Standardization and development of *Pasteurella multocida* inactivated adjuvanted vaccine against septic pasteurellosis in pigs

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In piggery, septic pasteurellosis caused by *Pasteurella multocida* (B:2) is an issue of concern, which needs an effective vaccine. Here, we prepared a double emulsified (DE) vaccine containing 2.5 mg inactivated antigenic mass of pig field strain (B:2) (named as soron) isolated from an outbreak of septicaemic death in pigs and *P. multocida* P_{52} cattle strain (B:2) and studied their efficiency in terms of immunity to direct challenge, duration of immunity and the role of humoral and cell-mediated immunity. Both of these strains showed presence of h*gbB*, p*fhA*, n*anH*, p*tfA*, and *tbPA* virulence genes. The sequence analysis of bands of 760 bp product using capsular primers were obtained for soron and P_{52} revealed 99.2% homology between these two strains, indicating differences at genetic level. *nanH* and *pfhA* genes of soron shared 99.2% and 92.7% homology with P_{52}, respectively suggesting differences between these two strains at genetic level. SDS-PAGE analysis of cell wall of both strains showed presence of about 15 major protein bands whereas Western blot analysis with 21 day soron immunized pig serum showed 16, 33, 47, 63 and 83 kDa polypeptides in both strains. The duration of immune responses were monitored at 3, 6 and 9 months post immunization in pigs. By direct challenge, pigs showed that the vaccines were protective at 21 days and up to 270th day post immunization. Vaccines induced a serum ELISA IgG response that peaked on 60 DPI which declined gradually up to 270th DPI in both vaccines. Stimulation index measured by lymphocyte proliferation test (LTT) indicated that the vaccine, induced cell-mediated immune response and in general percent stimulation index (SI) was higher in pigs immunized with soron vaccine at 15 days post challenge infection. The results showed that pig strain (soron) would be a potential homologous strain of *P. multocida* for the vaccine against pasteurellosis in place of use of cattle *P. multocida* P_{52} strain.

Keywords: Haemorrhagic septicaemia, Homologus Pig strain, Piggery, Soron, *Sus scrofa domesticus*, Swine

Pasteurella multocida is a small gram-negative coccobacillary organism having five sero-groups (A, B, D, E and F) and several serotypes. The organism is the causal agent of diseases in animals and bird species, generally called as "Pasteurellosis". Haemorrhagic septicaemia (HS) is a fatal disease of cattle and buffaloes caused by *P. multocida* (B:2) and it has also been suspected to cause HS in swine. Ghosh *et al.*[1] have shown that pigs are susceptible to HS infection by *P. multocida*, the major cause of pig mortality and sometimes outbreak in piggery. Several outbreaks of fatal *P. multocida* infections in pigs (sometimes with concurrent mortality in bovines and buffaloes and, with serologically or PCR diagnosed classical swine fever) have been reported in India and other pig-rearing countries during the last 30 years[2-4]. In Vietnam, the *P. multocida* isolates from concurrent outbreaks of HS in cattle, buffaloes and pigs were

found identical (ACIAR; Project ID AS2/1995/006). According to Roetzera *et al.*[5] vaccine development is aimed to prevent intensive infection or to control the disease from complications. Various types of *P. mutlcoida* vaccines and use of adjuvants have been reviewed[6]. Recently, the adjuvant effect of several bDNA was investigated on the humoral and cellular immune responses both induced by immunization of Balb/c mice with the iron-inactivated and formalin-inactivated vaccine, as well as the protection against murine experimental pasteurellosis after vaccination[7].

In India, cattle strain of *P. multocida* P_{52} has been used for production of vaccine against swine pasteurellosis. Still, there is a need for development of an effective vaccine against swine septic pasteurellosis (SSP). Here, we made an attempt to develop a vaccine containing the pig field isolate (named as soron) of *P. multocida* and studied its safety, protective efficacy and duration of immunity in pigs compared with vaccine prepared from cattle *P. multocida* P_{52} strain.

