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CONTENTS

Correlation between sero-conversion and clinical score in Peste des petits ruminants disease in goats

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ABSTRACT: A correlation analysis was conducted to evaluate the relationship between clinical signs and seroconversion. The results of the correlation analysis demonstrated a positive association between clinical signs and seroconversion in cases of PPR infection. Conversely, no correlation was observed between seroconversion and clinical score in PPR negative animals tested via RT-PCR. Therefore, it can be concluded that a combination of high clinical scores and high seroconversion serves as an indicator of the current ongoing PPRV infection, which can be valuable for surveillance purposes in areas where PPR is endemic.

Key words: Clinical score, sero-conversion, RT-PCR, PPRV, Infection

Peste des petits ruminants (PPR) is caused by *Peste des petits ruminants virus* (PPRV) belonging to the genus *Morbillivirus* of the family *Paramyxoviridae* within the order *Mononegavirales* (Amarasinghe *et al*., 2019). PPR mostly affects goats and sheep, but it also affects wild ruminants, pigs, dogs, and camels; cattle and buffaloes are infected asymptomatically with seroconversion, but other wild ruminants, pigs, dogs, and camels may show clinical signs and mortality (Albina *et al*., 2013; Rahman *et al*., 2016; Schulz *et al*., 2018). Goats are more susceptible than sheep with high mortality (Hussain, *et al*., 2003). The annual global economic losses are estimated to be USD 1.4 to more than 2.1 billion (Agrawal *et al*., 2023) and losses in India are estimated to be USD 2 million to USD 18 million which may go up to USD 1.5 billion (Bardhan *et al*., 2017) owing to morbidity, mortality, and productivity losses with trade limitations (Balamurugan *et al*., 2014). The goal of the global PPR eradication program, set to be accomplished by 2030, can be attained through timely diagnosis, limited movement of infected animals, and the strategic separation of sick animals from their herds (Agrawal *et al*., 2023). Hence, the regular screening of PPR in regional goats is needed. Therefore, the clinical samples from the suspected goats of Pantnagar were collected and screened for PPRV in the present study.

MATERIALS AND METHODS

Clinical samples for PPRV isolation and identification

The identification of these goats as suspected cases was based on the presence of specific clinical indications such as nasal discharge, sticky eyes, foul-smelling diarrhea, elevated body temperature (105 ^{0}F), and a considerable mortality in-contact goat. Each individual clinical sign was assigned a corresponding clinical score. The particulars regarding the animals, their exhibited clinical signs, the assigned clinical scores, and the outcome of the tests conducted can be found in Table 1.

The nasal, oral, and rectal swabs from PPR suspected goats were collected. The swabs were collected from the clinical outbreaks in 3 ml of PBS followed by gentle swirling and squeezing. The content was then filtered through a 0.22 µm Millipore syringe filter under aseptic conditions. The filtrate was used for RNA extraction and as inoculum for virus isolation. Anticoagulated blood in EDTA (Levram life sciences, India) and swabs (nasal, oral and rectal) in 3 ml of phosphate-buffered

saline (PBS) (pH 7.4) for virus identification were collected from the clinical outbreaks. The samples were immediately put inside the ice box and transferred to the lab. The clinical samples were stored at -30^0 C until used. Serum samples for testing anti-PPRV antibodies by c-ELISA were also collected from these suspected cases.

Identification of PPRV from clinical outbreaks

The RNA was extracted from clinical samples using TRIzol-S Reagent (SRL, India). The extracted RNA was stored at -20^0 C until further use. For the RNA extraction, the protocol of Chomczynski and Sacchi. (1987) was followed with slight modification. The detection of PPRV nucleic acid in the clinical samples (whole blood, nasal swab, oral swab, and rectal swab) was done by using a one-step RT-PCR kit (HIMEDIA) and amplicon was analyzed by agarose gel electrophoresis.

Competitive-ELISA for detection of anti-PPRV antibodies

Serum samples were tested by c-ELISA kit (ID Screen® PPR Competition, France) for detection of anti-PPRV antibodies in terms of competition percentage (S/N %). The test was performed as per the manufacturer's guidelines.

The results were expressed as competition percentage (S/N %) which was calculated as follows:

$$S/N\% = \frac{\text{OD sample}}{\text{OD negative control}} \times 100$$

The results were interpreted as follows-

Positive : S/N % $\leq$ 50 %

Doubtful : S/N % >50 % or $\leq$ 60%

Negative : S/N % > 60 %

Samples categorized as doubtful were retested by the above-mentioned c-ELISA kit

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Statistical analysis: The correlation between clinical signs and seroconversion was estimated by correlation coefficient using GraphPad Prism 9.

RESULTS AND DISCUSSION

Apparently healthy goats (Animal number 1, 5, 6, 18, 19) did not show any clinical sign. Out of 19 animals tested, 8 animals turned out to be positive by RT-PCR (Table 1). All RT-PCR positive animals showed a strong seroconversion except animal number 6 which did not show sero-conversion.

