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The effect of environmental changes on distribution of *Pasteurella multocida* in marsh buffaloes in south of Iraq.

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Abstract

The study was conducted to investigate the distribution of *Pasteurella multocida* in marsh buffaloes in south of Iraq and effect of environmental changes on distribution of *Pasteurella multocida*. A total 393 buffaloes of different ages and sexes, from May/2017 to April/2018, were clinically examined. Enrich and Selective medias ,biochemical test and Gram stain were used. All positive cases confirmed by polymerase chain reaction with specific primer to *P. multocida*. Statistically the distribution of *P. multocida* in infected buffalos (28%) were significantly higher than non-infected (14%).some buffalos with the clinical sings of hemorrhagic septicemia (HS). While other buffaloes showed clinical finding of Bovine respiratory disease (BRD) and other appeared normally. The isolation recorded in male 26% and in female 25%The number of infected buffaloes under one year of age(32.8%) showed significantly higher than 1-2 year (19.6%) and adult animal(18.9%). The distribution rate were 25.3% , 24.4 %,26.4% in Basrah. Dhi Qar, Misan respectively but there were no significantly variation between Provinces .Also The lower percentage of isolation in October /2017 (9%). and highest in February /2018 (37%), Significant correlations between distribution rates and mean of temperature at ($P \leq 0.01$) level but its non-significantly correlations between distribution rates and mean of humidity. We describe the *P.multocida* endemic in marsh buffaloes in south of Iraq, under 1 year old are more prone to *P. multocida*. There were a significant negative correlations between distribution rates and mean of temperatures

Key words; *Pasteurella multocida*, Buffaloes, environmental changes. Iraq

i. INTRODUCTION

Pasteurella multocida, a Gram negative pathogen bacteria and it can produce many type of diseases to many species of animals. hemorrhagic septicemia are important economic disease caused by *Pasteurella multocida* because it fatal to buffaloes in many cases, *Pasteurella multocida* also causes Bovine Respiratory Diseases in buffaloes but less than hemorrhagic septicemia and it is also etiological agent pneumonia in ovine and caprine also *Pasteurella multocida* causes fowl cholera in poultry , ([1;2]).

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Pasteurella multocida is found as a normal flora in respiratory tract but under stress or other infection like para influenza virus infection ,this will lead to enhance *Pasteurella multocida* infection so *Pasteurellosis* outbreak mostly occur in raining season ([3, 4])

Clinical finding of hemorrhagic septicemia (HS) which caused by *P.multocida* appear as an increase of temperature, difficult in respiration ,at same time serious then become mucoid discharge from nasal orifices , mouth frothing, and submandibular edema was observe, then after hours. animal will be recumbence then death in less than 7hours. While the infection with Bovine Respiratory Diseases: mostly include Respiratory disease (rhinitis to pneumonia.) and may death in some cases and it called Bovine Respiratory Diseases .(BRD).([5]).

The young buffalo and calves at age (6 Months to 2 years) most Susceptibility to *P.multocida* ([2])

Pasteurella multocida can be isolated from necropsy samples directly after death, like blood, lungs spleen or liver, also can be isolated from bone marrow after days of dead animals. In living animals blood or mucus from the nostrils or swabbing from the nasal cleft can be used for culturing ([6]).

In many Asian countries, outbreaks mostly occur during seasons of high humidity and high temperatures ([7,8]).

In Iraq, *Pasteurella multocida* causes many outbreaks and infections , thus there were many studies on cattle and buffaloes like ([8]) in marshes in south of iraq, ([9]) in Basrah ; and ([3,10,11]) in Baghdad , our country needed more studies required to investigation *Pasteurella multocida* distribution and the genotypes that cause the disease in buffaloes

Aims of study :

1. Investigate the presence of *P.multocida* in buffaloes in marshes in south of Iraq.
2. Effect of environmental changes on distribution of *P.multocida*

ii. MATERIAL AND METHODS

Animals of study and sample collection

The survey study to buffaloes in Marshes of south of Iraq in Thi-Qar, Basra and Misan provinces 393 buffaloes were examined clinically from marshes area ,different in age and sex ,between May/2017 to April/2018

Table (1) locations of taken samples from buffaloes in Marshes area according to Provinces

