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Case Report

Gangrenous mastitis with Hemolactia of local buffalo breed (A case report)

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Abstract

A five year local buffalo breed was brought to the Consultant Veterinary Teaching Hospital, College of Veterinary Medicine, University of Basrah, Iraq, with a patients history of swelling, pain and redness of the udder with coldness, blue black coloration, fluid exudation, and crepitating were detected on one quarter area. Moreover, edematous fluid was extended to the belly of the diseased buffalo. In addition, milk of the diseased buffalo were watery in its consistency with light red or deep pink color. Gangrenous mastitis was clinically diagnosed on the basis of clinical features caused by *Proteus mirabilis* which isolated on blood agar with its specific swarm forming character and confirmed by the VITEK system diagnosis on the basis of laboratory features. It has been concluded that the microorganism *Proteus mirabilis* could cause gangrenous mastitis in the local buffalo breed. Therefore, it needs more attention for prophylactic measure.

Keywords: Gangrenous mastitis, local buffalo breed, *Proteus mirabilis,* Iraq

INTRODUCTION

Parturition related to mammary gland pathologies in the domestic buffalo appeared to be common according to animal physiologic condition around the time of parturition, such as, lower milk production and lower cisternal storage of secreted milk. Moreover, according to anatomical feature, different pathologic conditions of the udder were also mentioned, such as longer teat, thicker streak canal which are always different in cattle and buffalo. Furthermore, Hemolactia, udder edema and hypogalactia might also occur in the cows and buffaloes at same circumstances, the time of parturition (Constable et al., 2017; Islam et al., 2008).

Farmers of dairy products are dearly methodizing the veterinary practitioners for the treatment of lactating cows or buffaloes producing milk which had some abnormal coloration such as reddish or pinkish due to the presence of blood since farmers suffer from the economic loss because bloody milk is often rejected by the consumers (Reddy et al., 2016). Although, The condition is usually sporadic in occurrence but rarely, several lactating animals may be affected at a time (George et al.., 2008).

There are several causes of blood in the milk as hemorrhage occurs via slinking, meaning passage of erythrocytes through capillary walls into the tissues in which the RBCs present in the alveolar cells of the mammary gland occurs more frequently after the time of calving. However, slinking may also occur at any stage during the lactation (Fagiolo and Lai, 2007). When the number of RBCs in the milk is numerous, it might give the milk a pinkish color, besides will have red cream and sediment. On the contrary, when the number of RBCs in the milk is low, milk might only have a slight reddish or pinkish tinge (Reddy et al., 2016). Several infectious agents, including those caused by some bacteria microorganism such as (*Brevibacterium eryth-rogenes,* leptospira spp, *Micrococcus cerasinus, Serratia*

marcescens, Micrococcus chromidrogenes rubber, Micrococcus roseus, Sarcina rubra Lactorubefaciens gruber, etc.), Although, some viruses and some species of yeast such as red yeast (*Monascus purpureus*) may cause systemic infections associated with intravascular hemolysis and capillary damage in udder leading to reddish or pinkish discoloration of milk (George et al.., 2008). On the other hand, trauma to udder and teat is one of the common causes of blood in milk due to hemorrhage. Though, the udder secretion will become more brownish without tendency to clot especially when hemorrhage originated from a major vein of the udder as the cause of bloody milk (Sarker et al., 20015).

Gangrenous mastitis is developed when the severe acute inflammation affect the udder with an obvious clinical manifestations of heat, redness, swelling and pain. However, progression with necrosis of the affected udder, coldness of the area, blue black coloration, fluid exudation, and crepitating were also detected (Islam et al., 2008; Pal et al., 2011). In addition, treatment of gangrenous mastitis is very difficult and possibly will be done through amputation of the gangrenous quarter(s) only to save the patient's life despite of doubtful prognosis (Fagiolo and Lai, 2007; Atyabi et al., 2006).

The present article was done to describe a case of local buffalo breed suffering from gangrenous mastitis at Basrah, Iraq with laboratory diagnosis and clinical management.

MATERIALS AND METHODS

Patient history

A five year local buffalo breed was brought to the Consultant Veterinary Teaching Hospital, College of Veterinary Medicine, University of Basrah, Iraq, with a patients history of swelling, pain and redness of the udder with coldness, blue black coloration, fluid exudation, and crepitating was detected on one quarter area. Moreover, Edematous fluid was extended to the belly of the diseased buffalo. In addition, milk of the diseased buffalo were watery in its consistency with light red or deep pink color.

