Haryana Vet. (October, 2022) **61(SI-2),** 122-124

Research Article

DETECTION OF ANTIBODIES TO PESTE DES PETITS RUMINANTS (PPR) VIRUS AND *MANNHEIMIA HAEMOLYTICA* FROM PNEUMONIC GOATS OF SOUTH GUJARAT REGION

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Received: 10.12.2021; Accepted: 22.02.2022

ABSTRACT

This study described infection of *Mannheimia haemolytica* and Peste des petits ruminants' virus (PPRV) antibodies in migratory goats. A total 43 nasal swabs and 43 serum samples were collected from goats exhibiting severe respiratory symptoms and processed for bacterial isolation and identification, whereas serum samples were assessed for detection of PPRV antibodies using c-ELISA. Based on Gram's staining, colony morphology and biochemical characteristics, from the tested samples, 25 (58.13%) samples were found positive for *Mannheimia haemolytica*. Out of 43 serum samples, 18 (41.86%)samples were found positive for presence of PPRV antibodies. This study revealed infection of *Mannheimia haemolytica* and PPRV antibodies in transitgoats and their role in severe pneumonia affected Goats.

Keywords: Antibodies, c-ELISA, Goats, *Mannheimia haemolytica*, PPRV

How to cite: Makwana, P.M., Kalyani, I.H., Patel, D.R., Desai, D.N., Sakhare, P.S., Muglikar, D.M. and Patel, N. (2022). Detection of antibodies to peste des petits ruminants (PPR) virus and *Mannheimia haemolytica* from pneumonic goats of South Gujarat region. *Haryana Vet.* **61(SI-2)**: 122-124.

Goat is the one of the most important livestock species in India due to its economic importance as it reared for meat, milk and leather purpose by poor community people. Compared to other animals, rearing of goats is easy, cheap, less laborious and highly profitable. In India, mostly goat farming is affected by outbreaks related with pneumonia and diarrhoea. Pneumonia is one of the most common upper respiratory distresses in goats throughout the world (Ackermann and Brogden, 2000).

Pneumonia in goat is clinically characterized by anorexia, fever (40-41°C), painful coughing, dyspnea, mucopurulent nasal discharge and depression (Rawat *et al.*, 2019). Pneumonia mainly occurs due to mixed infection having more than one etiological agent. It is the common illness in goats due to the seasonal change, transportation stress and migratory movement to new place (Sakhare *et al.*, 2019b). Among the infectious agents, *Pasteurella multocida* and *Mannheimia haemolytica* are more frequently associated with the outbreak of acute pneumonia and death of goats in all age (Falade, 2002). *Pasteurella* spp. is commensal in the upper respiratory tract of healthy goats but whenever stressful condition arises, it is responsible for disease condition. Factors like, poorly ventilated barns, exposure to bad weather, transportation stress and certain viral infections leads to pneumonic condition in goats. Similarly, PPR is a highly contagious, acute, febrile viral disease of goats characterized by fever, anorexia, depression, nasal discharge, ocular discharge, anorexia, abortion, erosion on nasal mucosa, stomatitis, diarrhoea, coughing and depression (Tanwar, 2013; Tariq *et al.*, 2014). In India, the disease has great economic importance on basis of mortality, morbidity, losses through body wastage, poor food efficiency, loss of the meat, milk and milk products, wool losses and offspring (Sakhare *et al.*, 2019a). The percentage of the livestock derived income loss due to PPR varies between 21-90% (FAO, 2009). The morbidity (65.66%) and mortality (34.34%) contributes mainly in overall losses due to PPR outbreaks and prevalence in India (Singh *et al.*, 2014). This study was carried out to investigate etiological agents for the pneumonic condition in goats.

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MATERIALS AND METHODS

Sample collection

A total of 43 nasal swabs and serum samples were aseptically collected from goats exhibiting pneumonic symptoms from Vapi Panjrapole, Gujarat. The history revealed that the herd of goats was transported to Panjrapole few days ago with no history of vaccination. The animals were continuously showing symptoms *viz*. fever, dyspnea, severe coughing and mucopurulent nasal discharge (Fig. 1). Based on clinical signs and symptoms, nasal swabs and serum samples were collected from selected goats irrespective of age and sex. After proper sample collection, samples were transported to the Microbiology department with ice pack.