Provinces		Location	Samples	North line	East line
Basrah 130 Samples	1	AlMdina	34	30.979587	47.208785
	2	Alhower	24	30.966508	47.315374
	3	Showarea	37	30.724765	46.819286
	4	Ghabishia&Nigamshi	35	30.700430	46.856965
DhiQar 127	5	Ghibash	35	30.973575	47.137087

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Samples	**6**	**Al Tar**	**34**	**30.935454**	**46.747348**
	7	**AlAmni**	**20**	**30.776862**	**46.656428**
	8	**Suq Al Shoukh\ Um Al Wadea**	**38**	**30.836915**	**46.564967**
Misan 136 Samples	**9**	**AlOmara/Al Misbaneah**	**36**	**31.862985**	**47.212388**
	10	**Al Musharah**	**32**	**31.854155**	**47.368365**
	11	**Al Salam**	**35**	**31.476647**	**46.887415**
	12	**Al Adil**	**33**	**31.512855**	**46954867**

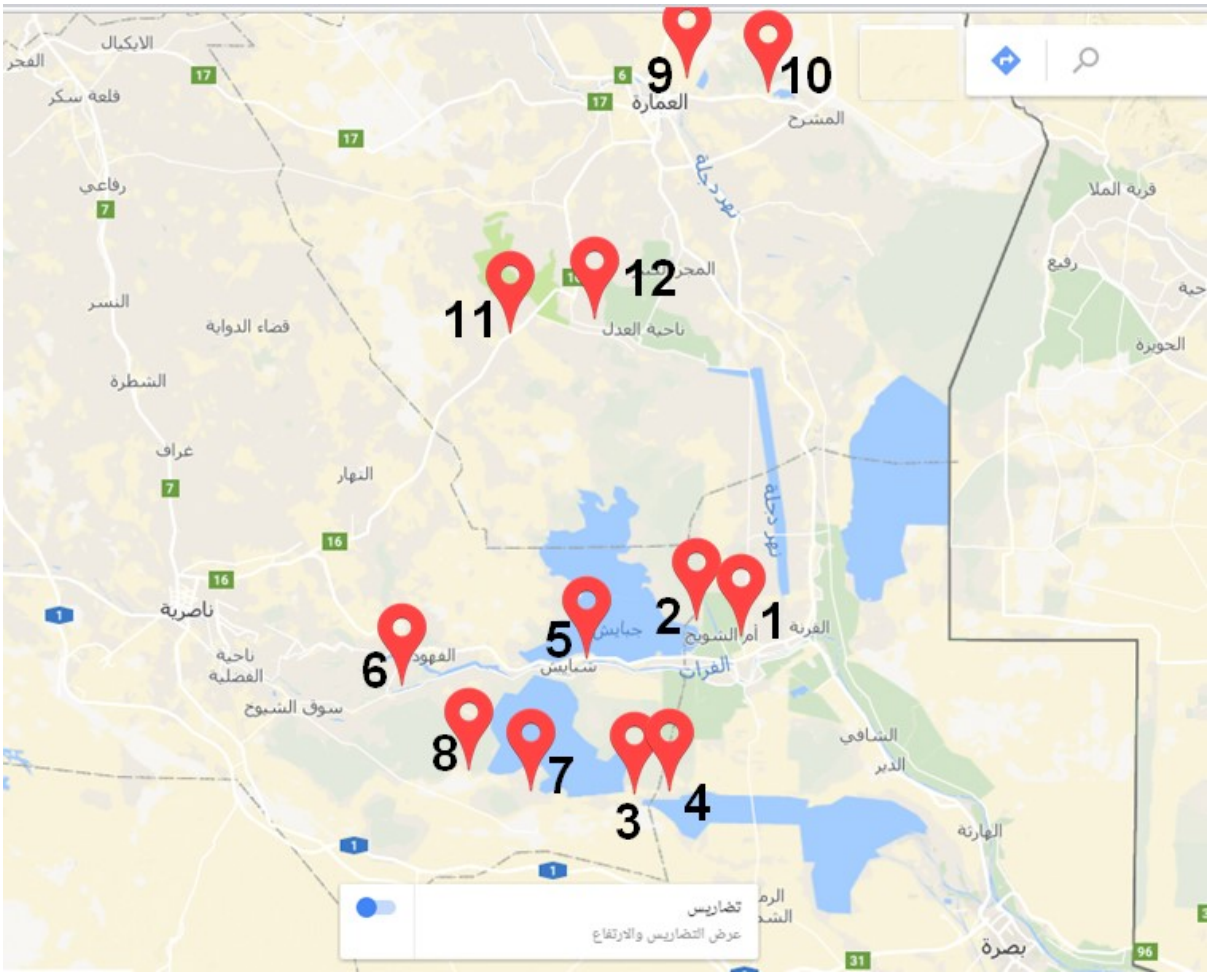

Figure. (1) Showed locations of taken samples from buffaloes in marshes area according to Provinces (Google earth)

Survey study

Nasal swabs were taken from each one

Culture and staining and biochemical

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Identification of *P. multocida by* following media (Brain heart infusion broth (BHI), Blood Agar, MacConkey Agar) ,

Gram stain and biochemical test catalase test ,oxidase , indole, urase ,triple sugar iron,

([6])

Conventional PCR study

Conventional PCR assay was used for detection of *Pasteurella multocida* by amplificated of universal primers ([12])

The experiment was carried out in the clinical pathology Laboratory of Internal and Preventive Medicine department in College of Veterinary Medicine-University of Baghdad

Table (2) PCR primer for detection of Pasteurella multocida

Primer	Sequence		PCR Amplicon
KMT-1	F	GCTGTAAACGAACTCGCCC	460bp
	R	ATCCGCTATTTACCCAGTGG	

Statistical analysis:

Statistical analysis were done by Chi-square test between parameters , and correlation between parameters ([13])

iii. THE RESULT AND DISCUSSION

- **The of *Pasteurella multocida* Distribution in Marshes**

distribution rate of *Pasteurella multocida* which was isolated from diseased buffaloes were(34%)and it was significantly higher at (P≤0.05) than non-Diseased buffaloes (14%) .

Tab. (3) Distribution rate of *Pasteurella multocida* in buffaloes according to clinical sings

Clinically	**Samples**	**Positive**	**Distribution rate %**

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Diseased	194	66	34*
Non Diseased	199	34	14*
Total	393	100	25.4

*Means a significant variation at (P≤0.05)

