EVALUATION OF LATEX AGGLUTINATION TEST FOR RAPID DETECTION OF GOAT POXVIRUS ANTIGEN AND ANTIBODIES

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Summary. – Soluble antigen fraction of goat poxvirus (GPV) separated from infectious viral particles by ultracentrifugation of skin scab suspensions prepared from experimentally infected goats was employed for the first time to diagnose goat pox. The antiserum raised against this fraction was found to be specific and not reactive with healthy goat skin extracts. Subsequently, a latex agglutination (LA) test has been developed and standardized for the rapid detection of GPV antigen or antibody in skin scab suspensions or serum samples, respectively. In comparison to the counter immunoelectrophoresis (CIE) test the LA test was more sensitive in the detection of GPV antibodies, but equally sensitive in the detection of GPV antigen. The LA test can be taken for a simple and quick diagnostic tool for primary screening of goat pox.

Key words: goat poxvirus; soluble antigen; antibodies; latex agglutination test; counter immunoelectrophoresis test

Introduction

Goat pox is one of the most severe diseases of goats among the pox infections of domestic animals characterized by pyrexia, generalized skin and internal pox lesions (Kitching and Taylor, 1985). Goat pox is usually diagnosed by clinical signs but its confirmation is made by serological tests like agar gel precipitation (Pandey and Singh, 1972), CIE (Sharma *et al.*, 1988a), or enzyme-linked immunosorbent assay (ELISA, Sharma *et al.*, 1988b; Datta and Soman, 1990). Although some of these tests are very sensitive and reliable, they require either costly chemicals or equipment or both, and are time-consuming. Moreover, these facilities are limited to a small number of central laboratories, and transportation of samples is difficult. Hence there is a need for a diagnostic test for goat poxvirus infection that can be simply and quickly performed under field conditions. Keeping in view these points, a LA test was developed and standardized, and its efficacy compared to that of the CIE test. The test was found simple and rapid, requiring less than 20 mins, and hence the test of choice for primary diagnostic screening of goat pox.

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Abbreviations: CIE = counter immunoelectrophoresis; GPV = goat poxvirus; LA = latex agglutination

Materials and Methods

Animals. Apparently healthy non-descript goats of either sex, about one-year-old, and having no history of goat pox, were used in this study.

Virus. The Sambalpur strain of GPV maintained by periodical skin-to-skin transfers in goats was used for experimental infection.

Soluble antigen. Twenty % (w/v) suspensions in sterile saline of skin scabs collected from the lesions of goats experimentally infected with GPV were frozen and thawed thrice, and clarified by low-speed centrifugation. The supernatants were used as positive antigen controls as well as for the preparation of soluble antigen. The latter was obtained by centrifugation at 85,000 x g for 2 hrs at 4°C. The clear supernatant was saved, tested for residual infectivity and concentrated in a dialysis bag using polyethylene glycol 20,000 before assaying for GPV antibody.

Antiserum was raised in two healthy goats by giving three injections of the partially purified soluble antigen (5, 8, and 10 mg

per dose, respectively), the first (intramuscular and subcutaneous) with complete Freund's adjuvant, the second (subcutaneous and intramuscular) with incomplete Freund's adjuvant, and the last injection (intravenous) without any adjuvant, on days 0, 21 and 35. Ten days after the last injection, the sera were collected and immunoglobulins were separated by 3 times repeated salting out at 40% ammonium sulphate saturation (Dubey, 1983). The final precipitate was resuspended in and dialyzed against saline before use in the tests for GPV antigen detection.

Controls. Clarified skin suspensions and sera obtained from uninfected, apparently healthy goats, as well as the suspensions prepared from the lesions and sera of experimentally infected goats were used as negative and positive controls, respectively.

Test samples. Goat pox-suspect skin scab specimens and sera obtained from the field, pre- as well as post-vaccination sera (frozen samples) of goats collected on various days were used as test samples.

CIE test was carried out according to Sharma *et al.* (1988a) with a modification that ionagar No. 2 (Oxoid) was used instead of agarose. Briefly, 5 ml of 1% melted agar in barbiturate buffer was poured over a clean glass slide. After solidification, 12 wells of 4 mm diameter and spaced 4 mm apart were punched in two rows. The antigen and sera were added to cathodic and anodic wells, respectively. Then, the electrophoresis was carried out at a rate of 20 V/cm for 45 mins to observe the lines of precipitation.

LA test was carried out as described by Hudson and Hay (1989) with slight modifications. Briefly, 100 µl of latex beads suspension (Sigma) was washed twice with 4 ml of 0.054 mol/l glycine-saline buffer pH 8.2 by centrifugation at 12,500 x g for 10 mins. After resuspension in 2.5 ml of the same buffer, 100 µl of 10 mg/ml solution of appropriate antigen or antibody was added and mixed for 20 mins in a mixer. The coated latex was then washed again twice with the same buffer and finally resuspended in 2.5 ml of 0.27 mol/l glycine-saline buffer pH 8.2 containing 0.1% of bovine serum albumin as a blocking agent and stored at 4°C.

