Short communication

Comparative study on peste des petits ruminants seroconversion level in sheep in Sudan

This paper (No. 25082020-00169-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has not yet been formatted for printing. It will be published in issue **39** (3) of the *Scientific and Technical Review*, with a cover date of December 2020.

A.M. Hassan [(1)*], K.H. Al Malik [(2)], A.M. Sheikh Deen [(3)], K.M. Al Faki [(3)], M.A. Abdel Aziz [(4)], H.Y. Mohammed Ahmed [(3)] & T.K. Angra [(5)]

(1) Epidemiologist, Consultant, Kafouri, Block 6, House 159, Khartoum North, Sudan

(2) University of Khartoum, Faculty of Veterinary Medicine, Department of Preventive Medicine and Veterinary Public Health, Shambat, Khartoum North, Sudan

(3) Ministry of Animal Resources, Abu Hamama, Khartoum, Sudan

(4) Veterinary Consultant, Azhari, Soba, Khartoum, Sudan

(5) University of Sudan for Science and Technology, Faculty of Veterinary Medicine, Kuku, Khartoum North, Sudan

*Corresponding author: alihassanahmed48@gmail.com

Summary

Peste des petits ruminants (PPR) is endemic in Sudan. It is considered as a priority disease in the list of transboundary animal diseases (TADs). The aim of this study was to assess the reliability of the detection of PPR antibodies by a competitive enzyme-linked immunosorbent assay (cELISA) in naturally infected or vaccinated sheep. Sera were collected from 3,186 sheep from flocks located in six states of Sudan and additionally 100 sheep were PPR vaccinated with

a PPR homologous vaccine (produced locally) in a quarantine station. The percentages of PPR antibody positive sera from the field varied between 72 and 100% according to state. All sheep vaccinated in the quarantine station were PPR seropositive. This study shows, first, that the percentages of PPR seropositive animals were all very high whether naturally infected or vaccinated. It also shows the reliability of the cELISA test used in this study.

Keywords

cELISA – Competitive enzyme-linked immunosorbent assay – Monoclonal antibody – Peste des petits ruminants – Peste des petits ruminants virus – PPR – PPRV – Quarantine station – TAD – Transboundary animal disease.

Introduction

Peste des petits ruminants (PPR) is a highly infectious disease of sheep and goats caused by peste des petits ruminants virus (PPRV) (1), a member of the genus Morbillivirus that includes the rinderpest virus.

Peste des petits ruminants is endemic in Africa between the Sahara and the Equator, in the Near and Middle East and in South Asia. The World Organisation for Animal Health (OIE) classifies PPR as a transboundary animal disease (TAD). The most effective tool for controlling the disease is through immunisation supplemented by other control measures including restricting the movement of affected animals (2).

The disease was observed in Sudan for the first time in February 1971, affecting both sheep and goats. The isolation of PPRV was carried out at the Central Veterinary Research Laboratory (CVRL) in Khartoum (Soba), in 1992 (3).

Live attenuated vaccines against PPR are available and they are safe and effective, but no method exists for differentiating between infected and vaccinated animals; hence awareness campaigns for

farmers and veterinary staff to promote recognition of the disease are of critical importance.

The aim of this study was to assess the reliability of the detection of PPR antibodies by a competitive enzyme-linked immunosorbent assay (cELISA) in naturally infected or vaccinated sheep.

Materials and methods

Sudan has a large population of small ruminants, recently estimated at 31 million goats and 41 million sheep. The two species are distributed in the 18 states with variable numbers. The states of Darfur and Kordofan in the west and River Nile in the north of Sudan have the highest numbers of sheep and goats.

Collection of serum samples

For screening for the prevalence of PPR antibodies, the Ministry of Animal Resources ([MOAR], Department of Animal Health and Epizootic Diseases Control) collected 3,186 blood samples from sheep in six states on a random sampling basis, as indicated in Table I.

Table I

Sheep serum samples collected from various states in Sudan

SN	State	Number of samples
1	White Nile	655
2	Blue Nile	376
3	Sennar	612
4	North Kordofan	623
5	River Nile	441
6	Al Jazeera	479
Total		**3,186**

SN: serial number

The following steps were taken during the collection of serum samples:

- animals (sheep) were bled for PPR antibody testing
- blood was kept overnight at room temperature (37°C)
- serum was separated from the clot using pipettes and centrifuged to remove any red blood cells
- serum samples were kept in bijou vials and placed in an icebox covered by ice
- the sera were sent quickly to the laboratory for analysis.

