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ANTIGENIC CHARACTERISATION OF *Pasteurella multocida* ISOLATES FROM RABBIT-II*

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Broiler rabbit rearing in Kerala is becoming an industry. Rabbits are prone to some diseases due to poor health coverage. Of the various afflictions of rabbits, Pasteurellosis, caused by *P. multocida* has been reported to be one of the common and serious problems causing heavy loss to the rabbit rearers. This infection has been reported in many rabbitries in Kerala state (Devi *et al.,* 1990), but so far serologic and antigenic relationship of the isolates have not been studied. Majority of the informations available on the serology of *P. multocida* are from the investigation of pasteurellosis in other species particularly cattle and birds. Recently serotypes of *P. multocida* infecting rabbits have received attention and much research on the serology are in progress. Serologic properties of the bacteria are based mainly on the capsular and cell wall antigenic characteristics.

In the present work, the serological characters of heat inactivated crude extract, Potassium thiocyanate (KSCN) extract and sonicated antigen of two isolates of *P. multocida* of rabbit origin (R_9S and $R_{23}S$) and a bovine vaccine strain (p-52) are reported.

Materials and methods

Antiserum

The antigens were prepared according to the procedure described - Heat inactivated crude extract (Syuto and Matsumoto, 1982), KSCN extract (Lu *et al.,* 1987) and sonicated antigen (Ireland *et al.,* 1989). *P. multocida* free healthy rabbits of 3-4 months were used for raising antiserum. Rabbits were injected subcutaneously with one ml of antigen with an equal quantity of Freund's Complete Adjuvant. Subsequently one ml of plain antigen was given intravenously at 5 days interval upto 50th day. Blood was collected in sufficient quantity and serum separated was stored at -60°C until further use.

Agar Gel Precipitin Test (AGPT)

The test was performed in slide/plate following the methods described by Heddleston *et al.* (1972). The central well was filled with antiserum and the peripheral wells were filled with homologous and heterologous antigens. The slides/plates were incubated at 37°C and results were recorded after 24 h after staining.

Immunoelectrophoresis

It was carried out by following the procedure of Heddleston *et al.* (1972). Wells and troughs were made on glass plate agar gel. First the wells were filled with antigens and run in electrophoretic chamber at a current rate of 3 mA per glass slide. After the completion of electrophoresis, the troughs were filled with a serum against a particular antigen. Then after 48 h the plates were stained and made permanent.

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Indirect Haemagglutination test (IHA)

It was carried out according to the procedure described by Sawada *et al.* (1982). In brief, the gluteraldehyde fixed sheep erythrocytes (SRBC) were used for sensitization with heat inactivated crude extract and sonicated antigen in IHA. In case of KSCN extract antigen, the gluteraldehyde fixed SRBC was treated with tannic acid before sensitization with the antigen. IHA was carried out in a 'U' bottomed micro titre plate. Serial two fold dilutions of antiserum in BSA-PBS were taken in 25 μl quantity in 24 wells. 25 μl of the sensitized SRBC was added to each well and plates were shaken and allowed to stand for 2 h at 25° C before reading the results. The IHA titre was expressed as the reciprocal of the highest dilution of serum showing a positive pattern. In every test known positive and negative controls were set.

Results and Discussion

Individually antiserum was raised in rabbits against the three antigenic preparations prepared from the three strains of *P. multocida* under study. Specific antibody could be detected in the serum of rabbits even at 10th or 15th day of antigen administration. Irrespective of the form of antigen, immunization was stopped at 50th day. Blood collected for serum contained sufficient level of antibody and could be detected well by serological tests employed in the present study. The findings in this present study are comparable with the findings of Manning (1984).

In the AGPT and immunoelectrophoretic analysis the antiserum against a particular for of antigen was tested against three homologous and two heterologous antigens. The antiserum against heat inactivated crude extract antigen from the three strains presented two identical precipitin lines with homologous and heterologous antigens (Fig. 1, A,B,C). The results were comparable with the results of Syuto and Matsumoto (1982) and Kajikawa and Matsumoto (1984) on crude extract antigen. The precipitin lines formed by heat inactivated crude extract antigen were comparable with the precipitin lines formed by phenol-water extract antigen from *P. multocida* of Penn and Nagy (1974).