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Materials and Methods

Animals

All the experimental protocols carried out on laboratory animals and piglets were approved by the Institutional Animals Ethics Committee (IAEC) of Indian Veterinary Research Institute (IVRI), Izatnagar-243122 (India). The animal experimentation was regulated as per standard operating procedure (SOP) outlined by Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Animal Welfare Division, Ministry of Environment and Forests, Government of India. Laboratory mice and rabbits were procured from Laboratory Animal Resource (LAR), IVRI, Izatnagar.

Bacteria

P. multocida isolated from pig during an outbreak (soron) and cattle standard strain of *P. multocida* P_{52} both of these B:2 serotype strains were used. The outer membrane protein profiles of the two strains were analysed by SDS PAGE[8] and Western blot analysis[9].

Virulence and pathogenicity

Virulence of these two strains for sero-negative pigs was determined by challenge of with ~5000 viable organisms through subcutaneous route. Five virulence genes, namely *ptfA*, *tbpA*, *nanH*, *pfhA* and *hgbB* were amplified[10] (Table 1 & 2). The PCR products were sequenced and further analyzed.

PCR products were sequenced at a custom sequencing facility. Gene sequences were submitted to NCBI as follows: KY053264 (*pfhB2* P_{52} strain gene), KY053265 (haemagglutinin gene, soron strain), KY053266 (*ptfA* gene, P_{52} strains), KY053267 (*ptfA* gene soron strain), KY053268 (*nanH* P_{52} strain), KY053269 (*nanH* gene, soron strain) KY053270 (capsule gene, P_{52}) and KY053271 (capsule gene,

soron strain). Mice minimum lethal dose (MLD) of soron was determined in mice[11]. The mice LD_{50} of P_{52} used have already been reported[12].

Vaccine

The experimental vaccines of *P. multocida* containing soron and P_{52} strains were prepared as water in oil in water (W/O/W) emulsion vaccines. Formalin (0.5% V/V) inactivated dense bacterial suspensions of the respective strains were obtained by

Table 2 — PCR conditions for amplification of virulence genes

Target gene	Temperature	Time (min.)	Cycle	Remarks
ptfA	94°C	4.00	1	Denaturation
	94°C	0.30 s		Denaturation
	55°C	0.30 s		Annealing
	72°C	0.60 s		Extension
	72°C	9.00 s	1	Final Extension
tbpA	94°C	4.00	1	Denaturation
	94°C	0.30 s	30	Denaturation
	57°C	0.30 s		Annealing
	72°C	0.60 s		Extension
	72°C	9.00 s	1	Final Extension
nanH	94°C	4.00	1	Denaturation
	94°C	0.30 s	30	Denaturation
	58°C	0.30 s		Annealing
	72°C	0.60 s		Extension
	72°C	9.00 s	1	Final Extension
pfhA	94°C	4.00	1	Denaturation
	94°C	0.30 s	30	Denaturation
	55°C	0.30 s		Annealing
	72°C	0.90 s		Extension
	72°C	9.00 s	1	Final Extension
hgbB	94°C	4.00	1	Denaturation
	94°C	0.30 s	30	Denaturation
	54°C	0.30 s		Annealing
	72°C	0.45 s		Extension
	72°C	9.00 s	1	Final Extension

Table 1 — Primer sequences for virulence gene

Target Gene	Primer name	Sequence of Primer	Product size (bp)
ptfA	Ptf A Forward	5'-AGCTGATCAAGTGGTGAAC-3'	488
	Ptf A Reverse	5'-TGGTACATTGGTGAATGCTG-3'	
tbpA	tbPA Forward	5'-TGCGACAACGGAAATTTCCTC-3'	789
	tbP A Reverse	5'-GGACAGTGCATATAACTTGTTTACTA-3'	
nanH	nanH Forward	5'-CACTGCCTTATAGCCGTATTCC-3'	964
	nanH Reverse	5'-AGCACTGTTACCCGAACCC-3'	
pfhA	PfhA Forward	5'-TCCATACACTCGGTAATATG-3'	1464
	PfhA Reverse	5'-TCTAATGTACCAGGCAGATC-3'	
hgbB	Hgb B Forward	5'-ACCGCGTTGGAATTATGATTG-3'	788
	Hgb B Reverse	5'-CATTGAGTACGGCTTGACAT-3'	