Microbiological findings

Milk sample was collected under aseptic conditions for bacteriological examinations and culture.

Gram's staining

Smears taken from the collected milk sample were stained with Gram's stain.

Culture media

Nutrient agar, blood agar, Staphylococcus Media, MacConkey agar, Eosin

Methylene Blue (EMB) agar, *Salmonella-Shigella* (SS) agar media and different sugar media were included for culture in the suspected milk sample since the sample was divided and inoculated separately in nutrient agar and blood agar to promote growth of the bacteria. Each group of these media was incubated at optimal temperature for overnight. The colonies were subcultured to obtain pure cultures with homogenous colonies. Characteristics, discrete colonies on the agar surface were observed. Their shape, size, consistency and color were studied. Gram stained slides of the isolates were examined microscopically to study their morphology.

Biochemical tests

VITEK 2 systems (Biomerrieux /France) which uses Advanced Colorimetry™**,** was used in this study to confirm the diagnosis of the isolated bacteria.

Collection of blood

The blood sample was collected from the jugular vein for a complete blood analysis (On an automatic full digital cell counter, Beckman, USA).

RESULTS

Diseased buffalo show signs of acute inflammation manifested with heat, redness, swelling and pain of the diseased udder. However, with coldness, blue black coloration, fluid exudation, and crepitating was detected on one quarter area. Moreover, edematous fluid was extended to the belly of the diseased buffalo (Figure 1 and 2). Milk of the diseased buffalo was watery in its consistency with light red or deep pink color (Figure 3).

Clinically, a systemic (vital) signs of high body temperature (40.5 C) with increase respiration (42/min) and heart rate (110/min) were detected on the diseased buffalo. Moreover, ruminal contractions were low (1/5 min) Table 1. Furthermore, increase total leukocyte count (14.56×10^3) with nutrophelia (62%) was revealed on (an automatic full digital cell counter, Beckman, USA), complete blood analysis Table 2.

Moreover, microbiological findings of the cultured media with confirmation of *VITEK 2 systems* result confirm that the causative organism belonged to *Proteus*

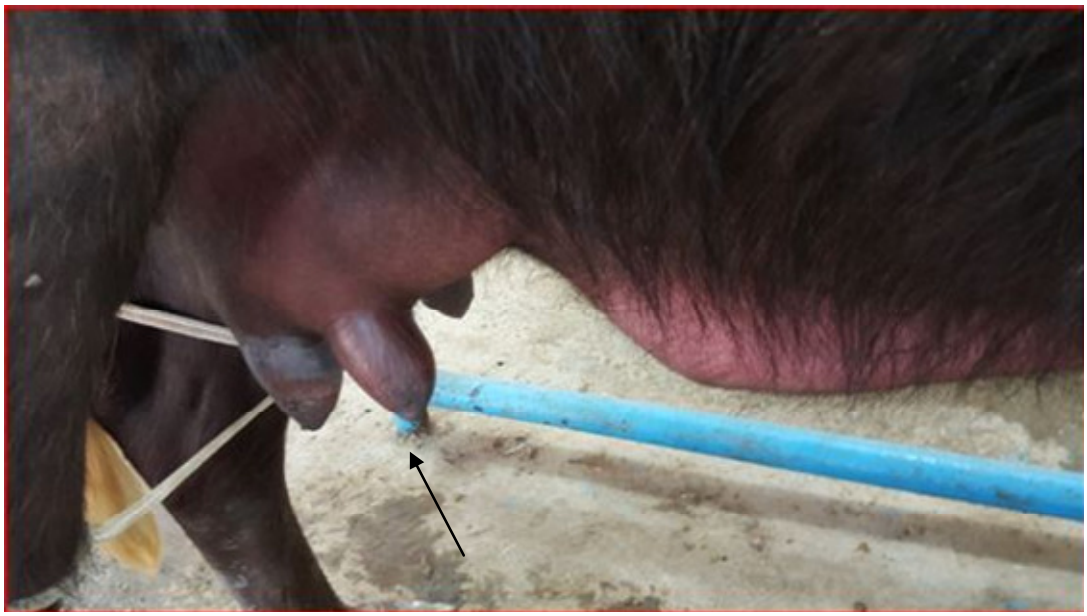


Figure 1. Diseased udder of buffalo show acute inflammation with redness and swelling, with coldness, blue black coloration, on one quarter (Arrow)