The antiserum against KSCN extract antigen from three strains developed multiple precipitin lines with homologous and heterologous antigens in which some were identical to other antigens tested (Fig. 1 D,E,F). The findings in the present study, were comparable with the findings of Gaunt *et al.* (1977) with avian isolates. The antiserum against sonicated antigen presented multiple precipitin lines (five) wherein few were identical to the homologous and heterologous antigens (Fig. 1 G,H,I).

From this study, it was observed that the sonicated antigen was more heterogenous and serologically more active.

In immunoelectrophoresis, antiserm against heat inactivated crude extract of three strains developed three precipitin arcs which were similar in their position and comparable between them (Fig. II A,B,C). The antiserum against KSCN extract antigen developed three precipitin arcs in R_9S and four in other two strains (Fig. II D,E,F). The results are comparable with the findings of Mukkur (1979) on KSCN extract antigen from cattle strain. The antiserum raised against sonicated antigen from three strains presented six precipitin arcs which were comparable between the homologous and heterologous antigens (Fig. II G,H,I). The immunoelectrophoresis presented more number of precipitin arcs compared to the AGPT indicating the sensitivity of the test. From the results of AGPT and immunoelectrophoresis it could be inferred that the R_9S and $R_{23}S$ were more closely related when compared to bovine strain P-52.

The three antigenic preparations from the three strains adsorbed on SRBC were specifically agglutinated by the antiserum raised in rabbits. Results of antibody titration in antiserum using homologous and heterologous antigens given in Table I. The KSCN extract and sonicated antigens showed same titres with homologous serum indicating the similar immunogenic potentiality. The results also revealed that the heat inactivated crude extract was less immunogenic. Irrespective of the antigen preparation methods, the $R_{23}S$ strain antigens could produce maximum antibody titre in the serum. The heterologous reaction were more prominent with antiserum raised against sonicated antigen. These results were in confirmity with the results of Sawada *et al.* (1982) with crude extract and KSCN extract antigen. The present results were in absolute confirmity with the results obtained by Azam *et al.* (1991) wherein they reported a dose-dependent immune response.

From the present results it is evident that the homologous reactions are predominant in the serological tests and the sonicated antigen functions as a better antigen irrespective of the strain of *P. multocida.*

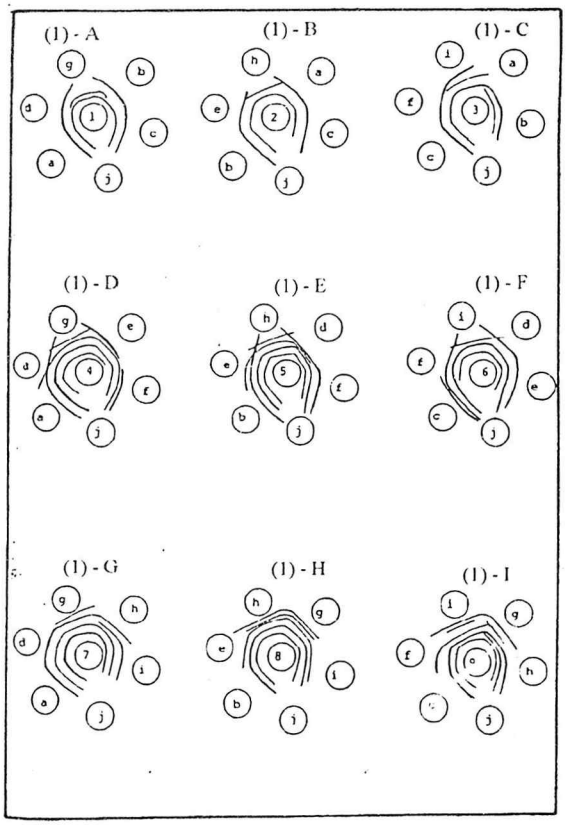
Table 1 Indirect haemagglutination titre of antisera raised against each antigen preparation from R_9S, $R_{23}S$ and P-52 strains of *P. multocida*

