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APPLICATION OF ELISA IN THE DETECTION OF GOAT POX ANTIGEN AND ANTIBODIES

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Summary. — Enzyme linked immunosorbent assay (ELISA) was standardized for detection of goat pox virus (GPV) antibodies and antigen using live and inactivated antigens and hyperimmune serum (HIS), convalescent, post-vaccinal, as also post-challenge sera. The ELISA was most sensitive in detection of antibody when compared with agar gel precipitation (AGP) and counte-immunoelectrophoresis (CIE) tests. There was no complete correlation between the antibody status of vaccinated goats and protective immunity as animals having detectable seroconversion were also solidly immune to virulent challenge. The application of ELISA in pox infections of goats has been discussed.

Kye words: ELISA, goat pox, goat pox virus, goat pox antigen, goat pox antibodies, immune response, CIE

Introduction

Goat pox (GP) and contagious ecthyma (CE) are caused among goats by two different pox viruses, i.e. goat pox virus (GPV) and contagious ecthyma (CEV) or orf virus. Although enzyme linked immunosorbent assay (ELISA) has been used for detection of CEV antibodies by Buddle and Pulford (1984) recently, there is no report available on the application of ELISA for the detection of GPV antigen and antibodies. Recently, we have reported the application of counterimmunoelectrophoresis (CIE) for detection of GPV antibodies and antigen (Sharma *et al.*, 1986). Although CIE was found to be more sensitive than agar gel precipitation test (AGPT), maximum seroconversions detected in goat pox immune goats by CIE were 40 per cent only. Thus, in order to have a more sensitive test, ELISA was standardized to the GPV antigen and antibody system. The results on the application of ELISA for the detection of GPV antigen and antibodies are reported in this communication.

Materials and Methods

Animals. Apparently healthy non-descript goat of either sex, 6 months to 1 year old having no history of goat pox were used for preparation of hyperimmune serum, propagation of virus and vaccination with killed goat pox vaccine.

Virus. Sambalpur strain of GPV (Bandyopadhyay *et al.*, 1984) was used in this study. The virus was maintained by skin to skin transfer in goat by intradermal (i.d.) inoculation.

Hyperimmune serum. Hyperimmune serum (HIS) against GPV was raised in goats as described earlier (Sharma *et al.*, 1986).

Purification of the virus. The GPV collected from skin lesion was purified essentially by the methods of Smadel *et al.* (1984) and Joklik (1962).

Preparation of GPV antigen. To preparate the crude antigen a 30% (W/V) suspension of skin papule of infected goat was used by grinding with sterile sand and PBS. The suspension was frozen and thawed three times before centrifugation at 2,000 rev/min for 20 min at 4 °C. The supernatant was concentrated using PEG-6000 by dialysis at 4 °C and used as crude antigen. The chloroform treated antigen was prepared by mixing of an equal volume of chloroform and crude antigen and shaken vigorously, kept at 4 °C for 1 hr with occassional shaking followed by centrifugation at 2,000 rev/min for 30 min. The upper acqueous phase was collected, stored at −20 °C till used.

Preparation of IgG. IgG from GPV HIS was prepared by precipitation with ammonium sulphate and by DEAE-Cellulose chromatography.

Preparation of $(Fab)_2$. $(Fab)_2$ was prepared by pepsin digestion of IgG.

Preparation of the conjugate. Anti-goat IgG peroxidase conjugate was prepared by Wilson and Nakane (1978) method whereas $(Fab)_2$ peroxidase conjugate was made following Avrameas and Ternyck (1971) two step method.

CIE and AGPT. These were carried out as described earlier (Sharma *et al.*, 1986).

Protein estimation. Proteins were estimated by Lowry's method.

ELISA for detection of antibodies. The sensitization of plates was carried with the pure, partially purified, chloroform treated and crude GPV antigens dissolved in PBS, distilled water, bicarbonate buffer or PBS containing 1 mg/ml sodium deoxycholate. The antigen concentration for coating varied between 5 μg/ml, while serum dilution was used between 1 : 10 to 1 : 400 in PBS tween (PBST). The optimum conjugate dilution was determined by a preliminary titration with plates coated with IgG. It varied from batch to batch (1 : 400 to 1 : 2,000).

ELISA for the detection of antigen. The ELISA plates were sensitized with GPV HIS diluted to 1 : 2,000 in PBS. The test antigen was prepared by triturating the skin lesions after washing with PBS to 30% (w/v) in PBS. This was frozen and thawed three times and centrifuged. The supernatant was collected and used as antigen. The conjugate used was $(Fab)_2$ peroxidase at a dilution of 1 : 1,000. Skin lesions of GPB tested for antigen detection included various stages, i.e. from 2 to 14 days after the appearance of lesions in response to experimental i.d. or natural goat pox infection.