Competitive enzyme-linked immunosorbent assay

The CVRL in Khartoum (Soba), which is part of the Animal Resources Research Corporation (ARRC) in the MOAR, followed the OIE recommendations to analyse PPR antibody seroconversion.

The cELISA technique was used to measure seroconversion to PPR, using the following procedure (3, 4):

- The freeze-dried PPR antigen was reconstituted with 1 ml of sterile water and further diluted to the manufacturer's recommended working dilution using 0.01 M phosphate buffered saline (PBS), pH 7.4.
- Samples of the diluted antigen (50 µl) were dispensed into an appropriate number of wells of a flat-bottomed, high protein-binding enzyme-linked immunosorbent assay (ELISA) microplate, using two wells per test serum.
- The sides of the microplate were tapped to ensure that the antigen was evenly distributed over the bottom of each well. The plate was sealed and incubated on an orbital shaker for 1 h at 37°C.
- The wells were washed three times with 0.002 M PBS, pH 7.4.

- Blocking buffer (40 µl; 0.01 M PBS, 0.1% [v/v] Tween 20 and 0.3% [v/v] normal bovine serum) was added to each test well, followed by 10 µl volumes of all test sera.

- Following the manufacturer's recommendations, a working dilution of the monoclonal antibody (MAb) was prepared in blocking buffer and 50 µl was added to each test well. The plates were sealed and re-incubated on an orbital shaker for 1 h at 37°C.

- Following the manufacturer's recommendations, a working dilution of rabbit anti-mouse immunoglobulin–horseradish peroxidase conjugate in blocking buffer was prepared and 50 µl was added to each test well. The plates were sealed and re-incubated on an orbital shaker for 1 h at 37°C.

- At the end of this period, the plates were washed as before, immediately refilled with 50 µl of substrate/chromogen mixture and incubated at room temperature for 10 min without shaking, followed by the addition of 50 µl of a stopping solution consisting of 1 M sulphuric acid.

- The test system included known rinderpest positive and negative serum samples (kept in the Veterinary Research Laboratory (VRL) in Khartoum [Soba]), a MAb control and a conjugate control.

- The resulting absorbance values were measured on an ELISA reader with a 492 nm interference filter, and the test results were expressed as percentage inhibition values compared with the value obtained using the MAb control.

- Inhibition values of 50% or more were considered positive and values below 50% were considered negative.

All 3,186 serum samples were analysed for PPR antibodies (to give the seroconversion prevalence) using the cELISA. The results were obtained on a positive–negative basis. The samples from Al Jazeera and Blue Nile were found to be 100% positive, so they were removed

from the analysis so as not to affect the overall reading and assessment of seroconversion. The samples from the remaining four states, White Nile, Nile River, Sennar and North Kordofan, were organised according to their serial number (field code), and approximately 5% of the samples were selected randomly from each state.

In addition, 100 ovine serum samples were collected from Al Khowai export quarantine station. This quarantine station is located in North Kordofan state in western Sudan, 700 km from Khartoum. This state is one of the richest states in terms of small ruminants, particularly sheep, and is the location of the famous Hamari and Kabashi sheep. Sheep are vaccinated using a PPR homologous vaccine (produced locally in CVRL, Soba, near Khartoum) and inspected by certified veterinarians for health before entering the quarantine station.

The above-mentioned serum samples, kept in an icebox and submitted to CVRL, Soba, were assessed for PPR antibody. Using the cELISA, the samples were analysed and the results were issued after seven days.

Results

The results of the analysis of the ovine serum samples are indicated in Table II. The results obtained from the sera collected from the six states revealed 100% PPR seroprevalence in two states, Al Jazeera and Blue Nile, while the PPR seroprevalence in the other four states ranged between 88% in Sennar and 72% in White Nile.

Table II

Results of screening of ovine serum samples for peste des petits ruminants seroconversion

	State	No. of serum samples	Results of analysis			
			No. +ve	% +ve	No. –ve	% –ve
1	White Nile	36	26	72	10	28
2	River Nile	32	25	78	7	22
3	Sennar	34	30	88	4	12
4	North Kordofan	36	29	81	7	19
Total and average		138	110	80	28	20
5	Blue Nile*	34	34	100	0	0
6	Al Jazeera*	34	34	100	0	0

*: 100% positive removed from the list
+ve: positive, detectable antibodies
–ve: negative, undetectable antibodies

The results of the analysis of the ovine serum samples from Khowai quarantine station for antibodies are indicated in Table III and revealed 100% seroprevalence of PPR.