Type of Antigen	Source of Antigen	IHA titre in the antiserum against								
		Heat inactivated crude extract			KSCN extract			Sonicated antigen		
		R_9S	$R_{23}S$	P-52	R_9S	$R_{23}S$	P-52	R_9S	$R_{23}S$	P-52
Heat inactivated crude extract antigen	R_9S	2048	256	32						
	$R_{23}S$	128	4096	64						
	P-52	64	64	2048						
KSCN extract antigen	R_9S				4096	256	512			
	$R_{23}S$				512	8192	256			
	P-52				128	128	4096			
Sonicated antigen	R_9S							4096	1024	1024
	$R_{23}S$							512	8192	256
	P-52							256	512	4096

Summary

Individually antiserum against three forms of antigens viz., heat inactivated crude extract, KSCN extract and sonicated antigen from the three strains of *P. multocida* was raised in rabbits. Serological tests such as AGPT, Immunoelectrophoresis and IHA were carried out to compare the antigenic relationship of the isolates. Each antiserum was tested against three homologous and two heterologous antigens and it produced multiple precipitin lines which were identical with the homologous and heterologous antigens. The sonicated antigen invariably presented more precipitin lines than the other forms of antigens. The AGPT results indicated close serological relationship between two rabbit strains than the bovine strain p-52. These results were further confirmed on immunoelectrophoresis wherein multiple precipitin arcs were developed and were comparable in their number and position.

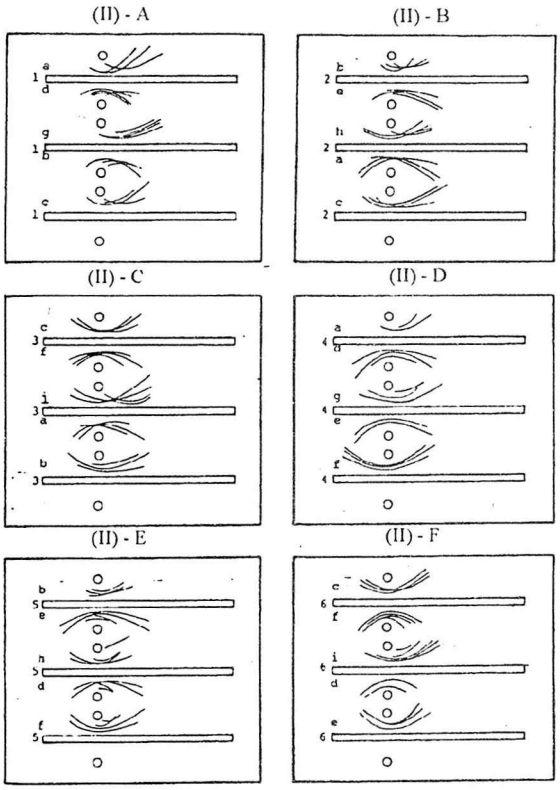
Fig. I A-I Diagramatic illustration of the precipitin patterns of different antigens of *P. multocida* from R_9S, $R_{23}S$ and P-52 strains



a	-	HICE Ag	-	R^9S,	1.	Antiserum against HICE Ag	- R^9S
b	-	HICE Ag	-	$R^{23}S$,	2.	Antiserum against HICE Ag	- $R^{23}S$
c	-	HICE Ag	-	P-52	3.	Antiserum against HICE Ag	- P-52
d	-	KSCN Ag	-	R^9S,	4.	Antiserum against KSCN Ag	- R^9S
e	-	KSCN Ag	-	$R^{23}S$,	5.	Antiserum against KSCN Ag	- $R^{23}S$
f	-	KSCN Ag	-	P-52	6.	Antiserum against KSCN Ag	- P-52
g	-	Son. Ag	-	R^9S,	7.	Antiserum against Son. Ag	- R^9S
h	-	Son. Ag	-	$R^{23}S$,	8.	Antiserum against Son. Ag	- $R^{23}S$
i	-	Son. Ag	-	P-52	9.	Antiserum against Son. Ag	- P-52
j	-	Blank					

HICE Ag - Heat Inactivated crude extract antigen
KSCN Ag - Potassium thiocyanate extract antigen
Son. Ag - Sonicated antigen

