COLOSTRAL IMMUNOGLOBULINS AGAINST *PASTEURELLA MULTOCIDA* (ROBERT TYPE-I) IN CROSSBRED COWS

Masood Akhtar, M. Ashfaque*, Hameed Afzal*, Musarrat Afaq & Sajjad-ur-Rahman*
Department of Veterinary Parasitology,
**Department of Veterinary Microbiology,*
University of Agriculture, Faisalabad

Antibody titre against *Pasteurella multocida* was determined by indirect haemagglutination test in the purified colostral immunoglobulins of ten crossbred cows. All cows had a varying amount (1:4-1:1024) of indirect haemagglutination antibodies in their colostrum. The geomean antibody titre from just after parturition to 72 hours (at 12 hour intervals) ranged from 3.24 to 181.00. Correlation between time intervals and geomean antibody titre against *Pasteurella multocida* was r = -0.9107 ($P<0.001$). The regression equation indicated a decrease of 2.58 in geomean titre per hour increase in time.

INTRODUCTION

The importance of colostrum to new born calves has long been realised. Apart from haemorrhagic septicaemia, its protective effects associated with the transfer of immunoglobulins have been demonstrated in the field and experiments (Gay *et al.*, 1965 and McGuire *et al.*, 1977). Despite the importance of colostrum, no literature is available on colostral antibodies against *Pasteurella multocida*. The present study assesses the colostral antibodies against *Pasteurella multocida* (Robert type-I) in crossbred cows.

MATERIALS AND METHODS

Pasteurella multocida (Robert type-I) isolated in the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, was used in the present study.

Preparation of antigen: A loopful of culture was inoculated in tubes containing 5 ml of Tryptose Yeast Extract (TYE) broth and incubated at 37°C. One ml inoculum (18 hours TYE broth culture) was seeded over three Roux flasks containing 125 ml TYE agar and incubated at 37°C for 24 hours. The growth was harvested in 15 ml, 2.5% sodium chloride per flask, according to the procedure recommended by Amies (1951). The harvested material was subjected to ultrasound waves (Rapid 600 Ultrasonic Ltd.) for 5 x 6 minutes. The sonicated material was centrifuged at 2,000 rpm for 30 minutes and the supernatent was used as antigen.

Colostrum samples: Seventy colostrum samples, seven from each of the 10 crossbred cows at 12 hour intervals upto 72 hours were processed for the purification of colostral immunoglobulins according to Akhtar (1990). The cows were vaccinated 2-4 months ago against haemorrhagic septicaemia.

Indirect haemagglutination test: Indirect haemagglutination (IHA) was performed to assess the antibody titres against *Pasteurella multocida* (Robert type-I) as described by Carter (1955). Human group "O" erythro-

cytes were used in the test. Washed packed erythrocytes (0.2 ml) were sensitized with 4 ml of the sonicated antigen in a water bath at 37° C for 2 hours. The sensitized erythrocytes were then washed twice with phosphate buffer saline (PBS) and pelleted by centrifugation. Finally, 2% suspension was made in PBS.

A two-fold dilution of the immunoglobulins was made with PBS. Equal volume (0.05 ml) of sensitized erythrocytes (2%) was added in each well of the microtitration plates. The plates were gently tapped to ensure even mixing of erythrocytes and kept at room temperature for 30-45 minutes and the degree of agglutination in each well was recorded in comparison with control.

are acquired from the dam's colostrum by ingestion and subsequent absorption from the small intestine.

