EVALUATION OF IMMUNOCAPTURE ELISA FOR DIAGNOSIS OF GOAT POX

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Summary. – An immunocapture enzyme-linked immunosorbent assay (IC-ELISA) for the detection of goat poxvirus (GPV) antigen in skin biopsy samples obtained from healthy and experimentally infected goats as well as from goats from field was evaluated. The assay was 80 – 100% specific and 70 – 86% sensitive, and was compared with a commonly used diagnostic test, namely, the counter immunoelectrophoresis (CIE) test in the efficacy for goat pox. Although IC-ELISA was marginally more sensitive than the CIE test, the diagnosis of goat pox could be successfully done provided both the tests were combined in screening scab suspensions. The IC-ELISA could also diagnose sheep poxvirus (SPV) infection.

Key words: goat pox; sheep pox; diagnosis; immunocapture ELISA; counter immunoelectrophoresis

Introduction

Goat pox is a highly contagious viral disease causing a great economic loss to the goat husbandry. The disease is usually diagnosed by clinical signs, however, laboratory tests are needed for confirmation. Despite the availability of various serological tests, CIE test (Sharma *et al.*, 1988a) is frequently used due to its relative simplicity and reasonable sensitivity. However, a few scab specimens suspected for goat pox were found negative in CIE test despite clear-cut signs of the disease in those goats. Moreover, the time required to isolate the causative agent of pox infections may delay the disease control. Thus, a rapid laboratory confirmation of the disease is crucial for its control. Hence, there is a need for a better diagnostic test for goat pox.

Though the existing ELISAs are more sensitive, the problems like a considerable background reaction (Sharma *et al.*, 1988b) and requirement of special reagents such as recombinant proteins (Carn, 1995) often limit their use as regular screening tests for goat pox. Hence, the present study was conducted to optimise and evaluate a relatively simple but efficient diagnostic test, namely the IC-ELISA for the detection of GPV antigen in scab suspensions. When compared, the assay in combination with the CIE test was found most useful for accurate and confirmative diagnosis of goat pox.

Abbreviations: EDTA = ethylenediamine tetraacetate; GPV = goat poxvirus; IC-ELISA = immunocapture enzyme-linked immunosorbent assay; PBS = phosphate-buffered saline; SPV = sheep poxvirus; Tris = tris-(hydroxymethyl)-aminomethane

Materials and Methods

Animals. Apparently healthy non-descript sheep and goats of either sex and about one year of age, and having no history of pox infection were used in the study.

Viruses. The Sambalpur strain of GPV and Jaipur strain of SPV passaged by periodical skin-to-skin transfers in goats and sheep, respectively, were used for experimental infection in homologous hosts by intradermal inoculation.

Hyperimmunisation of rabbits with purified SPV. SPV was purified from scab suspensions by ultracentrifugation at 85,000 x g for 90 mins at 4°C by the method described by Rao *et al.* (1997). An antiserum was subsequently raised (Hudson and Hay, 1989) by giving 4 injections of resuspended virus (200 μg of protein per dose) in TNE buffer (10 mmol/l Tris.HCl, 100 mmol/l NaCl, 1 mmol/l EDTA, pH 8.0), the first (intramuscular) with complete

Freund's adjuvant, the second and third (both intramuscular) with incomplete Freund's adjuvant, and the last (intravenous) without any adjuvant, on days 0, 14, 21 and 35. Ten days after the last injection, the serum was collected and stored at -20°C. The antiserum was used as a source of capture antibody in IC-ELISA for trapping the antigens in scab suspensions.

Homologous antisera. An antiserum raised in goats against partially purified soluble GPV antigens (Rao *et al.*, 1996), and a conventional homologous antiserum raised against scab suspensions (Sharma *et al.*, 1988a) were used separately as a source of detector antibodies.