Fig. II A-I Diagramatic illustration of immunoelectrophorogram of different antigens of *P. multocida* from R_9S, $R_{23}S$ and P-52 strains



a	-	HICE Ag	-	R^9S,	1.	Antiserum against HICE Ag	-	R^9S
b	-	HICE Ag	-	$R^{23}S$,	2.	Antiserum against HICE Ag	-	$R^{23}S$
c	-	HICE Ag	-	P-52	3.	Antiserum against HICE Ag	-	P-52
d	-	KSCN Ag	-	R^9S,	4.	Antiserum against KSCN Ag	-	R^9S
e	-	KSCN Ag	-	$R^{23}S$,	5.	Antiserum against KSCN Ag	-	$R^{23}S$
f	-	KSCN Ag	-	P-52	6.	Antiserum against KSCN Ag	-	P-52
g	-	Son. Ag	-	R^9S,	7.	Antiserum against Son. Ag	-	R^9S
h	-	Son. Ag	-	$R^{23}S$,	8.	Antiserum against Son. Ag	-	$R^{23}S$
i	-	Son. Ag	-	P-52	9.	Antiserum against Son. Ag	-	P-52
j	-	Blank						

HICE Ag - Heat Inactivated crude extract antigen
KSCN Ag - Potassium thiocyanate extract antigen
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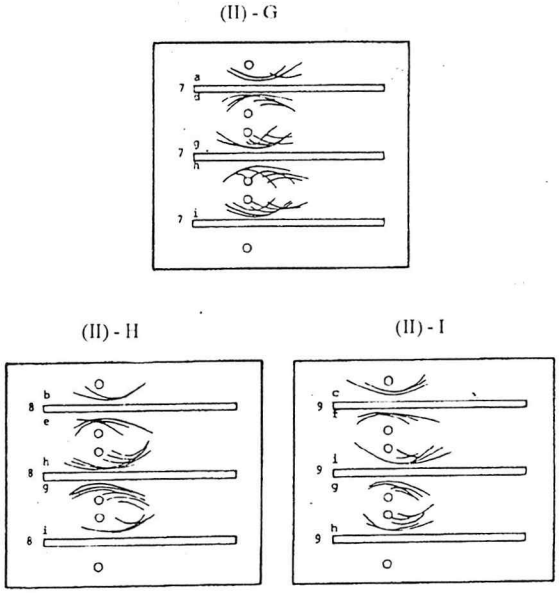
Fig. II A-I Diagramatic illustration of immunoelectrophorogram of different antigens of *P. multocida* from R_9S, $R_{23}S$ and P-52 strains



a	-	HICE Ag	-	R^9S,	1.	Antiserum against HICE Ag	- R^9S
b	-	HICE Ag	-	$R^{23}S$,	2.	Antiserum against HICE Ag	- $R^{23}S$
c	-	HICE Ag	-	P-52	3.	Antiserum against HICE Ag	- P-52
d	-	KSCN Ag	-	R^9S,	4.	Antiserum against KSCN Ag	- R^9S
e	-	KSCN Ag	-	$R^{23}S$,	5.	Antiserum against KSCN Ag	- $R^{23}S$
f	-	KSCN Ag	-	P-52	6.	Antiserum against KSCN Ag	- P-52
g	-	Son. Ag	-	R^9S,	7.	Antiserum against Son. Ag	- R^9S
h	-	Son. Ag	-	$R^{23}S$,	8.	Antiserum against Son. Ag	- $R^{23}S$
i	-	Son. Ag	-	P-52	9.	Antiserum against Son. Ag	- P-52
j	-	Blank					

HICE Ag - Heat Inactivated crude extract antigen
KSCN Ag - Potassium thiocyanate extract antigen
Son. Ag - Sonicated antigen

The antiserum was also subjected to IHA with homologous and heterologous antigens. The results presented a very high titre in the homologous antigens invariably with all the three forms of antigen. Among the three strains, the $R_{23}S$ gave highest antibody titres against the three forms of antigen. Though the KSCN extract and sonicated antigen showed same titre, the sonicated antigen preferably $R_{23}S$ gave good cross-titre indicating the superiority of this antigen for serological survey of *P. multocida* infection in rabbits.

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