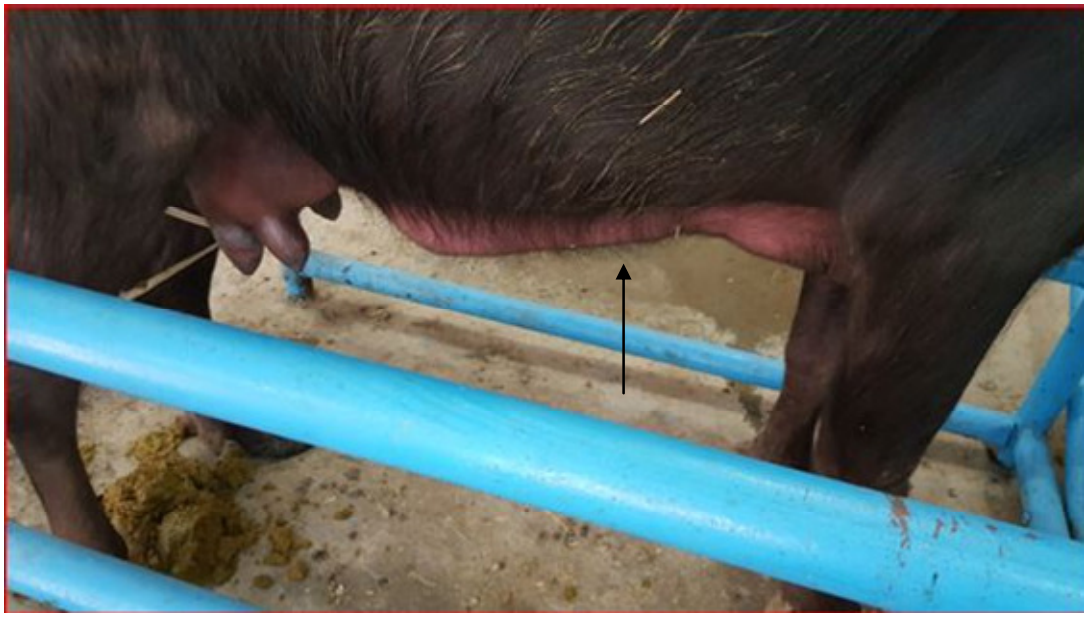


Figure 2. Edematous fluid (Arrow) was extended to the belly of the diseased buffalo.

Figure 3. Milk of the diseased buffalo is watery in its consistency with light red or deep pink color

Table 1. The Systemic (vital) signs of the diseased buffalo

Parameters	Diseased buffalo	Normal reference*
Body temperature C	40.5	37.8-39.2
Heart rate / min	110	55-80
Respiratory rate / min	42	10-20
Ruminal contractions / 5 min	1	3-5

**Normal reference (Smith, 2009)*

Table 2. Complete blood analysis of diseased buffalo

Parameters	Diseased buffalo	Normal reference*
TRBCs x 10^6	8.33	8.67
Hb g/dl	12.4	11.91
PCV	33	34
TLC x 10^3	14.56	11.34 *
Nutrophiles %	62	49 *
Lymphocytes %	33	44
Monocytes %	4	5
Eosinophiles %	1	2
Basophiles %	0	0
Total platelets count x10^3	408	412

**Normal reference (Smith, 2009)*

mirabilis. Furthermore, the gram stain from the milk smear showed a clear Gram-negative rods Figure 4.

Moreover, swarm forming character was also indicated of the blood smear culture of the causative *Proteus mirabilis* Figure 5

Furthermore, result of the VITEK 2 systems show that the final diagnosis of the biochemical result belongs to *Proteus mirabilis* Figure 6.