agar wash. Each suspension was standardized to contain ~1.4 mg antigenic mass/mL so that the adjuvanted finished preparation had ~2.5 mg antigenic mass in 3 mL[13-14], the final dose of vaccines to be administered. A water-in-oil (single emulsion) preparation was first prepared using appropriate quantities of sterile light liquid paraffin and lanolin, which was then re-emulsified with appropriate quantity of formal saline containing 2% Tween 80[11].

The sterility of the vaccines was tested as per section 2.2.11 Indian Pharmacopoeia (I.P.)[15]. The safety test was conducted in mice by injecting 0.2 mL of the preparation through intraperitoneal route and the mice were observed for 5 days for any untoward reaction or mortality[15]. The potency test was performed in pigs. Three weeks (21 days) post immunization along with two healthy pigs negative to *P. multocida* antibodies were challenged subcutaneously (s.c.) in the neck with one mL of 50 million mouse minimum infective dose. Animals were observed for 7 days.

Eighteen (18) apparently healthy pigs negative to *P. multocida* antibodies divided into two groups *viz*: soron and P_{52}, 3 pigs each at were immunized with 3 mL of each vaccine as a single dose by the intramuscular route (i.m.). One mL of 50 million mouse minimum infective dose was used as a challenge inoculum given s.c. in the neck. For direct challenge test, all pigs were challenged infected subcutaneously (s.c.) with respective *P. mutltocida* strain with the inoculum described above at 21 days, 3 months, 6 months and 9 months post-immunization along with control pigs. Pigs unvaccinated with either of the vaccine soron or P_{52} and free of *Pasturella* antibodies were included along with the immunized pigs at the time of challenge infection. At 3 months, no controls were challenged due to unavailability of pigs. The number of pigs controls have been restrictive due to paucity of space during challenge at different occasions.

Immune responses

Pre-vaccination, pre-challenge and 15 days post-challenge blood samples from each pig were collected to measure humoral and cell-mediated immune responses. Single dilution technique[16] was used for determination of antibody titers in sera samples collected on various days after immunization. Anti *P. multocida* (soron) immune pig serum (15th day post-challenge) and pre-vaccination serum were used as positive and negative controls, respectively. A 1:8

dilution of sonicated antigens of soron and P_{52} were used as antigens. 1:800 dilution of anti-pig IgG conjugate was used as per instructions of the manufacturer (Bethyl Laboratories, USA).

Cell-mediated immune response (CMI) in pigs at various days post immunization up to 15 days post challenge was assessed as per method of Mosmann[17] using [3-(4,5-dimethylthiazole-2-yl) 2,5-diphenyl tetrazolium bromide) (MTT) and peripheral blood mononuclear cells (PMNC), and the Stimulation Index (SI) values were determined as under:

$$\text{S.I (MTT)} = \frac{\text{Mean OD of stimulated culture}}{\text{Mean OD of unstimulated culture}}$$

Statistical analysis

To study the difference between soron and P_{52} vaccines OD and PN values were determined. Independent samples 't' test was applied to determine the significance of difference in OD values of two vaccines.

Results

Animals

The pigs were free from classical swine fever virus screened by PCR Kit (IDEXX-Part No. 99-43220) and these were seronegative to *Pasteurella* antibodies.

