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Full Article

Isolation of pasteurella multocida from cows and buffaloes in Urmia's Slaughter House

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ABSTRACT

Pasteurellosis is one of the important economic diseases in ruminants, especially in cows and buffaloes. It is caused by Pasteurella multocida and occasionally by Mannheimia haemolytica. The aim of this study was to isolate Pasteurella multocida from lungs with probable mentioned bacterial agents in cows and buffaloes in Urmia's slaughter house. 240 lung samples over a period of 12 months were cultured. The results have revealed 6 (2.5 %) Pasteurella multocida results suggest that the animal, its breed, sex, age and season can be effective in the occurrence of these positive cases. The positive samples were all from male beef cattle of hybrid breeds (4 samples) in winter and Holstein breeds (2 samples) in spring.

Keywords: *Pasteurella, Lung, Cow, Buffalo, Culture*

INTRODUCTION

Pasteurellosis is among the important contagious diseases in the world. As this disease has many similarities with respiratory diseases of cattle and sheep, scant attention is being paid to it. Mastitis and septicemia caused by Pasteurella is among the important diseases, which in case of a mistaken diagnosis can cause a high rate of mortality in cattle (Araghy Soure 2007). Since Pasteurellae, particularly *Pasteurella multocida* and *Mannheimia haemolytica*, are part of natural flora of the buccal-pharyngeal region, therefore in the animals which are under stress, like the ones that are being transferred, have respiratory infections, have bad nutrition and ventilation, and are being kept in overcrowded places, bacterial growth and proliferation occur in the region and later gets extended to the lower respiratory tract and causes pasteurellosis (Araghy Soure 2007, Shayegy 2007, Mustafa *et al* 1978). Within the Asia region, countries can be classified into three categories, based on incidence and distribution of the disease. These are countries where the disease is endemic or sporadic, clinically suspected but not confirmed, or free (Benkirane & De Alwis 2002). Outbreaks have been noted to occur at the beginning of the rainy season, or just before the lush season. However, in the endemic area, the disease occurs throughout the year (Mshknany Sadighi 1999). *Pasteurella multocida* is an

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endemic disease in most countries of Africa, central and South America, in addition to some countries in Europe such as: Portugal, Greece, Romania, Spain, Belarus, Russia, Estonia, Latvia, Ukraine, and Poland and Asia in the Iran such as West Azarbaijan, Mazandaran, Gilan, Khozestan (Tabatabaei & Firouzi 2002, Benkirane & De Alwis 2002, Borkowska-Opacka & Kedrak 2003). Within the Asia region, countries can be classified into three categories, based on incidence and distribution of the disease. These are countries where the disease is endemic or sporadic, clinically suspected but not confirmed, or free (Benkirane & De Alwis 2002). It was so serious that it was reported in other provinces of Iran such as Gilan, Mazandaran, West Azarbaijan, Fars during the Veterinary conference of the veterinary mangers of provinces in January 1937. This disease was frequent in West Azarbaijan. It was first reported from West Azarbaijan in 1933. It was reported that more than 95% of buffaloes and 40% of the total number of cows were died because of this disease. The next research was conducted in Urmia in 1994 (Mohammadi *et al* 2006). Dr. Tabatabai and Blorchiniand were reported a case of bovine mastitis caused by *Pasteurella multocida* pursuant papilloma surgery in 1979 (Songer & Post 2005). For this reason, recognizing such animals in a developing country like Iran is of even greater importance. In this study, based on the samples taken from lungs with pneumonia, the rate of infection with Pasteurella has been determined in Urmia.

MATERIALS AND METHODS

Animals. The species, sex, breed, and the live weight of the Cows and buffalos were recorded before slaughter. According to table (Modified & Roe 1982) the possibility that the expected prevalence of 10% and level of confidence 99% and Desired absolute precision 5% of 239 samples which is attempting to collect from 240 samples in 4 seasons to some equal (Modified & Roe 1982).

Specimens. After slaughter, using a sterile scalpel, samples were taken from suspicious lungs. The samples were transferred to microbiology laboratory in special ice-filled containers. As the organism is sensitive, the samples were delivered to the laboratory not later than 5-6 hours after sampling.

Bacteriological examination. The samples were cultured on blood agar, incubated in 37 °C for 48 hours. There were also two control cultures, one with *Pasteurella multocida* American Type Culture Collection (ATCC) 11039 and the other with *Mannheimia haemolytica* American Type Culture Collection (ATCC) 55518. Suspicious colonies from the initial cultures were brought in blood agar by conventional streaking techniques and were incubated in 37 °C for 48 hour **(**Figure 1).