Table III

Results of analysis of sheep serum samples from Khowai quarantine station for peste des petits ruminants seroconversion

Quarantine station	No. of serum samples	No. +ve	% +ve	No. –ve	% –ve	Remark
Khowai	100	100	100	0	0	vaccinated

+ve: positive, detectable antibodies
–ve: negative, undetectable antibodies

Discussion

Serum samples collected from sheep in four states of Sudan (White Nile, River Nile, Sennar and North Kordofan, as indicated in Table II) and those collected from a quarantine station (Table III), analysed for seroconversion against PPR, showed some variation in the results. The serum samples from the quarantine station, with animals vaccinated against the disease, showed 100% positive results for PPR seroconversion. There is no indication of whether these animals were infected with PPRV before vaccination or not. This result is in line with the observations made by Kumar *et al.*, the OIE and Anderson and McKay (4, 5, 6).

Regarding the samples from the field represented by the four states (White Nile, River Nile, Sennar and North Kordofan), positive results ranged between 72% in White Nile and 88% in Sennar, with an average of 80%. The results are similar to those reported by the MOAR from previous studies. Data extracted from surveillance carried out by the MOAR in Sudan indicated that the PPR prevalence in the country is almost 90% (personal communication).

The authors assume that these animals were either naturally infected with field virus or vaccinated with the homologous PPR vaccine. There are some factors that govern the efficiency of PPR vaccination. One of the most important factors is the thermosensitivity of the PPRV from which the live attenuated vaccine is produced, hence the significance of a proper cold chain. It is worth mentioning that, in a previous study, similar results were obtained for seroconversion to rinderpest and PPR in sheep, goats and cattle (3). In addition, there is a failure to achieve wide PPR vaccination coverage, which is sometimes limited by the persistence of unfavourable conditions, such as a lack of proper logistics.

Conclusion

This study confirms, first, that the percentages of PPR seropositive animals were all very high whether naturally infected or vaccinated. It also shows the reliability of the cELISA test used in this study.

The coming global campaign for the control and eradication of PPR, sponsored technically by the Food and Agriculture Organization of the United Nations (FAO), the OIE, and regional organisations including the African Union–Inter African Bureau for Animal Resources (AU–IBAR) and the Arab Organization for Agricultural Development (AOAD), and validated by Member Countries, is considered promising if properly implemented. For these initiatives to succeed, lessons can be borrowed from previous rinderpest eradication campaigns, including the availability of a potent vaccine and the international community appreciating the threats coming from PPR as a TAD, with its negative impact on livelihood, in addition to its impact on developing countries' economies and trade.

There is a strong case to justify asking the world as a whole to join forces and to encourage individual countries in particular to proceed with the implementation of the PPR progressive control pathway (PCP) and commit resources to that effect.

Acknowledgements

The authors acknowledge and appreciate the approval of Undersecretary MOAR to publish this paper and praise his efforts and those of his colleagues in the Ministry working hard to service the huge animal wealth in the country.

References

1. Diallo A. (2004). – Vaccination for the control of peste des petits ruminants. *Dev. Biol. (Basel)*, **119**, 93–98. PMID:15742621.

2. Gargadennec L. & Lalanne A. (1942). – La peste des petits ruminants. *Bull. Serv. Zootech. Épiz. AOF*, **5** (1), 16–21.

3. Ministry of Animal Resources (MOAR) of Sudan (2017). – Annual reports (annual statistical data, in hard copy form). MOAR, Khartoum, Sudan.

4. Kumar N., Maherchandani S., Kashyap S.K., Singh S.V., Sharma S., Chaubey K.K. & Ly H. (2014). – Peste des petits ruminants virus infection of small ruminants: a comprehensive review. *Viruses*, **6** (6), 2287–2327. https://doi.org/10.3390/v6062287.

5. World Organisation for Animal Health (OIE) (2018). – Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 8th Ed. OIE, Paris, France. Available at: www.oie.int/en/standard-setting/terrestrial-manual/access-online/ (accessed on 17 February 2020).

6. Anderson J. & McKay J.A. (1994). – The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiol. Infect.*, **112** (1), 225–231. https://doi.org/10.1017/S0950268800057599.