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Haemorrhagic septicaemia in bovine of Balochistan, Pakistan

Zahoor Ahmed[1], Muhammad Kamran Taj*[1], Ferhat Abbas[1], Deedar Ahmed[1], Ghulam Mohammad[1], Imran Taj[1], Aziz Ullah[1], Sana Arif[2], Attiya Aslam[2], Mriam Bilal[2], Asia Ali[3]

[1]*Microbiology Department (CASVAB), University of Balochistan, Quetta, Balochistan, Pakistan*
[2]*Zoology Department Sardar Bahadur Khan Women's University, Quetta, Balochistan, Pakistan.*
[3]*Computer Science Department Sardar Bahadur Khan Women's University, Quetta, Balochistan Pakistan.*

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Abstract

Total 200 blood samples were collected in which 76% were *Pasteurella multocida* positive and 24% were negative. While district wise results showed that Jaffer Abad, Nassir Abad and Jhal Magssi were the most effected regions of Balochistan. However age wise distributed in bovine was 22% in nine months, 35.5% in one year and 18.5% in two years. The incidence of Haemorrhagic septicaemia was more in buffalo (54%) as compared to cattle (22%). The pH and temperature is the most important factor which effects the growth of *Pasteurella multocida*. Our result revealed that *Pasteurella multocida* grows from 25°C to 45°C while pH results showed growth from 6 to 8 pH. The antibiotic result showed that *Pasteurella multocida* was sensitive to most of classes of antibiotic which were used in study whereas Metronidazole class showed multi drug resistance against *Pasteurella multocida*. In lab animal trial fallowing sign such as nasal discharge and laziness were observed in 24 hr while postmortem result revealed that trachea, lung and heart were hemorrhagic.

***Corresponding Author:** Muhammad Kamran Taj ✉ kamrancasvab@yahoo.com

Introduction

Pasteurella species are small, gram-negative bacilli that colonize mucous membranes of wild and domestic animals but are usually absent from the normal flora in humans (Ataei *et al.,* 2009). Most species can act as primary or opportunistic pathogens in their hosts and are responsible for significant losses to livestock (Blackall *et al.,* 2000).

Human infections are chiefly associated with some form of animal contact and occur predominantly following inflicted injuries (Unchitti *et al.,* 1992). This microorganism is pathogenic for a wide variety of mammals and birds (Ekundayo *et al.,* 2008). *Pasteurella multocida* cause Haemorrhagic septicaemia is cattle and buffalo (Kwon and king, 2003). Haemorrhagic septicaemia is one of the most common economically significant animal diseases occurring in both developed and developing countries (Jesse *et al.,* 2013).

This disease is particularly endemic in Asia and Africa but outbreaks have also occurred in Europe and North America (Rimler *et al.,* 2000). Pakistan has 31.8 million cattle population and a population of 29.0 million buffalo heads (Munir *et al.,* 2007). Haemorrhagic septicaemia is the most common and notable infectious disease of dairy animals in Pakistan, having mortality rate of about 70% and is causing several hundred million dollars annual losses to animal production (May *et al.,* 2001). The highest prevalence of Haemorrhagic septicaemia HS was recorded in Khanewal district and highest disease importance was recorded in Faisalabad district of Punjab (Farooq *et al.,* 2011). *Pasteurella multocida* is extensively involved in respiratory syndrome of commercial dairy farms all over the world particularly in countries having hot humid environment (Waheedullah *et al.,* 2009).

P. multocida is extensively involved in respiratory syndrome of bovine all over the world. In Pakistan it is the most important economic disease of livestock that has caused huge economic losses in Pakistan. Haemorrhagic septicaemia is the most common and foremost infectious disease of dairy animals (cattle and buffalo) in Balochistan, having high mortality rate and is causing several hundred million dollars annual losses to animal production. So therefore isolation of *Pasteurella multocida* from commercial dairy farm of Balochistan will significantly help scientists of Balochistan to control and develop good vaccine against Haemorrhagic septicaemia.

Materials and methods

Study area and samples collection

The samples were collected from 10 districts of Balochistan (Jaffer Abad, Nassir Abad, Jhal Magsi, Sohbatpur, Sibi, Kachhi, Lehri, Harnai, Barkhan, and Quetta. The research was conducted in center for advanced studies in vaccinology and biotechnology (CASVAB) university of Balochistan. Five milliliter blood was collected aseptically from the jugular vein of each diseased cattle and buffalo showing typical signs of Haemorrhagic septicaemia (clinically positive cases) and also from animals died due to Haemorrhagic septicaemia (Varte *et al.,* 2014).