A drop (25 µl) of two-fold dilutions (1:2 to 1:16) of tested sera prepared in glycine-saline buffer (0.27 mol/l) was mixed with a drop of antigen-coated latex on a clean glass slide and rocked gently while spreading the mixture for 1 min. The agglutination was read after 15 mins against a white background. Similarly, for antigen detection a drop of clarified skin scab suspension was mixed with a drop of antibody-coated latex. A clear-cut agglutination pattern with prominent blue particles against a white background was considered a positive, while an uniform bluish white colour was considered a negative result.

Effect of temperature. To study the effect of temperature on agglutination, the similarly charged slides were kept at 4°C, 37°C and room temperature (20-25°C). The results were read after every 5-min-interval up to 30 mins.

Stability of reagents. To investigate the stability of reagents stored at 4°C, the LA test was repeated with positive controls at weekly intervals for more than 5 months.

Results and Discussion

The lack of infectivity of soluble GPV antigen along with the histopathological findings indicated the removal of virus particles by ultracentrifugation. These results confirmed the report of Isloor and Negi (1995). The antiserum raised against partially purified soluble GPV antigen was found to be specific and not reactive with a healthy goat skin extract. The LA test also proved to be effective enough to detect soluble GPV antigen in skin scabs and GPV antibody in different kinds of sera. Hence, it can be suggested that the purified soluble GPV antigen represents a valuable diagnostic tool for goat pox. Its use in various tests can avoid the risk of spread of virus from the laboratory.

LA assay has been efficiently used in the detection of various antigen-antibody systems (Sidorovich *et al.*, 1990; Steinberg and Gershon, 1991; Trabelsi *et al.*, 1992; Meurman and Granberg, 1993) and proved to be simple as well as sensitive. Here also, the LA test was found to be by 50% more sensitive and rapid than the CIE test in the detection of GPV antibody. While only 20 of 101 (19.8%) samples were positive in the CIE test, 39 of 101 (38.61%) were positive in the LA test (Table 1). The McNemar analysis confirmed the significance of this difference ($X^2 = 16.0$, $p < 0.005$). The optimum temperature for agglutination was found to be 20-25°C. However, a prior dilution of sera was necessary in the LA test to avoid the prozone effect which results in falsely negative agglutination reactions (Stites and Rodgers, 1987).

Table 1. Comparative efficacy of the CIE and LA tests in the detection of GPV antibodies in sera

Kind of sera	No. of tested sera	No. (%) of positive sera	
		CIE test	LA test
Positive controls	5	5 (100)	5 (100)
Negative (healthy) controls	5	0 (0.0)	0 (0.0)
Field	12	2 (16.67)	4 (33.33)
Prevaccination	14	0 (0.0)	3 (21.43)
Postvaccination	65	13 (20.0)	27 (41.54)
Total	101	20 (19.8)	39 (38.61)

The higher sensitivity of the LA over the CIE test was probably due to the involvement of GPV-specific IgM, in addition to IgG, an extremely effective agglutinating antibody, about 750 times more efficient than IgG in agglutination (Stites and Rodgers, 1987). The IgM-type antibody with 10 binding sites (Edberg *et al.*, 1972) is a much more powerful agglutinator of relatively enormous latex particles than the smaller IgG-type

antibody with only two binding sites (Singh, 1983). In other words, the LA test is very useful in diagnosing early infections since IgM apparently precedes IgG in the phylogenesis of the immune response in vertebrates (Roitt, 1988).

The sensitivity of the LA test in the detection of GPV antigen in skin scab samples was comparable to that of the CIE test (Table 2). The McNemar analysis of these data did not reveal any significant difference. There were, however, only a few samples.

Table 2. Comparative efficacy of the CIE and LA tests in the detection of GPV antigen in skin lesions

Test	No. of samples (controls)		
	Total	Positive	
		No.	%
CIE	16(10)	9(5)	56.25(50)
LA	16(10)	8(5)	50.00(50)

Although it is obviously the most sensitive test, ELISA consumes more time and requires costly chemicals as well as skilled personnel. On the other hand, the LA test is not as sensitive as ELISA but has many other advantages: a simplicity, ease of operation, rapidity, economy and stability of the reagents for more than five months at 4°C. It can be successfully performed under field conditions provided coated latex beads are supplied. The CIE test needs costly equipment and cannot be performed under field conditions. The specificity of both the LA and CIE tests is similar (Martin, 1977). However, the relatively higher sensitivity of the LA test in the detection of GPV antibodies in prevaccination sera makes it practically more useful than the CIE test as a primary screening tool for goat pox.

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