Bacteria

The purity of cultures and their identification by conventional and molecular techniques have been reported by authors in their previous publication[15]. The P_{52} and soron strains were compared on the basis of cell wall and immunoreactive polypeptides by SDS PAGE and Western blot using hyperimmune rabbit and 15 day post-challenge pig sera against both strains. SDS PAGE profiles of sonicated extracts of the two strains did not reveal any difference (Fig. 1). Both strains showed presence of about 15 major protein bands with apparent molecular weights (MW) ranging 14-83 kDa. Based on staining intensity 16, 27, 33, 37, 47 and 83 kDa polypeptides were found prominent in both the strains. While in Western blot analysis, stain intensity revealed 16, 33 and 47 KDa proteins of soron and P_{52} strains (Fig. 2). MLD of soron was found as 0.2 mL subcutaneous injection of 10^{-8} dilution (approximately, 25 viable organisms). All subsequent challenge infections of pigs for potency determination of test vaccines were conducted with 50 million mice MLD of the two strains.
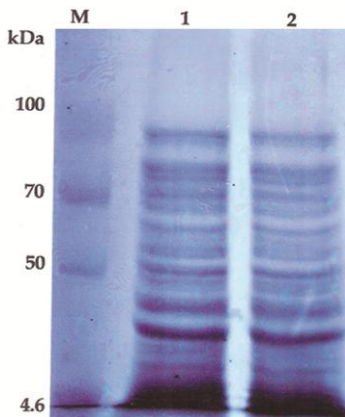
Fig. 1 — SDS-PAGE analysis of crude lysates of *P. multocida* of soron and P_{52}. [Lane M: Protein Marker Lane; 1. Soron strain; Lane 2. P_{52} strain]
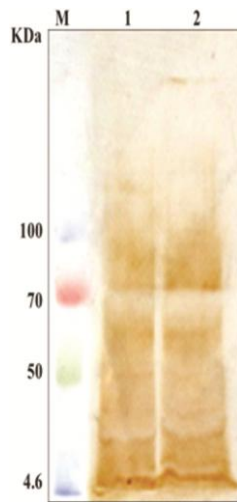

Fig. 2 — Western blot analysis of crude lysate of 21 days pooled serum. [Lane M: Protein Marker; Lane 1, Soron strain; and Lane 2, P_{52} strain]

Virulence and pathogenicity

Both P_{52} and soron strains produced a fatal septic shock in sero-negative piglets after subcutaneous administration of about ~5000 viable *P. multocida* organisms the death time of the two strains however differed. About 24-30 h after the challenge infection with soron, the piglet became off-fed and showed symptoms of restlessness, staggering gait, reddish-blue discolouration and slight swelling of neck area. After about 44 h, the animal became recumbent. Watery diarrahoea was seen for a brief period during the terminal stages and the animal died about 48 h after the challenge. No gross pathognomic changes could be visualized except accumulation of a small quantity of yellowish oedmatous fluid subcutaneously in the neck area. There was slight serous fluid
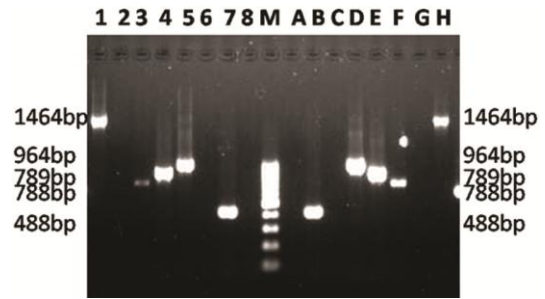

Fig. 3 — Virulent gene specific PCR of soron & P_{52}. [Lanes 1-8: represent soron strain; Lane A-H represents P_{52} strain; Lane M: Marker (1 K bp); Lane 1 & H: *pfhA* gene of 1464 bp of soron & P_{52}; Lane 2 & G; Negative Control; Lane 3 & F; *hgbB* gene of 788 bp of soron & P_{52}; Lane 4 & E: *tbpA* gene of 789 bp of soron & P_{52}; Lane 5 & D: *nanH* gene of 964 bp of soron & P_{52}; Lane 6 & C: Negative Control; Lane 7 & B: *ptfA* gene of 488 bp of soron & P_{52}; and Lane 8 & A: Negative control]