Table 1: Details of clinical samples, their clinical signs, clinical score, one step RT-PCR and c-ELISA results of clinically suspected goats for PPR

Animal number	RT-PCR	c-ELISA (S/N %)	Health status	Clinical score
1	negative	73.98	apparently healthy	0
2	negative	76.31	nasal discharge, diarhhoea,	2
3	negative	43.16	nasal discharge, diarhhoea,	2
4	negative	3.47	nasal discharge, diarhhoea,	2
5	negative	36.04	apparently healthy	0
6	positive	77.69	apparently healthy	0
7	negative	69.5	Diarrhoea	1
8	positive	15.91	nasal discharge, off-fed	2
9	positive	9.12	nasal discharge, lacrimation, diarrhoea	3
10	negative	74.15	nasal discharge	1
11	positive	9.32	nasal discharge, sticky eyes, diarrhoea	3
12	positive	8.77	nasal discharge, off-fed	2
13	negative	8.38	nasal discharge, off-fed	2
14	positive	6.1	nasal discharge, off-fed, fever, ulcer on mouth, diarrhoea	5
15	negative	75.22	nasal discharge,dull	2
16	positive	7.2	nasal discharge,dull	2
17	positive	7.29	fever, nasal discharge, coughing	3
18	negative	51.2	apparently healthy	0
19	negative	84.91	apparently healthy	0

Mean value of clinical score in RT-PCR positive animals=2.50, Mean value of clinical score in RT-PCR negative animals=1.09

Correlation analysis between clinical signs and seroconversion

Correlation analysis between clinical signs and seroconversion was done on the basis of:

1. Correlation between clinical signs and seroconversion in RT-PCR positive animals (infected) randomly taken samples on the basis of clinical signs versus sero-conversion.
2. Correlation between clinical signs and seroconversion in RT-PCR negative animals (past infection or never infected).

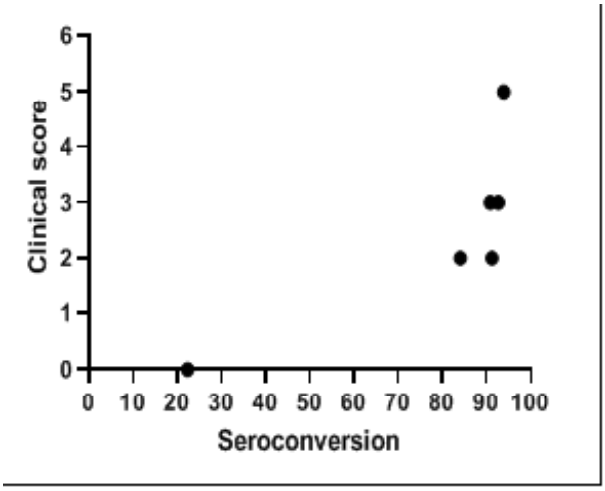

Fig. 1: Correlation between clinical score and seroconversion in RT-PCR positive animals (r=0.799, p=0.0172). A weak correlation with **r value 0.35 (p value=0.2903)** between clinical signs versus seroconversion in RT-PCR negative animals was obtained.

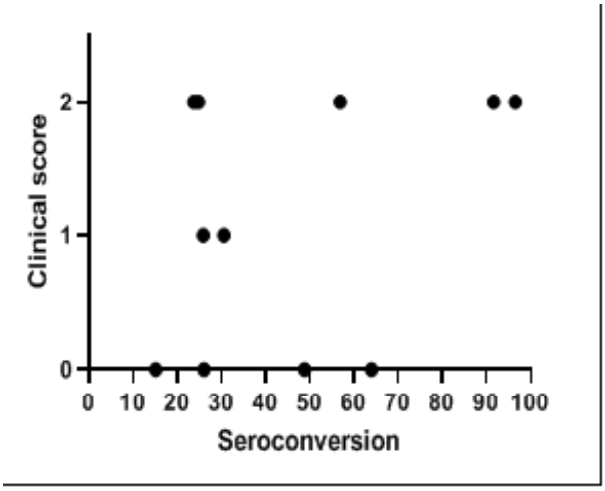

Fig. 2: Correlation between clinical score and seroconversion in RT-PCR negative animals (r=0.35, p=0.2903).

When correlation between clinical signs versus seroconversion in RT-PCR positive animals (PPRV infected animals) was made, a strong correlation of 0.799 was obtained which signifies that PPRV infected animals (ongoing infection) showed clinical signs which was positively associated with sero-conversion (p value=0.0172 significant).

One animal No 6 was found RT-PCR positive but negative in clinical score and sero-conversion, which could be because either the animal was sub-clinically infected or due to the early stage of infection the clinical signs and antibodies were not developed.

Thus, the correlation analysis shows that in an ongoing PPR infection, clinical signs and sero-conversion are positively associated and both are strong indicators of the ongoing infection. At the same time in RT-PCR negative animals no correlation was found between seroconversion and clinical score. Moreover, the RT-PCR positive animals showed higher clinical score (Mean value= 2.5) than the non infected animals (Mean value=1.09) (Table 1).

The study established a strong positive correlation (r = 0.799) between clinical signs and seroconversion in PPR- infected goats, reinforcing their interdependence. Notably, the absence of correlation in non-infected animals highlights the specificity of these indicators. The observed higher mean clinical score in RT-PCR positive animals (2.50) compared to non-infected (1.09) counterparts underscores the combined importance of visible symtoms and immune response as robust markers of active PPR infection. This correlation analysis provides valuable insights for designing targeted surveillance strategies , contributing to the global goal of PPR eradication by 2023.

CONCLUSION

Thus, it is concluded that high clinical scores with high seroconversion is an indicator of ongoing PPRV infection which can be used during surveillance of PPR in an endemic setting.

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