This result give to *Pasteurella multocida* an important cause of respiratory diseases as a primary pathogens or it may be play a secondary role in the pathogenesis of various diseases ([4]) .

Therefore, there are many studies were give same results on cattle and buffaloes as, ([14]) in Iran. which reported that more than 95% of buffaloes and 40% of the total number of cows were died because of *Pasteurella multocida* , and ([8]) in marshes in south of Iraq who recorded and isolated the causative agent of outbreak of HS in buffaloes during 2008. While ([7]) recorded distribution of (HS) in Sargodha division, Punjab province were isolated from healthy carrier (2%) and diseased buffaloes (4%) .Also ([3]) in Baghdad revealed (38.88%) isolations of *Pasteurella multocida* which were (60 %) from cows and (30.76%) from buffalo.

- **Clinical signs**

The clinical signs of affected buffaloes with Pasteurellosis were .Anorexia , elevated body temperature, pneumonia resulting associated with coughing and rapid breathing , depression and restlessness. respiratory distress with nasal discharge, and frothing from the mouth with throat edema in some cases

Inspiratory dyspnea was seen, subsequent to respiratory distress, due to upper respiratory tract obstruction following throat edema. Death of affected animals may be seen or occur due to asphyxia , Other than the release of pharmacologically active substances, there is another school of thought in the development and consequences of HS that the *Pasteurella multocida* produces endotoxins which are responsible for all manifestations ([15]). Death of the animals occurs due to hypoxia and toxemia ([16]).

pneumonic pasteurellosis or BRD is caused by more than one species of Pasteurella and serotype. On the other hand Pasteurella play a secondary act in disease process, and more than one of viruses have been convicted with BRD. The disease is different syndromes that caused under different conditions, but Pasteurella have important role (primary or secondary) to cause (BRD) complex. ([4]) Such clinical findings of hemorrhagic septicemia were recorded by many authors such as ([17]). In West Bengal, and ([18])

- **The colony *Pasteurella multocida* on blood agar appeared as two forms**

First colonies were roughly and small discrete tear shaped colonies. When the another colonies were mucoid and large colonies. also no haemolysis on the blood agar ,and No growth on MacConKey agar