accumulation in the thoracic and abdominal cavities. No apparent lesion on any parenchymatous organ including the lungs was noted. The animal challenged with soron died at about 48 h whereas the animal inoculated with P_{52} succumbed after 64 h. Both animals showed similar clinical symptoms and postmortem lesions and *P. multocida* organisms were isolated in pure culture from the heart blood. Presence of *hgbB* and *tbpA* (iron-acquisition factors), *pfhA*, *ptfA* (filamentous haemagglutinin, porin protein), and *nanH* (neuraminidase) genes could be detected in both the strains as specific primers showed amplification of 788, 789, 1464, 488 and 964 bp bands, respectively in PCR (Fig. 3).

The nucleotide sequence of HS Specific PCR (590 nucleotide) was analyzed with *P. multocida* soron and P_{52} strains along with gene sequences of other *P. multocida* (accession numbers such as AF_016260.1, AJ_421513.1, AY_948545.1) using Megalign tool of DNAStar programme. For HS specific PCR amplification, soron and P_{52} IVRI had 99.2% sequence homology with all the available sequences of *P. multocida* (Fig. 4A). The virulent genes nanH (789 nucleotide), PfhA (788 nucleotide) and PtfA (964 nucleotide) were analyzed with *P. multocida* of soron and P_{52} strains gene sequences of other *P. multocida* virulent genes by using Megalign tool of DNAStar programme. Virulent genes of soron and P_{52} had 99.2% sequence homology with all the nanH genes of *P. multocida* strains, for PfhA 98.2% sequence homology with all the available genes of *P. multocida* strains and for ptfA 100% sequence homology with all the available sequences of *P. multocida* strains (Fig. 4 B & C).

Vaccine

The vaccines were sterile and safe when tested in mice as per I.P.[15] All immunized pigs along with non-immune (control) pigs were challenged infected at different intervals and percent survival is shown in table overall results showed 66% protection with both vaccines except 100% protected in case of both vaccine at 3 months (Table 3). At 90 days the results

of the independent samples 't' test indicated that there was no significant difference in OD values of soron and P_{52} vaccines ($P > 0.05$). However, the OD mean was higher for soron (0.21067) vaccine as compared to P_{52} vaccine (0.17711). The results at 180 days of challenge indicated significant difference in OD values of soron and P_{52} vaccines ($P < 0.05$). However the OD mean was higher for soron (0.17867) vaccine
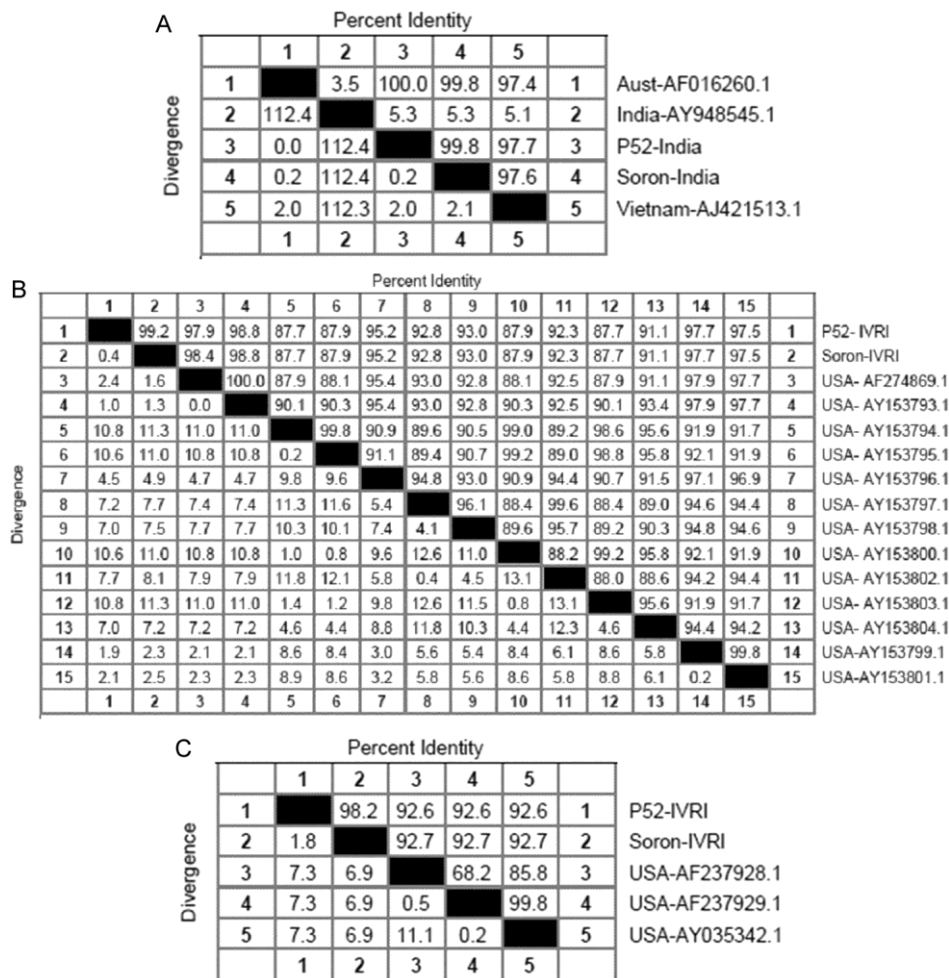

