The 2000 Bluetongue Outbreak in Italy


The aim of this experimental trial was to study viraemia and immune response in local breeds of sheep and goat, in order to evaluate their possible role in the epidemiology of bluetongue outbreak in Italy, and to assess the diagnostic value of commonly used antigen and antibody detection procedures.

Materials & Methods

A group of 5 ewes and 3 goats was challenged with 1 ml of tissue culture supernatant containing 105.6 TCID50/ml bluetongue virus (BTV) serotype 2 by the subcutaneous route. One ewe was inoculated with VERO cell suspension and was used as the control. The BTV-2 suspension used in this study was isolated from sheep during the 2000 Italian outbreak in Sardinia. Intravenous inoculation of embryonated chicken eggs followed by passages in VERO or BHK21 cells (3 and 4) was the isolation system used. Immunofluorescence using BTV monoclonal antibodies and electron microscopy was used as confirming assays. Immunofluorescence, electron microscopy, and ELISA (5,6, and 7) were also used to detect BTV antigen in infected embryo and in blood samples. A polymerase chain reaction (PCR) using group-specific and type-specific primers was used for detection of BTV nucleic acid in blood samples (8). The duration and level of viraemia in the challenged animals were also determined. The antibody responses were monitored by ELISA (Bluetongue Virus Antibody Test Kit, c-ELISA - VMRD, Inc., USA), agar gel immunodiffusion AGID, VMRD, Inc., USA, and serum neutralization (SN) assays (9). Sensitivity values of tests employed were estimated and compared using a bayesian approach (10). Probability of the various possible sensitivity values were estimated using the Beta distribution. Different Beta distributions were calculated on the basis of different periods on infection.

Results

The inoculated ewes and goats suffered mild clinical symptoms of bluetongue disease whereas the control animal did not show any clinical sign. Starting from the third day post-inoculation (d.p.i.), BTV was isolated from the blood of challenged animals by intravenous inoculation of embryonated chicken eggs followed by two passages in cell culture (BHK 21 or VERO cells) while a PCR technique was able to detect the virus only from the fifth d.p.i. (fig. 2). The sensitivity distributions of the in vitro isolation system was higher than those of the PCR technique (fig. 2 and 4). BTV was recovered from sheep blood samples up to 45 d.p.i. in contrast, in goat blood samples BTV was not more recovered after 24 d.p.i (fig. 5 and 6). In many animals the viraemic titres were often above 103DI50/ml which is the minimum level necessary to infect feeding Culicoides spp. The c-ELISA and SN’s detected BTV antibodies in the inoculated animals starting from the 7th d.p.i. whereas the sera tested positive on AGID only on 9 d.p.i (Fig. 1). The c-ELISA showed the highest sensitivity distributions in both the periods considered in this study (Fig. 1 and 3).

Conclusions

- According to this study, the level of viraemia was earlier and higher in goats although it lasted longer in sheep.
- For a period of 42 days, at least one infected animal would have been able to infect feeding Culicoides spp.
- The VMRD c-ELISA was the test which showed the highest sensitivity distribution.