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Dr. Abhilash B
Assistant Professor, Department of Veterinary Clinical Complex, Veterinary College. Bidar, Karnataka, India

Basavaraj A
Professor and Head, Department of Veterinary Microbiology, Veterinary College, Bidar, Karnataka, India

Shrikant MB
Veterinary Officer, Dept. of AH & VS, Govt. of Karnataka, India

Correspondence
Dr. Abhilash B
Assistant Professor, Department of Veterinary Clinical Complex, Veterinary College. Bidar,

Identification of goat poxvirus by agar gel immunodiffusion, counter immunoelectrophoresis and indirect enzyme-linked immunosorbent assay from an outbreak in Bidar

Dr. Abhilash B, Basavaraj A and Shrikant MB

Abstract
Fourteen serum samples from osmanabadi goat breeds suspected for goat pox cases in Islampur village of Humnabad taluk, Bidar district were investigated by three different serological assays: agar gel immunodiffusion (AGID), counter immunoelectrophoresis (CIE) and indirect enzyme-linked immunosorbent assay (i-ELISA). All the sera samples collected were positive by i-ELISA and 9 were positive by CIE and 6 by AGID. The i-ELISA sensitivity was 100%, while that of CIE and AGID was 64% and 42%, respectively. The i-ELISA described combines high specificity and sensitivity in detection of goat poxvirus. The suspected samples were positive for goat pox disease and an i-ELISA was therefore shown to be the method of choice for goat poxvirus diagnosis directly from serum samples during an outbreak.

Keywords: goat pox outbreak, AGID, CIE, i-ELISA

Introduction

Goat pox is characterized by pyrexia, generalized skin and internal pox lesions, and lymphadenopathy. The disease is caused by goat poxvirus (GPV) which is enveloped, double-stranded DNA virus, classified in the genus Capripoxvirus of the family Poxviridae and known to cause significant economic losses in regions where they are endemic. On anamnesis, introduction of newly purchased osmanabadi breed of goat from neighbouring state in to the herd was found to be the reason for this outbreak. Although experienced veterinarians readily diagnose these diseases in their acute forms, laboratory confirmation has been reliant upon serological detection of virus. In the present outbreak the serological assays used for identification were AGID, CIE and i-ELISA. These tests are difficult and time consuming and not readily available in countries that do not hold live viruses.

Materials and Method

Antigen: 20% (w/v) suspension in sterile saline of goat poxvirus infected skin scab collected from the lesions of affected goat was clarified after freezing and thawing three times by centrifugation. The supernatant was used and soluble antigen was prepared according to Rao and Negi, (1997) [5] and used for coating the plates.

AGID: Agar gel immunodiffusion test was performed according to the method of Bhambani and Krishnamurthy (1963) [2] with slight modifications.

CIE: Counter immunoelectrophoresis test was performed according to the method of Basavaraj (1992) [1].

i-ELISA: 96 well micro-titre plates (Nunc) were coated with 100 µl of coating buffer, containing 1:50 diluted antigen and incubated overnight at 4^0 C. Test samples were diluted in blocking buffer (2% porcine gelatin in 1X PBS) at ratio of 1:100 separately in perplex plates. Diluted sera samples (100µl) were transferred to micro titre plates after washing thrice with PBS-T and incubated at 37^0C for one hour on shaker at 300 rpm.100µl of Anti goat IgG HRP conjugate was added per well (1:8000 dilution) and incubated at 37^0 C for one hour on shaker at 300 rpm. The plates were again washed thrice with PBS-T, colour was developed with OPD substrate, after 10 minutes of incubation in dark at room temperature. Enzyme activity was stopped by 1M H_2SO_4 and plates were read at 492nm. The OD values were expressed in percent positivity (PP) and the sample which gave a PP value of more than 54% was considered

Positive and below 54% was considered as Negative.

Results and Discussion

A gel diffusion technique for the diagnosis of goatpox was introduced as early as the 1960's using either homologous (Bhambani and Krishnamurthy, 1963) [2] or heterologous (Uppal and Nilakantan, 1967) [8]. Immuno-electrophoresis is commonly used for the detection of various antigens or antibodies. In CIE, tris-barbital buffer was used as an alternative of normal saline solution to make it more sensitive (Basavaraj, 1992) [1]. A precipitation line had appeared between the wells of test sera samples and GPV antigen in AGID and CIE, where in 9 samples were positive by CIE and 6 samples by AGID.

It has been described that the enzyme immunoassays have proved highly efficient in detection of viral antibodies (Carn *et al.,* 1994) [3]. Many have used either infectious scab material (Sharma *et al*., 1988b) [7] or soluble proteins from infectious scab (Rao *et al*., 1999) [6] or heterologous recombinant SPV P32 antigens (Carn *et al*., 1994) [3] as coating antigen. The mean±SE OD values of test samples in i-ELISA were ranging from 0.987±0.02 to 1.466±0.03 respectively. The average OD value of positive control was 1.45 and i-ELISA has proved to be highly specific for identification of GPV antibodies. Here all the sera samples were shown positive making the test 100% sensitive and endorsing the results with Hosamani *et al.* (2006) [4].

References

1. Basavaraj A. Kenyan Safety and potency tests of strain of sheep pox virus vaccine in goats. PG. thesis, U.A.S., Bangalore, 1992.
2. Bhambani BD, Krishnamurthy D. An immunodiffusion test for the diagnosis of sheep and goat pox. J. Comp. Path. 1963; 73:349.
3. Carn VM, Kitching RP, Hammond JM, Chand P. Use of a recombinant antigen in an indirect ELISA for detecting bovine antibody to capripoxvirus. J. Virol. Methods. 1994; 49:285-294.
4. Hosamani M, Singh SK, Mondal B, Sen A, Bhanuprakash V, Bandyopadhyay SK, *et al.* A bivalent vaccine against goat pox and Peste des Petits ruminants induces protective immune response in goats. Vaccine. 2006; 24:6058-6064
5. Rao TVS, Negi BS. Evaluation of different serological tests for the diagnosis of goat pox using soluble antigens. Trop. Anim. Hlth. Prod. 1997; 4:235-239.
6. Rao TVS, Poonam M, Asgola D. Evaluation of avidin-biotin ELISA for the detection of antibodies to goatpox virus using non infectious diagnostic reagent. Acta Virologica. 1999; 43:297-307
7. Sharma B, Negi BS, Yadav MP, Shankar H, Pandey AB. Application of ELISA in the detection of goatpox antigen and antibodies. Acta Virologica. 1988b; 32:65-69
8. Uppal PK, Nilakantan PR. Serological reactions in sheep pox II – Agar gel diffusion test. Indian Vet. J. 1967; 44:374-382.