Fig. 4 — Nucleotide sequence alignment of (A) soron and $P_{52}HS$; (B) soron and P_{52} *nanH*; and (C) soron and P_{52} *PfhA* gene with other *P. multocida* strains

Table 3 — Protection in pigs immunized with soron or P_{52} vaccines and challenged with *P. multocida* soron or P_{52}

Total vaccinated animals	Dose of vaccine & route	Challenge dose & route	No. Survived/No. Challenged							
			Soron				P_{52}			
			21 days	90 days	180 days	270 days	21 days	90 days	180 days	270 days
24	3 mL, i.m.	1 mL, 50 mouse mld, s.c.	3/3	3/3	2/3	2/3	3/3	2/3	2/3	2/3
% Protection			100	100	66	66	100	66	66	66
Control (Non- immune) 06		1 mL, 50 mouse mld, s.c	0	0	0/1	0/1	0	0	0/1	0/1
% Protection			-	-	0	0	-	-	0	0

Table 4 — Analyses of ELISA OD values in pigs after challenge and vaccination

Days	Soron vaccine	P_{52} vaccine	Mean difference	t	P value
After vaccination					
60	0.15167	0.11800	0.03367	1.158	0.264
90	0.11822	0.13778	-0.01956	-0.745	0.467
170	0.13778	0.14156	-0.00378	-0.133	0.896
After challenge					
90	0.21067	0.17711	0.03356	1.267	0.223
180	0.17867	0.12956	0.04911	2.318	0.034
270	0.10422	0.15400	-0.04978	-1.310	0.209

Fig. 5 — Each bar in histogram represents results obtained from group of 3 pigs at different intervals

as compared to P_{52} vaccine (0.12956). The results at 270 days and challenged lastly analyzed that there was no significant difference in OD values of soron and P_{52} vaccines $(P <0.05)$. However the OD mean was higher for P_{52} (0.15400) vaccine as compared to P_{52} vaccine (0.10422) (Table 4).

Immune responses

A classical pattern of antibody response with initial rise over the pre-vaccination levels and achievement of a peak between 21st to 35th DPI, and then a gradual decline over the period up to 270 days was observed against both vaccines. Both vaccines (soron or P_{52}) induced cell mediated immunity in pigs. It was evident from results in general the perecent stimulation index in pigs immunized with sorona nd P_{52} vaccine was on higher side in case of pigs immunized by soron vaccine and 15 days post infection (Fig. 5).