Controls. Twenty % skin suspensions prepared in sterile phosphate-buffered saline (PBS) from uninfected, apparently healthy goats (10) were used as negative controls. Similarly, the scab suspensions prepared from frozen skin lesions collected over the years from experimentally GPV-infected goats (15) were used as positive controls. The specimens (29) originating from field goats suspected for goat pox were used as test samples. All the skin/scab suspensions were frozen and thawed thrice, clarified at 2000 rpm for 30 mins, and the supernatants were subjected to screening for GPV antigen by both the CIE test and IC-ELISA after its optimisation.

Scab suspensions prepared from healthy and experimentally SPV-infected sheep were also screened in IC-ELISA. GPV antigen fractions like soluble antigens and virus materials obtained by ultracentrifugation of clarified scab suspensions were also tested.

CIE test was carried out by the method described by Rao *et al.* (1996).

IC-ELISA was performed as follows. ELISA microplates (Corning) were coated with 75 µl per well of anti-SPV rabbit serum dilutions of 1:10, 1:25, 1:50 and 1:100 in a coating buffer (0.01 mol/l carbonate-bicarbonate pH 9.6). The plates were incubated overnight at 4°C. Following 3 washes with PBST (0.03 mol/l PBS, 1% Tween-20, pH 7.4), 50 or 75 µl of skin/scab suspensions at various dilutions ranging from concentrated suspension to dilutions of 1:2, 1:5, and 1:10 in a blocking buffer (2% bovine serum albumin, 0.15 mol/l PBS, 1% Tween-20, pH 7.4) was added per well in quadruplicate and kept at 37°C for 75 mins with intermittent shaking. After a thorough washing with PBST, 75 µl of detector antibodies of either source at dilutions of 1:50, 1:100, 1:200 and 1:400 in the blocking buffer was added per well and kept at 37°C for 1 hr with intermittent shaking. Again after washing, 75 µl of rabbit anti-goat IgG-horseradish peroxidase conjugate (Dakopatts) at dilutions of 1:2,500, 1:5,000, 1:7,500 and 1:10,000 in the blocking buffer was added per well and kept at 37°C for 75 mins. The plates were again washed and 75 µl of a chromogen was added per well. The plates were kept at 37°C for 15 mins and the reaction was stopped by addition of 75 µl of 1 N sulphuric acid per well. The plates were read at 492 nm (A_{492}) in a spectrophotometric ELISA reader (Molecular Devices).

After titration of the assay, all the healthy and positive controls as well as test samples were screened. In the assay, a cut-off value was determined as the average A_{492} value from all the healthy skin suspensions read 3 times.

Indirect ELISA was carried out by the method described by Sharma *et al.* (1988b).

Results

During titration of the IC-ELISA, it was found that an 1:25 dilution of capture antibody, 50 µl of undiluted skin/scab suspension, 1:100 dilution of detector antibody and 1:5,000 dilution of the conjugate yielded satisfactory results. Moreover, a cut-off value higher by 20% than A_{492} from any of the 10 healthy skin suspensions gave an 80 – 100% specificity and a 70 – 86% sensitivity (Martin, 1977) among the controls. The detector antibody, namely the homologous antiserum against soluble GPV antigens fared slightly better

Table 1. Efficacy of CIE test and IC-ELISA alone and in combination in the detection of GPV antigen in skin lesions

Samples	No. of samples	Number (%)						
		Positive in CIE test	IC-ELISA-positives out of CIE-negatives	Positive in IC-ELISA	CIE-positives out of IC-ELISA-negatives	Positive in both tests combined[a]	Positive in both tests	Negative in both tests
Negative (healthy) controls	10	0 (0.0)	0/10 (0.0)	0 (0.0)	0/10 (0.0)	0 (0.0)	0 (0.0)	10 (100.0)
Positive controls	15	10 (66.7)	5/5 (100.0)	11 (73.3)	4/4 (100.0)	15 (100.0)	6 (40.0)	0 (0.0)
Test (field) samples	29	12 (41.4)	10/17 (58.9)	18 (62.1)	4/11 (36.4)	22 (75.9)	8 (27.6)	7 (24.1)
Total	54	22	15/32	29	8/25	37	14	17
(%)		(40.7)	(46.9)	(53.7)	(32.0)	(68.5)	(25.9)	(31.5)

[a]An initial screening by CIE test followed by IC-ELISA testing of CIE-negatives.

than the conventional antiserum in the detection of GPV antigens. The assay also detected SPV antigen successfully in scab suspensions prepared from SPV-infected sheep.

