A COMPARISON OF WHOLE VIRUS AND RECOMBINANT TRANSMEMBRANE ELISA AND IMMUNODIFFUSION FOR DETECTION OF OVINE LENTIVIRUS ANTIBODIES IN ITALIAN SHEEP FLOCKS

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ABSTRACT


Sera from two sheep experimentally infected with ovine lentivirus (OLV) and from 186 sheep selected from flocks with known high or low prevalence of infection or on the basis of virological or histopathological examination were simultaneously tested by whole virus (WV) ELISA, recombinant transmembrane (r-TM) ELISA and AGID assay. Antigens for both the WV ELISA and AGID were prepared from an Italian field isolate; recombinant antigen was derived from the N'-terminal region of the transmembrane envelope protein of strain K1514. The WV ELISA detected the highest number of seropositives, followed by the r-TM ELISA and AGID test. The sensitivity and specificity of the r-TM ELISA relative to the WV ELISA were 0.66 and 0.95, respectively. Immunoblot analysis of 14 WV ELISA-positive and r-TM ELISA-negative sera showed that the major core protein was immunodominant on WV antigen. It is concluded that the r-TM ELISA was more sensitive than the AGID test but less sensitive that the WV ELISA, particularly for detecting antibodies in the early stages of infection.

Keywords: antibody, antigen, diagnosis, lentivirus, sheep, serology

Abbreviations: AGID, agar gel immunodiffusion; bp, base pair; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; IB, immunoblot; kD, kilodalton; OLV, ovine lentivirus; MEM, minimal essential medium; p.i., post-infection; r, recombinant; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TM, transmembrane; WV, whole virus

INTRODUCTION

Ovine lentivirus is the causative agent of a disease that is distributed worldwide, with various clinical signs, pathological findings and local names (Cutlip et al., 1977).

The antibody response in infected animals confers no resistance and the course of the infection is always fatal. Nevertheless, detection of specific antibodies is useful for diagnostic purposes and represents the only practical foundation for eradication programmes. Several serological tests have been employed to detect OLV antibodies in infected animals. The widely used AGID test shows high specificity (Cutlip et al., 1977). It is suitable for determining the infection status of flocks, but it is too
Insensitive to efficiently facilitate the eradication of the infection. The ELISA, performed with purified virus as antigen, is the method of choice for specificity and sensitivity but it requires expensive and time-consuming tissue culture systems (Houwers et al., 1982; Vitu et al., 1982; Tolari et al., 1992). Moreover, some cellular proteins may co-purify with viral proteins and adversely affect the specificity of the test. Western blot assay requires large amounts of viral proteins and is too expensive to be used routinely.

Recombinant OLV proteins from Icelandic strain K1514 have recently been expressed in a prokaryotic system (Kwang and Cutlip, 1992a). The hydrophilic region of the TM envelope protein was found to be a primary immunodominant marker (Kwang and Cutlip, 1992b). The recombinant transmembrane protein (r-TM) has been used in serological assays with sera from North American sheep and goats (Kwang et al., 1993, 1994).

The r-TM protein represents a hydrophilic region of 94 amino acid sequence coded by a 282 bp fragment of the env gene derived from the Icelandic strain K1514. Unfortunately, very little information is available on the genetic homology of this region among different isolates of small-ruminant lentiviruses. The published sequences for British and South African OLV strains show 84% and 87.2% amino acid homology, respectively, with the strain K1514, with values ranging from 91% to 76% (British OLV) and 89% to 87% (South African OLV) when the amino-terminal and carboxyl-terminal segments are compared (Querat et al., 1990; Sargan et al., 1991). It is therefore not clear whether the r-TM encoded by the K1514 strain can be used as a universal antigen to detect ovine infection in different geographical areas. We have therefore used the r-TM ELISA to detect OLV infection in Italian sheep and compared the results with those from both the AGID test and a whole virus (WV) ELISA, performed with viral antigen from an Italian strain of OLV.

MATERIALS AND METHODS

Serum samples

A panel of 194 ovine serum samples was used (Table I). Some of the sera were known to be positive by AGID. One flock with a high prevalence of infection had >20% of sheep positive by AGID; three flocks with a low prevalence of infection had <5% positive by AGID. Other sera came from animals with histological lesions characteristic of OLV or from animals from which OLV had been isolated. In addition, four serial samples were obtained from two animals 0, 14, 28 and 38 days after they had been experimentally infected by the intratracheal route with Italian isolate OLV-130/91. The six negative control sera for ELISA tests were from a flock continuously serologically monitored for OLV infection during the last 3 years. The two known positive sera were AGID-positive sera from sheep naturally infected with OLV.