Isolation and identification of bacteria

All the nasal swabs were streaked on blood agar and MacConkey agar media and incubated at 37°C for 24-48 hours for bacterial isolation. Identification of bacteria was done by Gram's stain, cultural properties and biochemical tests. For identification, typical colonies were subjected to

Gram's staining to observe staining characteristics and cellular morphology. From primary culture, mixed and gram -negative bacteria were further sub-cultured on Blood and MacConkey agar plates (Quinn *et al.*, 1994) for hemolytic properties and pure colony analysis. Identification of the bacteria was confirmed by primary and secondary biochemical tests. (Quinn *et al.*, 1994).

Competitive ELISA (c-ELISA)

Serum samples were screened for the detection of PPRV antibodies using c-ELISA kit (ID Vet France) as per user manual. Briefly, 25 µl of dilution buffer was taken to each well, 25 µl of positive control, 25 µl of negative control and 25 µl of sample to be tested added to the wells. The plate was incubated at 37°C for 45 minutes and washed 3 times with 300 µl of the washing solution. After washing 100 µl of 1-X conjugate was added to each well of plate and incubated at 21°C for 30 min. Then 100 µl of the substrate solution was added to each well and incubated at 21°C for 15 min (Yellow colored development in negative serum samples). To stop the reaction 100 µl of the stop solution was added to each well. O.D. values were recorded at 450 nm and interpretation of each sample was done as positive, negative as per the kit formula.

RESULTS AND DISCUSSION

Examination of 43 nasal swab samples revealed the recovery of 25 isolates of *Mannheimia haemolytica* giving (58.13%) infection rate. Identification of *M. haemolytica* was performed on the basis of Gram staining, colony morphology and biochemical characteristics. Examination of Gram-stained smears from isolates revealed small Gram-negative bacilli. On blood agar, small gray, round colonies with α-hemolytic zones were observed, while on MacConkey agar pink colonies were developed. The organisms which were exhibiting catalase and oxidase tests positive, and narrow zone of hemolysis on blood agar, able to grow on MacConkey agar, but unable to produce indole, were interpreted as *M. haemolytica* (MacFadinn, 2000; Marru *et al.*, 2013) (Fig. 2).

Out of the tested samples, 18 (41.86%) samples were found positive for PPRV antibodies by c-ELISA (Fig. 3). The mean OD values of samples showing 50.00% inhibition were considered as positive for PPR antibodies as per the formula provided by the manufacturer. Similar to this, Thakor *et al.* (2016) and Sakhare *et al.* (2019b) reported 33.33%, 25.70%, 47.14% and 65.77% seropositivity in goats of south Gujarat, respectively. However, Singh *et al.* (2006) reported 27.80 per cent and 42.76 per cent seropositivity in apparently healthy goats from Delhi and Haryana state, respectively. It has been

