10
Kunting 5	0/5	2/5

Table 2. *Rinderpest and PPR C-ELISA results from the examination of Gambian sheep and goat sera collected in Niamina District before and after vaccination against rinderpest*

Sample number	Rinderpest C-ELISA		PPR C-ELISA	
	Pre-vaccination	Post-vaccination	Pre-vaccination	Post-vaccination
1	–	+	–	–
2	–	–	+	+
3	–	–	+	+
4	–	+	–	–
5	–	–	+	+
6	–	–	+	+
7	–	–	+	+
8	–	–	+	+
9	–	–	+	+
10	–	–	IS*	IS
11	–	–	+	+
12	–	+	–	–
13	–	–	+	+

* Insufficient sample.

Table 3. *Antibody status of Nigerian cattle, sheep and goat sera as detected by rinderpest and PPR C-ELISA*

Location (species)	No. tested	Vaccination status	RP & PPR positive		RP* positive		PPR positive		% protected (inc. PPR)†
			No.	(%)	No.	(%)	No.	(%)	
Government farm (cattle)	38	Annual	21	55	11	29	3	8	84 (92)
Baruchi state (cattle)	265	Annual	99	37	35	13	67	25	50 (75)
Baruchi state (sheep and goats)	75	No vaccination	0	0	2	3	20	27	3 (30)

* RP, rinderpest.
† Total immunity to both rinderpest and PPR.

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seropositive for both rinderpest and PPR viruses, but only 8% (3/38) had antibody against PPR virus alone. In contrast 13% (35/265) of the cattle from Baruchi State were seropositive against rinderpest virus alone, 37% (99/265) were seropositive for both rinderpest and PPR viruses and 25% (67/265) of the cattle had antibody against PPR virus alone. The sheep and goats from Baruchi State showed a similar antibody prevalence i.e. 27% (20/75) against PPR virus alone.

The 80 cattle sera from Ghana were collected as part of the PARC sero-monitoring programme following rinderpest vaccination. When tested by C-ELISA, 52% (42/80) of the sera were rinderpest positive. Those sera which were negative for rinderpest were tested for antibody to PPR by C-ELISA and 96% (37/38) were found to be PPR positive.

DISCUSSION

In this study, the first indication of the interference by antibodies against PPR virus with the immune response to rinderpest virus arose from the examination of sera from sheep and goats collected in Gambia before and after vaccination against rinderpest. The unusually low response to rinderpest vaccination in some districts was explained by the presence of antibodies against PPR virus before vaccination. Antibodies against PPR virus are both cross-neutralizing [4] and cross-protective [5, 6] against rinderpest virus and presumably prevent replication of the attenuated virus in the rinderpest vaccine.

Experimental transmission of PPR virus from sheep and goats to susceptible cattle has been demonstrated previously. Dardiri and colleagues [2] reported that cattle infected experimentally with PPR virus showed no clinical signs but developed a humoral antibody response to PPR virus and were protected against challenge with virulent rinderpest virus. Also, cattle infected by contact with PPR infected sheep and goats developed humoral antibody against PPR virus and were protected against challenge with virulent rinderpest virus. The results presented here show serological evidence for the transmission of PPR virus from sheep and goats to cattle under natural conditions.

The rinderpest C-ELISA has been shown to be specific for rinderpest, giving no cross-reactions with antibodies against PPR virus (3). In contrast, the PPR C-ELISA although utilizing a PPR-specific monoclonal antibody, does detect some cross-reactive antibodies against rinderpest virus (3). Therefore, where sera are positive by both PPR and rinderpest C-ELISA, the sera are regarded as positive for rinderpest, due to the specificity of the rinderpest assay. Those sera which are PPR positive and rinderpest negative are regarded PPR positive. Using these criteria when examining the Nigerian results, and assuming that the antibodies in cattle against PPR virus would protect against rinderpest infection, the true immunity level of the cattle in Baruchi State was 75% not 50%. These results were substantiated on examination of the sera from Ghana. The antibody prevalence against rinderpest virus was 52%. However, the combined antibody prevalence against both rinderpest and PPR viruses was found to be 98%, which probably reflects the true immunity level of the cattle. It is worth noting that a number of countries in West Africa whose cattle populations have a low prevalence of antibody against rinderpest appear to be free of the disease.

Although originally confined to West Africa, PPR has been reported recently in East Africa and India. Further serological surveys are essential to determine the distribution of PPR infection throughout Africa and Asia. Recent tests on 500 sera from other West African countries (unpublished data) have shown areas where there is a high prevalence of antibodies against PPR virus in cattle. Some of these countries have never reported the presence of PPR.

The presence of cross-neutralizing antibodies against PPR does not explain the low levels of post-vaccination immunity in all countries. Animals in countries such as Ivory Coast have been shown to have high levels of immunity against rinderpest despite the reported high incidence of PPR in sheep and goats (7). The transmission of PPR from small ruminants to cattle may be dependent on the type of animal husbandry and possibly the strain of PPR virus present and further seroepidemiological studies are planned to examine these factors. Experiments to determine the duration of immunity against rinderpest conferred by antibodies against PPR virus are also essential in evaluating the importance of these serological data.

If these preliminary findings are substantiated, they may have far reaching implications in evaluating the immune status of any national herd. Vaccination teams have been severely criticised for the poor immune response in animals following vaccination. However, this may not have been due to poor vaccination, but to the presence of antibodies against PPR virus before vaccination commenced. Continued vaccination against rinderpest in such situations may never improve rinderpest immunity levels and thus, may be a waste of national funds and resources.

These results suggest that in regions where PPR virus is endemic, all rinderpest negative sera should be tested by the PPR C-ELISA to give a better indication of the immune status of the cattle.

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