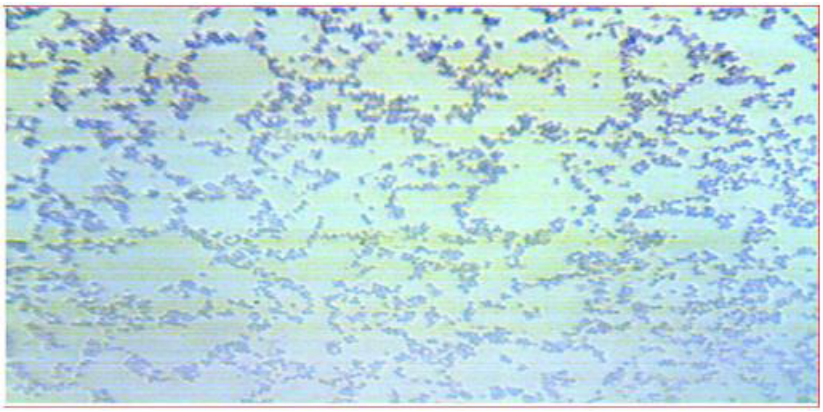
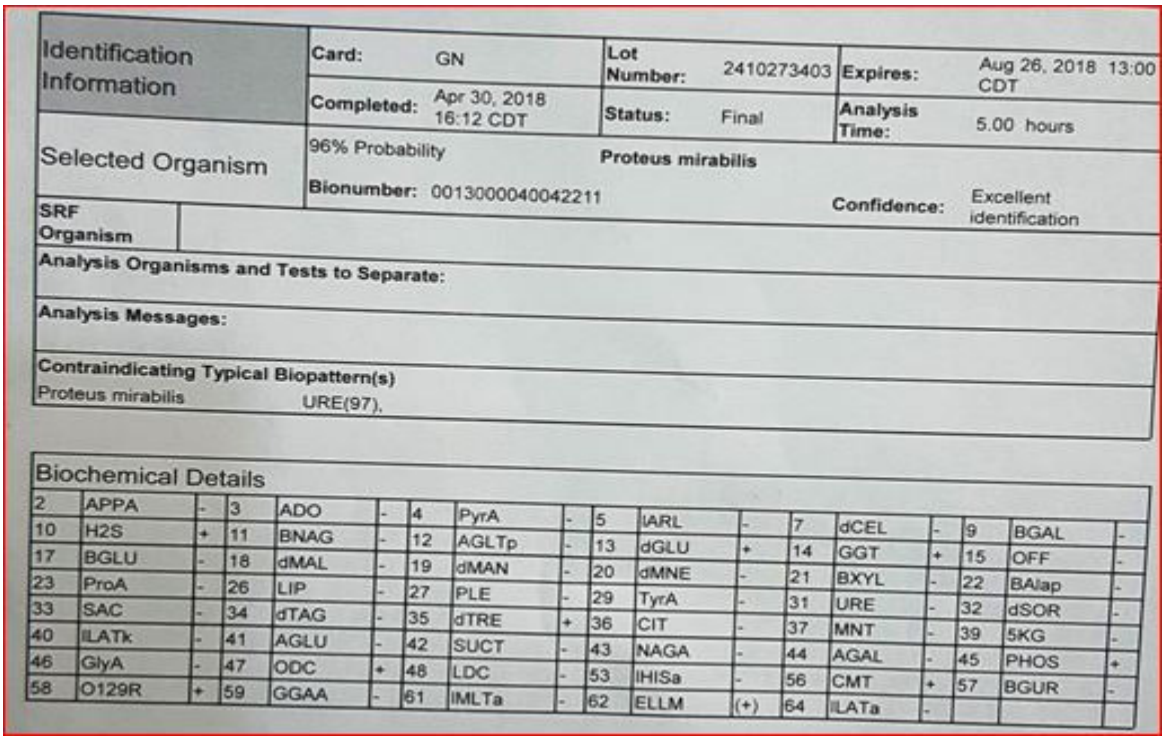


Figure 4. Gram stain from the milk smear show a clear Gram-negative rods

Figure 5. Swarm forming character was indicated of blood smear culture for the causative Proteus mirabilis.



Identification Information	Card: GN	Lot Number: 2410273403	Expires: Aug 26, 2018 13:00 CDT
	Completed: Apr 30, 2018 16:12 CDT	Status: Final	Analysis Time: 5.00 hours
Selected Organism	96% Probability	Proteus mirabilis	
	Bionumber: 0013000040042211		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			
Proteus mirabilis	URE(97),		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(+)	64	ILATa	-			

Figure 6. The result of VITEK 2 systems

DISCUSSION

To our knowledge, acute gangrenous mastitis caused by *Proteus mirabilis* is rarely reported in buffalo mastitis and rarely identified in the diagnostic laboratory. Although it has been isolated in pure culture from cases of UTI (Hogan et al., 1999; Różalski et al., 2012).

Several infections including those caused by some gram negative or positive bacteria and even some viruses and red yeast might cause systemic infections associated with intravascular hemolysis and capillary damage in udder leading to reddish or pinkish discoloration of milk (George et al.., 2008; Hogan et al., 1999). Leptospirosis is one of the common causes of blood in milk in dairy animals. When leptospirosis is the cause of bloody milk, the milk from all four teats would be red in color, thick in consistency and it contains blood clots and milk clots (Reddy et al., 2016; Fagiolo and Lai, 2007; Atyabi et al., 2006). On the other hand, