Optimization of an Indirect ELISA for Detection of Orf Virus Antibody in Goats

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ABSTRACT
An indirect ELISA for detection of orf virus antibody in goats was optimized by using various coating and blocking buffer combinations during the standardization process at various levels of pH. The plates with 0.02 M PBS and antigen (1:10) kept overnight at 4°C and pre blocking with 0.002 M PBS containing 0.1% Tween 20 and 3% skim milk powder for 1 h was suitable for carrying out the test when used with 0.002 M PBS containing 0.05% Tween 20 as wash buffer.

Keywords: Contagious ecthyma, goat, I-ELISA, orf

Orf or contagious ecthyma has a zoonotic significance and has been reported as a natural disease in human though it occurs mostly in lambs between 3-6 months of age (Gokce et al., 2005; Ndikuwera et al., 1992; Lewis, 1996). A large number of methods can be used to confirm the diagnosis of orf. ELISA and immunofluorescence were found to be the most sensitive tests for diagnosis and confirmation of parapoxvirus infection (Hartmann et al., 1985). An indirect ELISA was optimized and evaluated using purified goat pox virus (GPV cell culture attenuated) antigen and different groups of serum samples from goats with known and unknown immune status for the detection of GPV antibodies (Bhanuprakash et al., 2006).

The main objective of the present study was to optimize an indirect ELISA for detection of orf virus antibody in goats. A total of 266 goat sera samples (grouped according to sex and age) from four districts of Assam namely Kamrup, Jorhat, Udayguri and Mangoldoi were evaluated and cell culture attenuated orf virus vaccine (procured from Indian Veterinary Research Institute, Mukteswar, Uttar Pradesh) was used as the test antigen.

An indirect ELISA was optimized by making slight modification in the method described by Balamuragan et al. (2007). Briefly, the I-ELISA was carried out using cell culture attenuated orf virus vaccine as coating antigen (procured from Indian Veterinary Research Institute, Mukteswar, Uttar Pradesh) at 1:10 dilution in 0.02 M PBS in 96 well flat bottomed, polystyrene microtitre plates (Polysorp, NUNC, Germany) and incubated at 4°C overnight. After incubation, the plates were washed 3-4 times with wash buffer PBS-T (0.002 M PBS containing 0.05% Tween 20) to remove unbound antigen and pre-blocking was done with 50 µl of washing buffer, serum was serially diluted in blocking buffer (0.002M PBS containing 0.1% Tween 20 and 3% skim milk powder) at 37°C for 1 h. After incubation and proper washing for 3-4 times with washing buffer, serum was serially diluted in blocking buffer with 1:10 initial dilution and plate was incubated for 1 h at 37°C. After incubation and washing the plate 3-4 times with washing buffer, anti-goat HRPO conjugate (SIGMA-ALDRICH, St.Louis, USA) diluted in blocking buffer (1:1000) was added.
and incubation was done at 37°C for 1 h. After proper washing, substrate solution (ortho phenylenediamine) and H₂O₂ was added in each well and then the plate was incubated at 37°C for 15 min. The reaction was stopped with 1M H₂SO₄.

Appropriate negative serum control, tracing antibody and conjugate controls were used in the test. A volume of 50 µl was used for all types of reagents throughout the test. The absorbance values were measured at a wavelength of 490 nm in ELISA reader (Bio-Rad, USA, Model: 680 Microplate Reader). The cut off OD value was obtained by subtracting the mean OD value ± SE of negative control from that of test samples. Highest dilution of the serum showing difference of OD values >0.1 was considered as titre of the sera (Balamuragan et al., 2007).

The use of 0.02 mol/L PBS and antigen (1:10) as coating buffer overnight at 4°C and preblocking done with 0.002 M PBS containing 0.1% Tween-20 and 3% skim milk powder for 1 h was found suitable for light colour development of the plates when used with 0.002M PBS containing 0.05% Tween-20 as wash buffer. This optimization was in contrast to the generally used carbonate bicarbonate buffer as the coating buffer (Balamuragan et al., 2007), which resulted in dark background colour of the plates when tried during the study process. This optimized indirect ELISA was used for the study on seroprevalence of the disease and may be considered as a diagnostic test for further study of the disease. Although there are many existing diagnostic tests available for determination of orf virus, but there is no any standard test to distinguish the vaccine strains with that of the wild-type viruses (Hosamani et al., 2009).

A standard diagnostic test will be useful to assess the prevalence of orf virus and other parapoxvirus associated disease in a certain country, helping to overcome the difficulty in quantifying the impact of orf virus on livestock production (Hosamani et al., 2009). There has been however, a very strong recommendation in the literature for the use of an indirect-ELISA for the detection of Orf virus specific antibody (McKeever et al., 1987; Yirrell et al., 1994; Azwai et al., 1995; Inoshima et al., 1999).

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References