Isolation and identification

The isolation of organism was done from blood on brain heart infusion blood agar (BHIBA) and tryptose yeast extract agar. The isolates were identified on the basis of cultural, morphological and biochemical characteristics (Cheesbrough *et al.,* 2006).

Antimicrobial susceptibility test

Mueller hinton agar using Disc Diffusion Bauer technique and Mc. Farland turbidity (0.5) was used for standardized antibiotic sensitivity test were performed on standard method following clinical and laboratory standards institute (CLSI, 2008) protocol. Isolates organism were considered as sensitive, and resistant to a particular antimicrobial agent on the basis of inhibitory zone (Sugun *et al.,* 2013).

Pathogenicity of isolated bacteria in mice

0.5 ml growth suspension of isolated having 1×10^9 CFU/mL was *subcutaneously* injected in to the mice. The dead mice postmortem was carried out to observe localized lesion on different part of body and observed disparate organs (Sanchez *et al.*, 2004).

Result

Total 200 blood samples were collected in which 76% were *Pasteurella multocida* positive and 24% were negative as shown in Fig. 1. The district wise result reveled that, 14% samples were positive from Jaffer Abad, 13.5% were from Nassir Abad, 13% were from Jhal Magsi, 13% were from Sohbatpur, 8.5% were from Sibi, 07% were from Kachhi, 04% were from Lehri, 1.5% were from Harnie, 01% were from Barkhan, and 0.5% were from Quetta, as shown in Fig. 2. The age wise distributed was 15% in nine months 19.5% in one year and 11% in two years in buffalo while 07% in nine months, 16%. In one year and 7.5% in two years in cattle as shown in Fig. 3.

Results about species wise incidence of Haemorrhagic septicaemia exposed that the incidence of Haemorrhagic septicaemia in buffalo was 54% and in cattle was 22%. The buffalo of all districts were more effect as shown in Fig. 4.

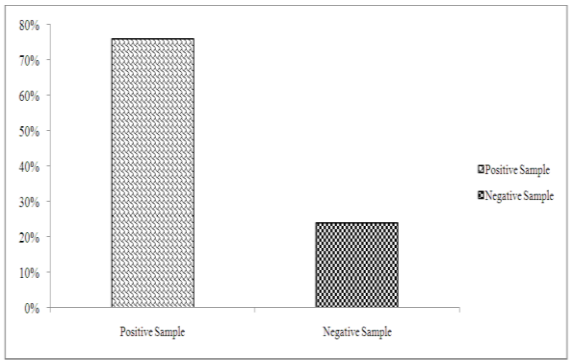

Fig. 1. Overall percentages of positive and negative samples of *Pasteurella multocida*.

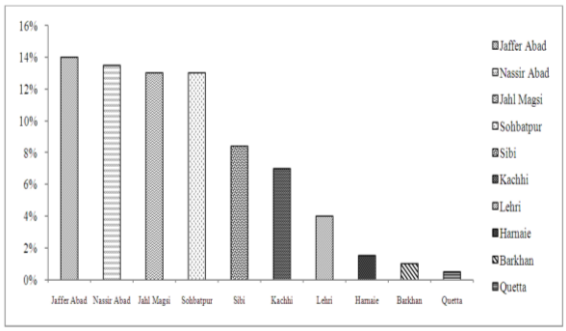

Fig. 2. *Pasteurella multocida* positive samples from different districts of Balochistan.

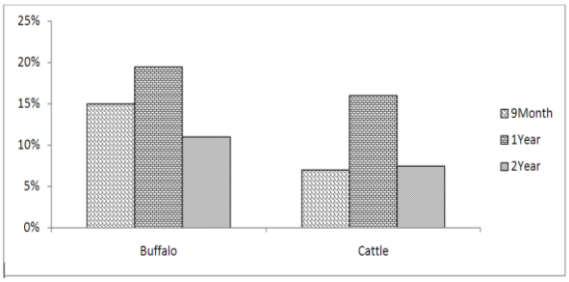

Fig. 3. Positive samples age wise from different districts of Balochistan.