Staining by Gram stain appeared as Gram negative, coccobacilli or short-rod singly or in pairs

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The identification of *Pasteurella multocida* isolates was done according to biochemical tests of (oxidase +ve), (catalase +ve) , (indol +ve) where (urea -ve) and (Triple sugar iron result)

Tab. (4)Showed the Biochemical tests and its results for *P. multocida*

No	Biochemical test	Result
1	Urea test	-ve
2	TSI Agar	slant color (yellow) bottom (yellow) gas (-ve) H2S (-ve)
3	Indole test	+ve
4	Oxidase test	+ve
5	Catalase test	+ve

These findings were agreement with more than one authors like ([8,3]) whom found same characters to *P. multocida*

- **Polymerase chain reaction assay.**

All positive isolation were confirmed as *P.multocida* by the PCR method and they all match with species-specific 460 bp with the KMT1T7 and KMT1SP6 primers. In PCR assay, amplification was observed from all the isolates , applied to confirm the serotypes of *P.multocida.* This primers discovering by ([12])

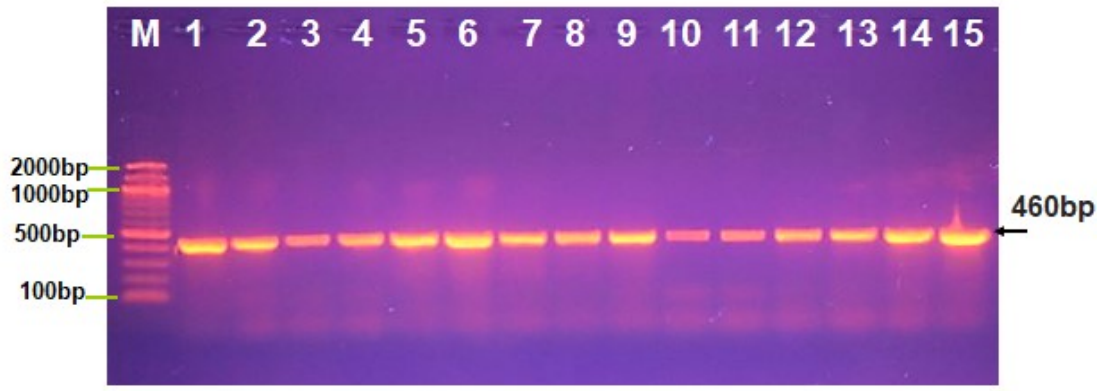

Figure (2): Agarose gel electrophoresis image that showed the PCR product analysis of diagnostic gene in *Pasteurella multocida* isolates. Where M: marker (2000-100bp), lane (1-15) positive isolates at (460bp) PCR product size.

These findings confirmed the results obtained by ([10,19,20]), whom reported approximately 460 bp amplified product from all *P.multocida* isolates.

- **The distribution according to sex .**

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As well as the infection recorded in male 26% were higher but non-significant higher than female 25 % ,Tab. (5);

Tab. (5) distribution of Pasteurella multocida in buffaloes according to sex

sex	Sample	Positive	Distribution %
Female	202	51	25
Male	191	49	26
Total	393	100	25.4

This result is in agreement with .([9]) and disagreed with ([10]) who recorded the percentage of female cases was higher than in male and may be due to that stress factors like pregnancy, milking and calving occurred in female

- **The distribution according to age**

The number of infected buffaloes under one year of age(27.1%) was significant higher than 1-2 year (16.1%) and adult animal(16.1%) at (P≤0.05) Tab. (6);

Tab. (6) distribution of Pasteurella multocida in buffaloes according to Age

Age (year)	Sample	positive	Distribution(%)
Under 1	180	59	32.8%
1-2	102	20	19.6%
Above 2	111	21	18.9%
Total	393	100	25.4%

*Means a significant variation at (P≤0.05)

Radostits *et al .,* (2007)([21]). mentioned that all age groups are susceptible to infection, but in endemic regions, older animals previously exposed to the pathogen may have antibodies providing some protection. In these regions the most susceptible age group is 6 months to 2 years of age. Colostral immunity of calves from cows vaccinated against hemorrhagic septicemia peaks at 8 to 16 weeks of age and then declines, also there is no difference in susceptibility between breeds

This result is in agreement with ([22,23])

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- **The distribution according to Provinces**

The Distribution rate were 25.3% , 24.4 %,26.4% in Basrah. Dhi Qar, Misan respectively but there were no significant variation between Provinces.