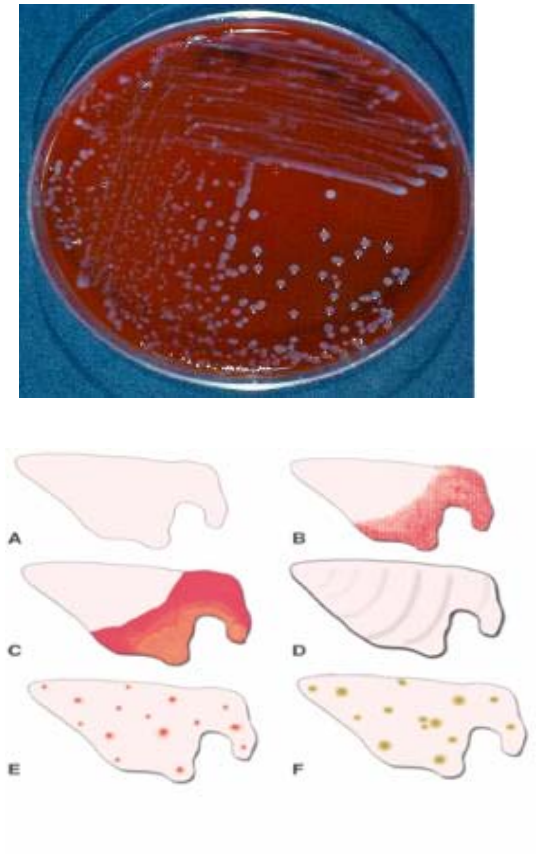

Figure 1. Culture and pathologic evaluation of Pasteurella from upper respiratory**.**

In these pure cultures, *Pasteurella multocida* and *Mannheimia haemolytica* colonies could be studied

more easily. In this stage, MacConkey agar medium was also used. The colonies in both culture media were studied for their shape, size, color, and hemolysis capacities. The colonies were also gram-stained. Oxidase and catalase tests were also applied to the colonies. TSI, SIM, Urea Agar, Litmus Milk, and some other culture media containing sugars were also used (under the same 37 °C and 48 hours incubation conditions).

Statistical estimation of results. The results were analyzed statistically using chi-square and Fishers exact tests with confidence level 95%. (Collier *et al* 1998, Mustafa *et al* 1978, Zahra Salehi & Shaig 2007, Shayegy 2007).

RESULTS

A total of 240 buffalo and cattle lungs (78 from male cattle, 52 from female cattle, 64 from male buffaloes, and 46 from female buffaloes) were studied during a 12-month period (figure 2). Following microbiology tests, 6 samples were *Pasteurella multocida*-positive. The samples were all from male beef cattle of hybrid breeds (4 samples) in winter and Holstein breeds (2 samples) in spring. 4 of the positive samples were from the samples taken in the winter and 2 from the samples taken in the spring (Table 1).

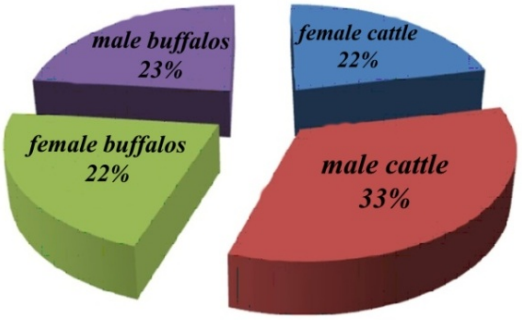

Figure 2. shows the dispersal of some male cattles and female buffaloes.

Among the cows studied we saw the most frequency in male cows and female buffaloes and the most frequency in female buffaloes. In this study, most flock cattles and buffaloes were limited to 1 year old and 2 year olds . male cattle and buffaloes were more frequent than female cattle and buffaloes.

Table 1. Shows the dispersal of some cattles and buffaloes isolated because of their lung diseases during the 4 seasons.

season	Breed	Sex	samples	Positive and negative	Total sample
spring	cattle	female	9	-	60
		Male	21	2+	
	buffalo	female	14	-	
		Male	16	-	
summer	cattle	Female	13	-	60
		Male	20	-	
	buffalo	Female	13	-	
		Male	14	-	
fall	cattle	Female	19	-	60
		Male	15	-	
	buffalo	Female	6	-	
		Male	20	-	
winter	cattle	Female	11	-	60
		Male	22	4+	
	buffalo	Female	13	-	
		Male	14	-	

DISCUSSION

Isolation of pasteurella multocida from cows and buffaloes has been the subject of studies, particularly in South- Eastern Asia. The theory of infection of susceptible hosts subsequent to the dissemination of the resting pathogens from the respiratory tract of convalescents, as well as non-clinical carriers, together with the rigours of nature, has been given wide acceptance (Kumar *et al* 2004).