Table 1. Cut-off values in ELISA with different GPV antigens

Antigen	Negative serum range	Positive serum range	Cut-off value
Purified antigen 10 μg/ml in PBS	0.04 — 0.14	0.24 — 1.5	0.30
Partially purified antigen 20 μg/ml in PBS	0.05 — 0.14	0.22 — 1.5	0.40
Chloroform treated antigen 25 μg/ml in PBS	0.08 — 0.24	0.25 — 1.5	0.45
Chloroform treated and membrane filtered 25 μg/ml in PBS	0.08 — 0.24	0.22 — 1.5	0.45
Crude antigen 25 μg/ml in PBS	0.08 — 0.30	0.25 — 1.5	0.50

Table 2. Comparative efficacy of AGPT, CIE and ELISA for detection of goat pox antibodies on identical samples

Test	No. of sera		
	Tested	positive	% positive
AGPT	262	2	0.76
CIE	262	70	26.71
ELISA	262	196	74.80

Results and Discussion

Different antigens used for sensitizing the plates gave various background. The cut-off values arrived arbitrarily at a serum dilution of 1 : 200 in PBST for these antigens are given in Table 1. The purified and partially purified antigens gave considerably less background stain than the crude and chloroform treated antigens. There was no significant effect of different coating medium used for sensitizing the plates. However, the sodium deoxycholate (1 mg/ml) considerably reduced the intensity of final colour development.

The comparative efficacy of AGPT, CIE and ELISA in detecting goat pox antibodies is presented in Table 2. It is obvious from the results that ELISA is significantly more sensitive than AGPT and CIE in detecting GPV antibodies.

The comparative efficacy of AGPT, CIE and ELISA in detecting antibodies reacting with GPV antigens in the prevaccination, post- vaccination

Table 3. Detection of goat pox virus antibodies in pre-vaccination, post-vaccination and post-challenge sera by AGPT, CIE and ELISA

Serum	AGPT	CIE	ELISA
Pre-vacination	*0/136	4/136 (2.94%)	12/60 (20%)
7 DPV	0/51	11/51 (21.56%)	48/51 (94.11%)**
14 DPV	0/54	11/54 (20.37%)	48/54 (88.88%)**
21 DPV	1/45 (2.22%)	18/45 (40%)	33/45 (73.33%)**
35 DPV	0/35	10/35 (28.57%)	30/35 (85.71%)**
20 DPC	1/28 (2.57%)	9/28 (32.14%)	25/28 (89.28%)**
55 DPC	0/19	11/19 (57.89%)	6/19 (31.57%)**
Total	2/368 (0.54%)	74/368 (20.10%)	202/292 (69.17%)

* animals positive/total tested (% positive)
** AGPT, CIE and ELISA were conducted on identical samples
DPV = day post- vaccination
DPC = day post- challenge

and post- challenge sera is presented in Table 3. It is also clear from this table that the sensitivity of ELISA was consistently higher than AGPT and CIE in all categories of sera screened.

Since with the ELISA it is not possible to differentiate between goat pox, sheep pox and contagious ecthyma virus antibodies (unpublished data), it is possible that 20% prevaccinated sera showing positive reaction in ELISA against GPV antigen, might show CEV antibodies as some of the goats used in this study had previous history of CE infection.

Although challenge experiments showed that all animals were protected against virulent GPV (Sambalpur strain), ELISA showed about 73 to 85% seroconversions at the time of challenge, i.e. 21 to 35 days post-vaccination (DPV). In our earlier studies it was observed that positive or negative sero-conversions to goat pox vaccination did not reflect the immunity against virulent challenge (Anon, 1986). Thus the presence of antibodies has no correlation with the protection in goat pox. This findings concur with the observations of Buddle and Pulford (1984) who have shown in case of contagious ecthyma that the presence of antibodies in lambs does not correlate with the protection against challenge.

We have recently standardized delayed type hypersensitivity (DTH) allergic test for goat pox which could detect up to 75% immune goats (Negi *et al.*, 1987) but this test also could not distinguish between goat pox, contagious ecthyma or sheep pox antigens. Although about 94% vaccinated goats were positive for antibodies at 7 DPV, the antibodies declined very fast in the serum of vaccinated and challenged animals. Since goat pox and contagious ecthyma occur in goats and ELISA is quite sensitive in detecting antibodies to both these viruses using GPV antigen, this test can be used in conjunction with DTH test for screening of goats for natural exposure to these viruses and also for determination of sero-conversions to vaccination on herd basis.

The ELISA for the detection of GPV antigen was carried out by the antibody sandwich method using $(Fab)_2$ conjugate. The standardization results showed that with the use of $(Fab)_2$ conjugate the background noise was considerably less. However, this test cannot be used for differenting between CE, GP and sheep pox viruses as all these viral antigens gave significant cross-reactions (unpublished data).

When ELISA and CIE were compared for detection of GPV antigen in goat pox skin lesions cellected 2—14 days after their appearance, the antigen was detected in 44.7% (17/38) and 53.5% (30/56) of lesions, respecspectively. The samples examined by ELISA were common in both tests. The slightly less sensitivity of ELISA as compared to CIE for antigen detection could be due to the high (0.5 OD) cut-off values. In ELISA we consistently got a high background with crude antigen. This was because of possible presence of antibodies against host tissue proteins in the HIS. While standardizing CIE it was observed that the Triton-treated negative skin tissue antigen gave positive reaction while chloroform treated and crude antigens did not (Anon, 1986). It is possible that background reaction in

ELISA may be decreased by raising HIS against purified virus. This aspect needs further study.

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