Statistical analysis

The results of vaccination analysed at 60 days by the independent samples t test indicated that there was no significant difference in OD values of soron and P_{52} vaccines $(P >0.05)$. However, the OD mean was higher for soron (0.15167) vaccine as compared to P_{52} vaccine (0.11800). At 90 days of vaccination there was no significant difference in OD values of soron and P_{52} vaccines $(P >0.05)$. However, the OD mean was higher for P_{52} vaccine (0.13778) vaccine as compared to soron vaccine (0.11822). The results at 180 days analyzed indicated significant difference in OD values of soron and P_{52} vaccines $(P <0.05)$. However, the OD mean was higher for P_{52}vaccine (0.14156) vaccine as compared to soron vaccine (0.13778) (Table 4).

Discussion

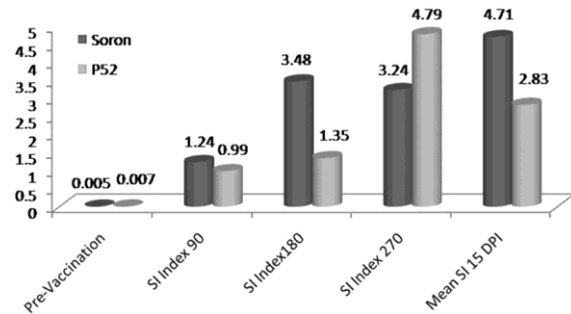It is pertinent to mention that both A and D are the major *P. multocida* serogroups detected in the respiratory tract of pigs worldwide. Serogroup A plays a more important role in the pathogenesis of pneumonia than serogroup D, while serogroup D produces several virulence factors (hsf-1 and nanB) and a dermonecrotic toxin[19]. Perusal of the literature on pig pasteurellosis reveal that serotype B:2 of the organism causes HS-like, systematic septic pasteurellosis in pigs inflicting heavy losses to the pig industry[2]. The first detection of type B:2 *P. multocida* in Hungary, isolated from cases of generalised pasteurellosis in backyard pigs, with phenotypic and genotypic characterisation of the isolates[20]. We also encountered septic pasteurellosis in pigs and isolated *P. multocida* B:2 named as soron. Currently, in India for the prevention of pig pasteurellosis and when needed, the vaccine made from cattle strain *P. mutlocida* P_{52} is being used in pigs. We, therefore, studied efficacy of water-in oil-in water adjuvanted vaccine against pig septic pasteurellosis in pig made up of a virulent *P. multocida* field isolate B:2 (soron) obtained from an outbreak of pig septic pasteurellosis.

Both soron and *P. multocida* P_{52} strains were analysed by SDS-PAGE analyses to look into their identity. Johnson *et al*[5] has reported a high degree of homogeneity between the protein profiles of 14 *P. multocida* strains associated with HS and could not differentiate isolates on the basis of SDS-PAGE. Similar results were have been reported by other workers on cell wall peptides of P_{52}. Several attempts have also been made to identify immunogenic proteins of HS-causing *P. multocida* employing Western blot analysis. In our investigation, 16, 33 and 43 kDa proteins could be identified as dominant immuonogens against pig sera showed presence of IgG titers. On the basis of immunoaffinity chromatography reported these three polypeptides as major immuonogens against which a host mounted predominant response[19-20]. The association of purified OMPs with induction of clinical protection against HS

has been widely investigated[21-22]. The general conclusion of all these attempt revealed that no single component of *P. multocida* is entirely responsible for protective immunity. All the cell wall fractions, LPS, proteins and polysaccharides contribute towards protection of animals.