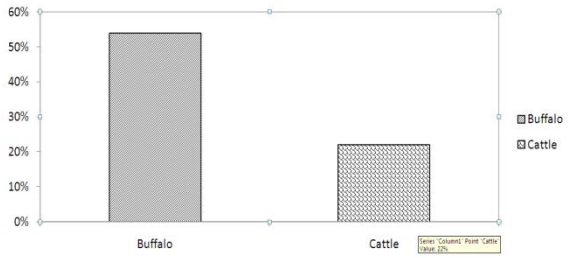

Fig. 4. *Pasteurella multocida* in cattle and buffalo from different districts of Balochistan.

Temperature and pH effect on the growth of Pasteurella multocida

Temperature and ph effect on the growth of *Pasteurella multocida*. The pH and temperature is one of the most important environmental factors that affect many aspects of the biological system of *Pasteurella multocida*. The *Pasteurella multocida* grows from 37ºC to 45ºC while pH result showed growth from 6 - 8 pH as shown in Table 1.

Table 1. Growth of *Pasteurella multocida* on different temperature and pH.

Temperature °C	
0	-
4	-
10	-
15	-
20	+
25	+
30	+
35	+
37	+
40	+
45	+
50	-
pH	
2	-
3	-
4	-
5	-
6	+
7	+
8	+

Biochemical tests

Pasteurella multocida was identified by gram staining and through different biochemical tests (IMIVIC, sugar fermentation tests, catalase test, oxidase test, gelatin liquefaction test, and H_2S production tests) as shown in Table 2.

Table 2. Biochemical tests and sugar fermentation test for confirmation of pathogens.

Pasteurella multocida																
Gram Staining Shape	Gram Negative Bacteria Coccobacilli															
Biochemical Test																
Hemolysis	Growth On MacConkey's Medium	Simmon Citrate	Gelatin Hydrolysis	Methyl Red	Voges-Proskauer	Motility Test	Urease Test	Lysine Decarboxylase	Lysine Decarboxylas	Growth On Potassium Cyanide	Hydrogen Sulfide	Nitrate Reduction	Indole Production	Ornithine Decarboxylas	Catalase Test	Oxidase Test
-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
Sugar Fermentation Tests																
Glucose	Fructose	Galactose	Mannose	Sucrose	Maltose	Trehalose	Arabinose	Xylose	Mannitol	Sorbitol	Inositol	Dulcitol	Salicin	Lactose		
+	+	+	+	+	+	+	+	+	+	+	-	-	-	-		

Antimicrobial test

Twenty-three antibiotics has been used against *Pasteurella multocida* and result showed that *Pasteurella multocida* was sensitive to classes Penicillin, Tetracycline, Quinolones, Cephalosporin, and Aminoglycosides, while only resistance to class Metronidazole as shown in the Table 3.

Table 3. *Antibiotic* resistance and sensitivity test against *Pasteurella multocida*.

	Class	Antibiotics	*Pasteurella multocida* Zone of inhibition (mm)
I.	Penicillin	Penicillin-G	(11mm)
		Amoxycillin	(17mm)
		Ampicillin	(16mm)
		Cloxacillin	(08mm)
I.	Tetracycline	Oxytetracycline	(22mm)
I.	Quinolones	Ciprofloxacin	(18mm)
		Oflaxcin	(16mm)
		Rifampicin	(16mm)
		Lincomycin	(08mm)
		Bacitracin	(07mm)
		Enrofloxacin	(20mm)
V.	Cephalosporin	Cepharadin	14mm)
		Cefepime	(12mm)
		Cephalexin	(14mm)
V.	Aminoglycosides	Kanamycin	(15mm)
		Gentamycin	(16mm)
		Amikacin	(07mm)
I.	Maerolides	Erythromycin	(09mm)
I.	Glycopeptides	Vancomycin	(12mm)
I.	Polypeptide	Colistin Sulphate	(09mm)
X.	Flagyl	Metronidazole	Resistant
X.	Miscellaneous	Chloramphenicol	(19mm)
		Sulphamethoxazole	(12mm)
		Trimethoprim	(12mm)

Pathogenic effect in mice

The *Pasteurella multocida* was inoculated in to mice, with direct intra peritoneal injection of the purified bacterial species. After inoculation of pathogens in to mice was observed to checked different clinical symptoms of mice. In 24 hours nasal discharge, laziness, and reluctant to movement was noted in inoculated mice. After 24 hours the inoculated animal was noted as dead in cage. The postmortem of mice showed hemorrhagic lesion on trachea, heart, lungs and kidneys as shown in Fig. 5.