Vergol et al (2001) and nakaya et al reported that Pasteurella multocida was most frequent (32% and 64.3% respectively) isolated organism from infected lungs .Whereas Sedeek et al (2001) isolated Pasteurella multocida with less freguent (8.3%) from nasal cavity of infected cattles. Said organism did not isolated from the same cattle's lung . During the study of Magwood et al (1969) on bacterial flora of nasal cavity in healthy and infected to pneumonia cattles did not find any relationship between nasal cavity flora and pneumonia prevalence. Also Araghy Soure et al,

because of limited sampling just from lung, did not isolate Pasteurella multocida and mannheimia haemolytica from healthy look cattle but the previous genus of Pasteurella (actinobacillus) is isolated from a bull cattle with pus discharge from nasal cavity and high fever (38.1) In past time the said organism was isolated only from human and usually from respiratory tract of healthy people. However, there are some reports about prevalence of septicemia and meningitis in result of this organism in human (Araghy Soure 2007). It is noticeable that Bar Bar et al (1997) reported that this organism is one of the organisms that isolated with low frequent from cattle lung and is one of the potential pathogens of respiratory disease complex. In study of Sadighi Meshkati (1995) on pathologic evaluation of pasteurellosis on cattle in Urmia, 100 cattle during the 8 months, 3 Pasteurella multocida and 1 mannheimia haemolytica positive sample were isolated .In plus in study of Mohamadi et al (2005) in Mashhad region on isolation and recognition of Pasteurella from upper respiratory tract of healthy and infected cattle to respiratory disease, they found that the prevalence of Pasteurella multocida and mannheimia haemolytica is higher in infected cattle(Araghy Soure2007).

Allen et al (1983) show that clinical signs, macroscopic injuries and bacteriological founding of shipping fever were in result of Pasteurella multocida and mannheimia haemolytica . Also Rio Johnson (1999) in experimental method show shipping fever was in result of mannheimia haemolytica type 1 with Mycoplasma bovis.

Fern et al (1977) did the same statistical investigation to evaluate the relationship of nasal cavity fluid antibody with the intensity of lung injury. They did not found any significant relationship. But they found a positive relationship between intensity of lung injury and serum antibody in vaccinated cattles (Mohammadi *et al* 2006).

Mannheimia haemolytica, is the most frequent isolated organism from lung in cattle with pneumonia. And Pasteurella multocida were found in respiratory disease simultaneously with other bacterial and viral disease (Mohammadi *et al* 2006). It is stabilized in experimental study that however the organisms regularly are transferring from their local site in nasopharynx to lung via air flow, but in a healthy animal, with this high exposure to nasal flora and contaminated air, the lung keeps sterile with defensive mechanisms (Irisk 2007). The both of the Pasteurella multocida and mannheimia haemolytica are the flora but only Pasteurella multocida could be isolated from nasal samples and mannheimia haemolytica rarely could be isolated from cattle and buffalo nasal samples (Irisk 2007).

In present study Pasteurella multocida was the major isolated bacteria from lungs. Pasteurella multocida are main isolated bacterial cause of the pneumonia in young cows. So it can be a reflex of opportunist content of this organism in secondary invasion. This organism has high reproduction following the initial invasion of other opportunists like mannheimia haemolytica in injured respiratory system. The synergistic relationship of mycoplasma and mannheimia haemolytica is reported (Irisk 2007). Some of scientists e.g. Ferry Hoskins 1993 reported that additional to Pasteurella some other bacteria are attend in disease. Webster and Smith confirmed the result of the Hoskins and isolated staphylococcus aurous from nasal cavity of a rabbit with pasteurellosis. But Pasteurella multocida and mannheimia haemolytica are the main cause of pasteurellosis and other bacteria are just common with Pasteurella in nasal cavity (Jafar Gahzar 1993). In confirmation of this topic Hoskins shows that Pasteurella before and after disease are existing in nasal cavity (Quinn *et al* 2002). In study of Saini, Sharma and De Alwis (DeAlwis 1992), they demonstrate that pasteurellosis can be occurred in any season and it has highest occurrence in winter and it is because of environmental stresses like cold weather and high concentration .The results of Rio Johnson (2001) study shows that the illness index and respiration rate could be a useful scale for bacterial

prediction .But isolation of bacteria could make the results unclear. Because the hepatization of lung in not followed by mannheimia haemolytica isolation. Also bacterial infection plus to macroscopic injuries are followed by other signs like signs of reparatory disease due to leakage of endotoxins and pyrogens (Mohammadi *et al* 2006).

Pasteurellosis in cattle and buffalo is not in the center of veterinary attention in Urmia's Slaughter House and it is, however, a potential source of economic loss. According to the results, it can be concluded that, due to a weaker immune system in lower ages, it is higher in cattle than in buffalo and, due to the overcrowded and stressful keeping conditions, it is higher in cold seasons. And, since all positive samples from cattle show *Pasteurella multocida*, it is probably higher than *Mannheimia haemolytica*. The 6 *Pasteurella multocida*-positive samples in this study, account for a probable rate of 2.5% in Urmia.

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