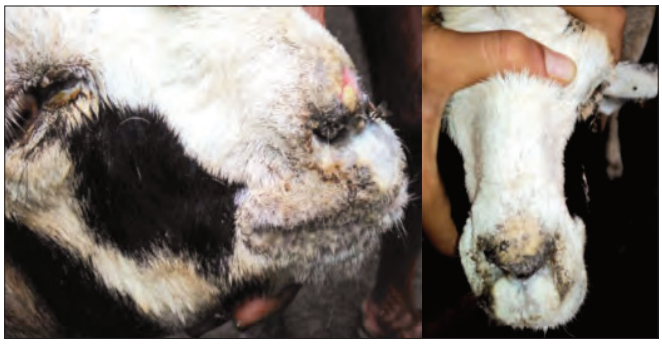

Fig. 1. Pneumonic Goats

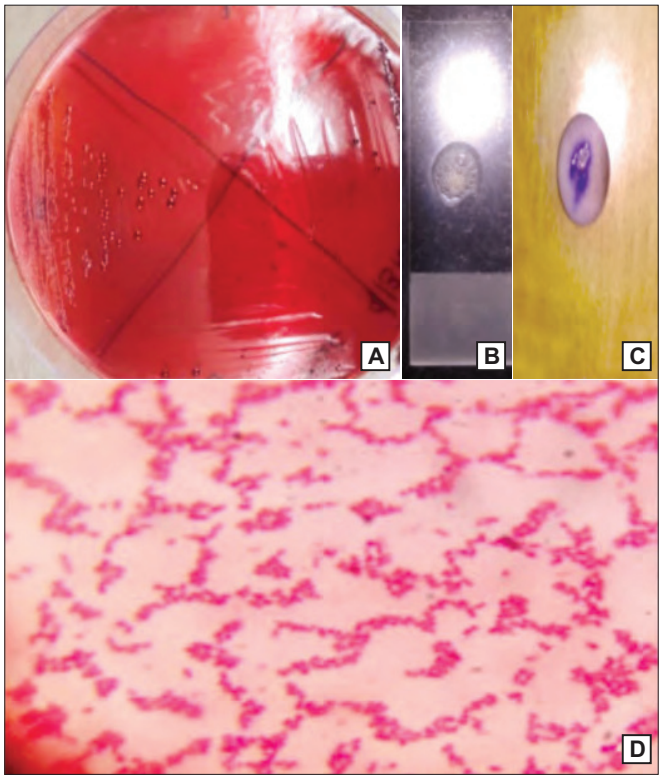

Fig. 2. Characterstics of *Mannhemia haemolytica* (A) Colonies on MacConkey agar plate, (B) Catalase positive test, (C) Oxidase positive test, (D) Gram staining (Gram negative bacilli)

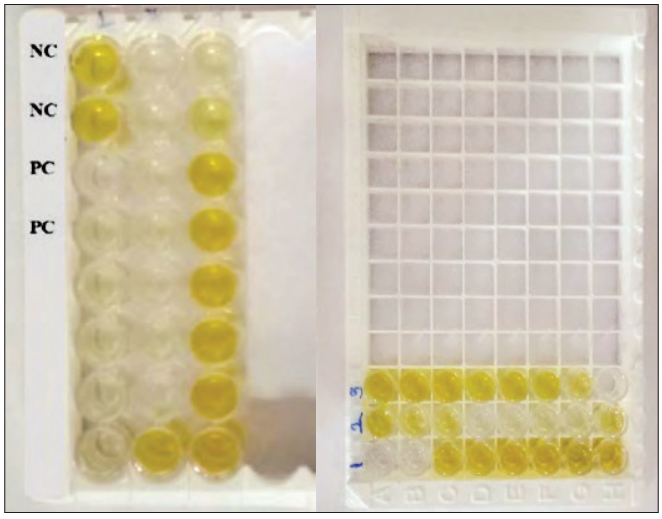

Fig. 3. Samples positive for PPRV

concluded that, as there was no vaccination at the time of sample collection, PPR infection might be spread in healthy flocks due to the transit movement from one place to other.

In the present study, most of the animals were exhibited clinical signs like, dullness, fever, dyspnea, sneezing, serous discharge from eyes and nose as well as mucopurulent nasal discharge. Collected samples were screened for bacterial isolation and PPR Vantibodies detection and it showed presence of *M. haemolytica* and anti PPRV antibodies in affected animals. On the basis of history, previous study carried out in this area and as farmers are not aware about vaccination in goats as well as no any vaccinations against PPRV in this region, which is declared by animal owners, it might be inferred that there might be the infection of PPRV due to migratory movement. As, there was no vaccination, there might be possibility that animals were already infected by PPRV during their transportation. In addition, presence of PPRV infection was confirmed by others in this area as well (Sharma *et al.*, 2015; Sakhare *et al.*, 2019a). All the animals transported to Panjrapole were in group so, they might be in stressful condition. As the pneumonic pasteurellosis is one of the important conditions that is associated with stress and concurrent infection with respiratory viruses, animals acquired bacterial infection due to the stressful condition and close contact with each other during transportation. *M. haemolytica* is an opportunistic bacterium that causes pleuropneumonia in goats in stressful conditions such as transportation (Taunde *et al.*, 2019).

The incubation period is typically 4–6 days but may range from 3–10 days. In most cases, clinical signs appear in 3-6 days. (OIE, 2021). In this case, all the animals were transported few days before to Panjrapole. So, might be animals were already having infection of PPRV. As PPR virus is excreted and secreted from body of affected animals, transmission of PPR requires close contact between animals and mainly it transmits through inhalation of aerosols.

The present study illustrates the involvement of *M. haemolytica* and PPR virus in pneumonic cases associated with stress condition due to shipment of goats. In conclusion, under inadequate welfare conditions, stressful factors may favor the development of pneumonia in goats of all age group.

ACKNOWLEDGMENTS

We want to acknowledge Dr. V.B. Kharadi, Dean, for providing necessary funds to carry out the work. We also want to extend our thanks to Mrs. Amisha Chaudhari, Mrs. Akruti Patel and Akruti Desai, Lab technicians of our department for supportive work.