A comparative and combined efficacy of the CIE test and IC-ELISA in the detection of GPV antigen is shown in Table 1. While only 22 of 54 (40.7%) samples were positive in the CIE test, 29 of 54 (53.7%) samples were positive in the IC-ELISA. However, in the CIE-negatives (32), 15 (46.9%) were positive in the IC-ELISA, whereas in the IC-ELISA-negatives (25), only 8 (32.0%) were positive in the CIE test. Thus, in combination of both the tests, i.e. in an initial CIE screening followed by IC-ELISA screening of CIE-negatives, 37 of 54 (68.5%) samples were found positive.

It has been observed that about one-third of the samples responded positively in both the tests while in the remaining samples, most of the clear CIE-positives gave negative results in the IC-ELISA. In the indirect ELISA, the CIE-positives but IC-ELISA negatives gave substantial A_{492} readings and thus further confirmed the CIE results. Although the IC-ELISA seemed to be marginally more sensitive than the CIE test, the McNemar's analysis did not detect any significant difference.

It has been also observed that, in the IC-ELISA, a virus suspension gave a positive reaction whereas the corresponding soluble GPV antigen fraction was found negative. However, the indirect ELISA confirmed the presence of the antigen in the soluble fraction.

Discussion

Antigen trapping enzyme immunoassays have proved highly efficient and promising for the diagnosis of many viral diseases (Livesay *et al.*, 1993; Libeau *et al.*, 1994). In this study, also an IC-ELISA was shown to be more effective than a CIE test for diagnosis of goat pox in scab suspensions. However, these two tests gave contradictory results in some of the positive controls and field samples. It was apparently due to the fact that only soluble antigens were involved in the CIE test leaving the intact virus particles immobilized in agar gel (Westwood *et al.*, 1965; Rao *et al.*, 1997). In the IC-ELISA, these soluble antigens were probably inhibiting/competing with virus particles for limited binding sites available on capture antibodies, hence, a relatively larger quantity of soluble antigens in scab suspensions occupied most of the sites. However, they might not in turn possess other binding sites for detector antibodies resulting in a poor or zero A_{492} reading. Alternatively, the intact virus particles might have many binding sites, some for capture antibodies and others for detector antibodies, producing a satisfactory A_{492} reading. Therefore, the scab suspensions which were rich in soluble antigens reacted strongly in the CIE test but turned out negative in the IC-ELISA. The indirect ELISA, however, further confirmed the presence of GPV antigens in these samples. Moreover, weak positives and some negatives in the CIE test turned out positive in the IC-ELISA because of the presence of a relatively higher quantity of virus particles than that of soluble antigens.

The ability of a capture antibody to successfully detect a GPV antigen demonstrated in this study further confirmed the finding that SPV and GPV are closely related (Kitching and Taylor, 1985; Subbarao *et al.*, 1984). The present study also supports our previous data (Rao *et al.*, 1996) on the utility of polyclonal antiserum raised against soluble GPV antigens to diagnose goat pox. As this reagent could detect also SPV antigens effectively, it can be suggested that an IC-ELISA may be regarded as a common diagnostic test for goat pox and sheep pox which are caused by two different but serologically cross-reactive viruses (Pandey and Singh, 1972; Subbarao and Malik, 1983).

In conclusion, to demonstrate the presence of either soluble antigens or virus particles in a scab suspension that is considered positive for infection, a combination of both the tests, i.e. a preliminary screening of suspected samples by a relatively simple and rapid CIE test followed by IC-ELISA testing of CIE-negatives, is recommended for a confirmative and efficient diagnosis of goat pox.

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