With the capsular primers, bands of 760 bp was obtained for soron and P_{52} and the sequence analysis of this product revealed 99.2% homology between two strains, indicating differences at genetic level. The virulence genes in soron and P_{52} investigated by the PCR amplification showed *nanH* and *pfhA* genes of soron showed 99.2 and 92.7% homology with P_{52}, respectively which suggested differences at genetic level between these two strains. Till date, the roles of any of these identified virulence factor(s) remain yet to be elucidated with the clinical syndrome and the ultimate outcome of infection in bovines and pigs, which is an LPS-mediated fatal septic shock[23]. PCR amplification of the virulence genes may help in differential identification of *P. multocida* strains. *P. multocida* has been accepted as an extracellular pathogen. Protection against an extracellular pathogen is predominantly antibody mediated. Cell-mediated immune responses (CMI) plays only a minor role, if any[24]. The general agreement on the question of correlation of antibody titer with protection against challenge is that both are directly correlated[25]. But the lowest minimum antibody titer for protection is still not known for these species. This value is not only important from the point of view of determining the immune status of an animal, but may also help in developing an *in vitro* test procedure for potency evaluation of vaccines. The CMI response of immunized pigs, assessed using LTT assay showed that both the vaccines induced cell-mediated immune response. The role of CMI response for protection against *P. multocida* has been largely discounted initially[10]. However, several workers later reported involvement of both humoral and cell mediated responses in defense against the pathogen[25-26]. Our results indicated variation in SI index in soron and P_{52} (Fig. 1) which may be due to individual host response of pigs. For ruminants, it has been reported that antibody mediated immunity plays a pivotal role in suppression of infection in early stages, whereas, CMI is probably required for complete elimination of organism later[27]. The specific roles of humoral and cell mediated components in monogastric animals like pigs, need further investigations.

The vaccine made up from soron strain induced 66% comparable protection by direct challenge up to 9 months to that of cattle P_{52} strain. There was 100% protection at 3 months in pigs immunized with soron vaccine on challenge whereas 66% in case of P_{52} vaccine. Overall, we observed 66% protection in both vaccines at 6 and 9 months which is acceptable as per I.P.[15]. A study of cross reaction between two local Sudanese *P. multocida* strain B and E, the active cross protection test in rabbits showed that vaccine of strain E protected 50% of the rabbits against challenge with strain B, while the vaccine of B could not protect the rabbits against the challenge with E strain (0%). Each vaccine of B and E could protect the rabbits 100% against the challenge with the same strain (homologously)[27-28]. In our study, both soron and P_{52} were B:2 serotypes, thus, there was no question of cross-reactivity between these two strains of *P. multocida*, but the interest was to understand that soron strain was a better candidate against septic pig pasteurellosis so that this study could establish it as a novel strain for vaccine in pigs instead using cattle strain *P. multocida* P_{52}. Further, it has been held in a bacterial disease caused by *C. chauvoei* that among the relevant factors in the development of immunity, the most important is protection provided by vaccines made from strains that are homologous to those in the local environment[29]. Vaccination with homologous strains gives better protection against a homologous challenge than against a heterologous challenge, though some strains produce immunity with a wider spectrum of protection than do others[30]. Type A strains of *P. multocida* were investigated for identification of possible protective antigens in mouse model. It was stated that protective antigens might be only poorly expressed or not expressed at all on bacteria cultured in normal laboratory media. The expression *in vivo* might be significantly different from *in vitro*[31]. It would appear that the ideal situation would be introduction of regional field strains for the production of commercial products, along with improved epidemiological vigilance[32]. We could not subject soron and P_{52} strain to whole genome sequence. However, the results of soron and P_{52} did indicate differences like the pig challenged with soron died at about 45 h, whereas the animal inoculated with P_{52} succumbed after 64 h, thus it was also attributed a difference in virulence of soron as compared to P_{52}. Our results strengthen the view point

that vaccine made homologous strain of organisms to those in the local environment is the most important for protection[33].

Conclusion

These results demonstrate that *Pasteurella multocida* strain "soron" isolated from a septic pasteurellosis was found effective as a vaccine in pigs. The vaccine prepared from soron strain offered 66% comprable protection to cattle strain P_{52}. Soron strain was more virulent since pigs challenged with this strain died in 45 h whereas pigs challenged with cattle strain P_{52} sucumbed at 64 h. The study suggest *P. multocida* a field local (soron) B:2 would be potential vaccine candidate strain for pig septic pasteurellosis.

Conflict of interest

There is no conflict of interest.

Acknowledgement

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