REFERENCES

Ackermann, M.R. and Brogden, K.A. (2000). Response of the ruminant respiratory tract to *Mannheimia* (*Pasteurella*) *haemolytica*. *Microbes. Infect.* **2**: 1079-1088.

Falade, S. (2002). Further Pasteurella isolates from the republic of Zambia. *Trop. Vet.* **20**: 130-131.

FAO. (2009). *Peste des petits* ruminants: an increasing threat to small ruminant production in Africa and Asia. *EMPRES Transboundary Animal Disease Bullettin.* No. 33.

MacFadinn, J.F. (2000). Biochemical tests for identification of medical bacteria. (3rd Edn.), New York: Williams and Wilkins Lippincott; ISBN: 0683, pp. 05318-05183.

Marru, H.D., Anijajo, T.T. and Hassen, A.A. (2013). A study on Ovine pneumonic pasteurellosis: Isolation and Identification of Pasteurellae and their antibiogram susceptibility pattern in Haramaya District, Eastern Hararghe, Ethiopia. *BMC Vet. Res*. **9(1)**: 1-8.

OIE (2021). Chapter 3.8.9. Peste des petits Ruminants (Infection with small ruminant morbilli virus). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp. 1-16.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994). Clinical Veterinary Microbiology. Mosby-Year Book. pp. 254-258. Dublin.

Rawat, N., Gilhare, V.R., Kushwaha, K.K., Hattimare, D.D., Khan, F.F., Shende, R.K. and Jolhe, D.K. (2019). Isolation and molecular characterization of *Mannheimia haemolytica* and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. *Vet. World.* **12(2)**: 331-336.

Sakhare, P.S., Kalyani, I.H., Vihol, P., Sharma, K.K. and Makwana P. (2019a) Symptomatology and Pathomorphological changes in Outbreak of Peste des petits Ruminants (PPR) in Small Ruminants. *Intas Polivet.* **20(1)**: 118-122.

Sakhare, P., Kalyani, I., Vihol, P., Sharma, K., Solanki, J. Desai D. and Makwana. P. (2019b). Seroepidemiology of Peste des petits Ruminants (PPR) in Sheep and Goats of Southern Districts of Gujarat, India. *Int. J. Curr. Microbiol. App. Sci.* **8(11)**: 1552-1565.

Sharma, K.K., Kshirsagar, D., Kalyani, I., Patel, D., Vihol, P. and Patel, J. (2015). Diagnosis of Peste despetits ruminants infection in small ruminants through in- house developed Indirect ELISA: Practical considerations. *Vet. World.* **8(4)**: 443- 448.

Singh, B., Bardhan, D., Verma, M.R., Prasad, S. and Sinha, D.K. (2014). Estimation of economic losses due to Peste des petits Ruminants in Small Ruminants in India. *Vet. World.* **7(4)**: 194-99.

Singh, S., Jindal, N., Nain, S.P.S. and Khokhar, R.S. (2006). Seroprevalence of Peste des petits ruminants in sheep and goats in and around Haryana state. *Haryana Vet.* **45**: 11-14.

Tanwar, V.K. (2013). An Outbreak of Peste des petits Ruminants in a Goat Flock. *Haryana Vet.* **52**: 143.

Tariq, A., Aqil, K., Akabaar, Z., Mahboob, K., Sarfraz, A., Rafique, R., Nasir, F. and Parveen, S. (2014). Peste des petits ruminant (PPR) in small Ruminants- A clinical hematoserological and pathological aspects. *Int. J. Vet. Sci.* **3(4)**: 206-209.

Taunde, P.A., Argenta, F.F., Bianchi, R.M., Cecco, B.S., Vielmo, A., Lopes, B.C., Siqueira, F.M., Caroline, P.A., Gustavo, G.M.S., Claudio, S.B., Luciana, S., Saulo, P.P. and Driemeier, D. (2019). *Mannheimia haemolytica* pleuropneumonia in goats associated with shipping stress. *Cienc. Rural.* **49**: e20180621.

Thakor, R.B, Patel, M.D., Patel, R.M. and Kalyani, I.H. (2016). Seroprevalence of Peste des petits ruminants in goats of south Gujarat. *Ind. J. Small Rumin.* **22(2)**: 252- 254.