The purified colostral immunoglobulins from 0 to 72 hours (at 12 hour intervals) after parturition were processed for the titration of haemagglutinating antibodies against *Pasteurella multocida* (Table 1). The IHA titre at 0, 12, 24, 36, 48, 60 and 72 hours interval after parturition ranged from 1:32 to 1:1024, 1:32 to 1:1024, 1:16 to 1:256, 1:8 to 1:128, 1:4 to 1:32, 1:4 to 1:32, 1:4 to 1:8, respectively. The geomean IHA antibody titre against *Pasteurella multocida* from 0 to 72 hours ranged from 3:24 to 181.00. The geomean titre (GMT) at 0, 12, 24, 36, 48, 60 and 72 hours after parturition was 181.00,

Table 1. Distribution of antibody titres against *Pasteurella multocida* determined by IHA test

Hours	Reciprocal dilutions											Geomean titre
	0	2	4	8	16	32	64	128	256	512	1024	
0	-	-	-	-	-	1	1	3	3	1	1	181.00
12	-	-	-	-	-	1	3	2	2	1	1	147.02
24	-	-	-	-	3	1	2	2	2	-	-	59.71
36	-	-	-	4	1	2	2	1	-	-	-	22.61
48	-	-	1	7	1	1	-	-	-	-	-	9.18
60	2	-	3	4	-	1	-	-	-	-	-	4.92
72	3	-	4	3	-	-	-	-	-	-	-	3.24

RESULTS AND DISCUSSION

Indirect haemagglutination has been commonly used for studying the humoral antibody titres in the serum against *Pasteurella multocida* (Carter, 1955). Numerous research workers have shown that adequate levels of serum immunoglobulins are essential for the survival of a new born calf which normally is born virtually agammaglobulinaemic (McEwan *et al.*, 1970 and Villouta *et al.*, 1980). The immunoglobulins

147.02, 59.71, 22.61, 9.18, 4.92 and 3.24, respectively. The results indicate that as the time increases after parturition, the geomean IHA antibody titres against *Pasteurella multocida* decrease (Fig. 1).

There is a significant but negative correlation between time intervals and geomean IHA antibody titres against *Pasteurella multocida* ($r = -0.9107$, $P < 0.001$). The regression equation (GMT = 151.09 - 2.58 x time) indicates a decrease of2.58 in GMT per hour increase in time.

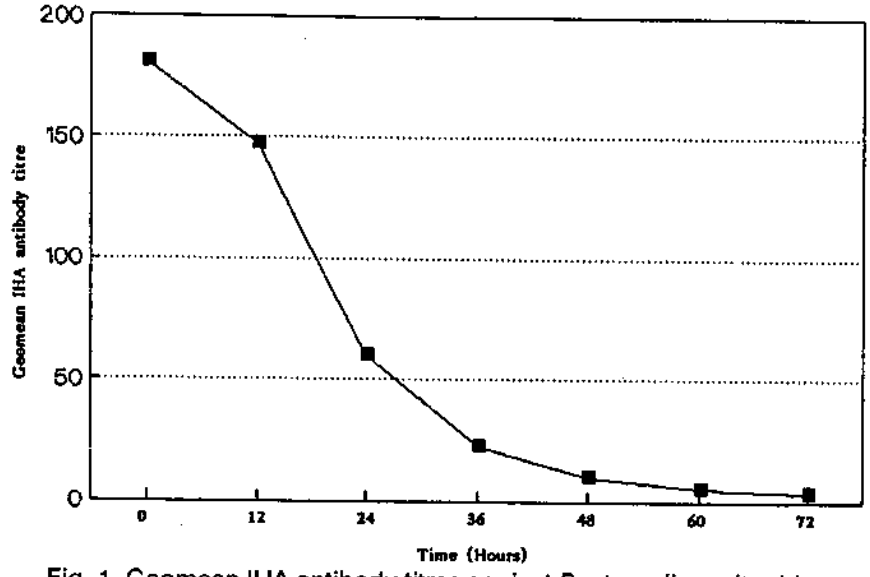

Fig. 1. Geomean IHA antibody titres against *Pasteurella multocida* in purified colostral immunoglobulins

The results of this study indicate that there is need for increased emphasis on evaluation of immune status of cows as this knowledge will encourage changes in management practices, directed at increasing the immunoglobulin contents in the colostrum.

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