Tab. (7) distribution of Pasteurella multocida in buffaloes according to Provinces

Provinces	Samples	Positive	Distribution %
Basrah	130	33	25,3
Dhi Qar	127	31	24.4
Misan	136	36	26.4
Total	393	100	25.4

The Mesopotamian marshes, consist of three large marsh complexes: Al-Hawizeh, Central, and Al-Hammar in southern Iraq (29°55'00"N to 32°45'00"N and 45°25'00"E to 48°30'00"E) are the biggest wetlands in Iraq and one of most important natural aquatic systems in region of Middle East ,also , one of the largest centers of buffaloes concentration in Iraq, up to 60% of buffaloes herd. Water supply to the marshes has fluctuated with the discharge of the Tigris and the Euphrates Rivers through the centuries and with the ability of Iraqi rulers to control water distribution, given the area's unique geology and seasonality . ([24]).

So these marshes have same environment therefore there were no significant variation between provinces.

- **Result of distribution according to time**

The distribution of *Pasteurella multocida* infection, in clinical cases, through the months of the study, reported the highest number of infection during February /2018 (37%), and decline in October /2017(9%) Tab. (8) ;

Tab. (8) distribution of Pasteurella multocida in buffaloes according to month

Year	Month	Location	samples	positive	Distribution %

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2017	May/	AlAmni	20	7	33%
	June	Alhower	24	7	29%
	July	AlMdina	34	8	24%
	August	Um Al Wadea	38	9	24%
	September	Al Musharah	32	7	22%
	October	Al Tar	34	3	9%*
	November	Al Misbaneah	36	5	14%
	December	Ghabishia	35	7	20%
2018	January	Showarea	37	11	30%
	February	Al Salam	35	13	37%*
	March	Ghibash	35	12	34%
	April	Al Adil	33	11	33%
	Total		393	100	25.4%

*Means a significant variation at (P≤0.05)

- **Correlations between Distribution rates and environmental changes**

There were significant correlations between distribution rates and Mean of temperatures significant at the 0.01 level (2-tailed). But its non-significant Correlations between Distribution rates and Mean of Temperatures & Humidity at Tab. (9). Tab. (10)

Tab. (9) Showed Correlations between Monthly distribution of *Pasteurella multocida* and environmental changes

Year	Month	Distribution rates %	Mean of Temperatures	Mean of Humidity
2017	May/	33	32.3	27

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	June	29	36.6	16
	July	24	39.2	18
	August	24	39.7	22
	September	22	34	24
	October	9	30	29
	November	14	20	38
	December	20	15.2	42
2018	January	30	12.5	63
	February	37	14.4	55
	March	34	19.5	37
	April	33	25.8	32

Tab. (10)Correlations between Distribution , Temperatures & Humidity

	Correlation	**Distribution**	**Temperatures**	**Humidity**
Distribution	Pearson Correlation	1	-.931-**	.251
Temperatures	Pearson Correlation	-.931-**	1	-.186-
Humidity	Pearson Correlation	.251	-.186-	1

**Means a significant variation at (P≤0.01)

Although clinical disease can occur at any time of the year, close herding and wet conditions clearly contribute to the spread of the disease. Outbreaks of the disease are often associated with wet, humid weather during the rainy season. Stressors such as inadequate feed supply or exhaustion are considered important predisposing factors that not only increase the susceptibility to clinical disease, but also stimulate shedding of the bacterium from subclinically infected animals . ([4,11])

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Iraq one of countries outside tropical region ,there for the rainy season in winter only . so there are correlations between distribution rates and mean of temperatures.

This result agreement with ([10]) and ([25]) ,but these finding was disagreement with the studies of India HS occur most commonly in the month of June- September (rainy season).The organism (*Pasteurella multocida*) does not survive outside the animal to any significant degree so as to be a source of infection. Moist condition prolong its survival outside the animal making an outbreak more likely. Thus the disease tends to spread more during the wet season ([26])

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