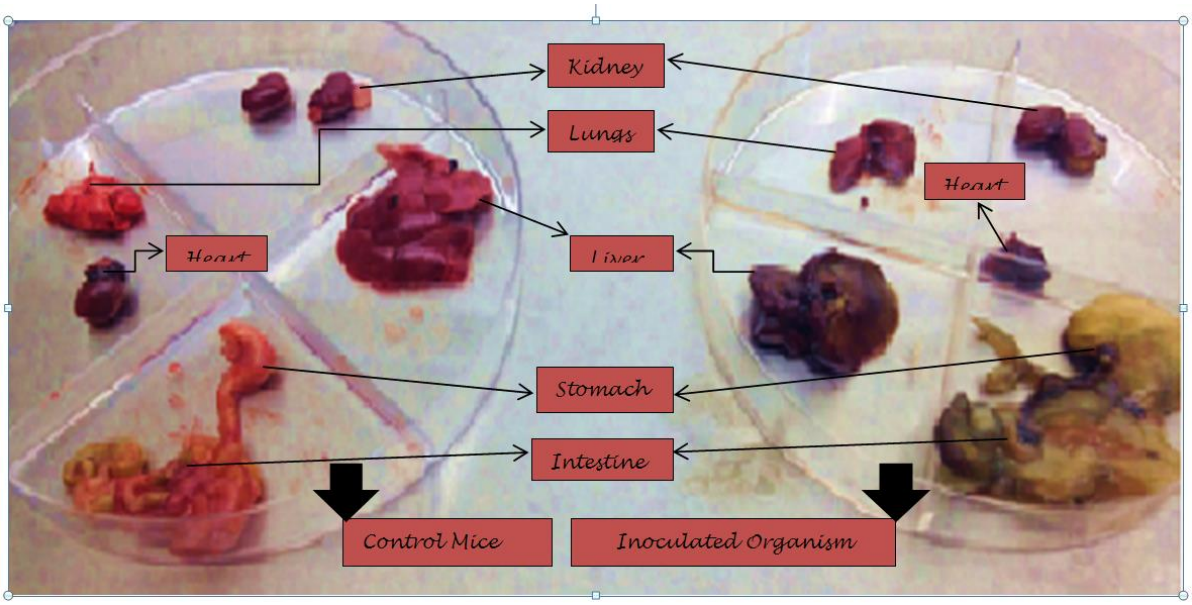

Fig. 5. Pathogenicity of *Pasteurellamultocida* in mice.

Discussion

Total 200 samples were collected in which 76% were positive and 24% were negative for *Pasteurella multocida*. Districts wise results showed that Jaffer Abad, Nassir Abad and Jhal Magssi, were the most effected regions of balochistan while Harnai, Barkhan and Quetta, were less effect region of balochistan for haemorrhagic septicaemia. The incidence of Haemorrhagic septicaemia in buffalo was 54% and in cattle was 22%. Buffalo was the most effect specie then cattle in all ten districts of the province. The bovine of 1 year (35.5%) was more affected as compared with other age groups.

The effect of different range temperature and pH on *Pasteurella multocida* has been characterized. The result showed that the *Pasteurella multocida* yield was highly depended on temperature low temperature and high temperature didn't allow *Pasteurella multocida* to grow on media. While the tremendous growth of *Pasteurella multocida* was recorded at 35°C to 37°C where as poor growth was observed at 20°C to 25°C.

pH of media was also found affecting *Pasteurella multocida* survival. Our result demonstrate that apart from ideal values of pH been demonstrated the growth of *Pasteurella multocida* in maximal at various other values of pH. Our result showed that the best pH for the growth of *Pasteurella multocida* was from 6 to 8 pH. The antibiogram results revealed that the best choice of drugs for the control of *Pasteurella multocida* was Tetracycline. In animal trial nasal discharge and hemorrhagic lesion were observed at heart, liver and kidney.

Conclusion

Haemorrhagic septicaemia is an important disease in the tropical area of balochistan Pakistan. This disease is most devastating to smallholder farmers where husbandry and preventive practices are poor and free-range management is common. So proper management & vaccine will provides protection to the bovine of the region.

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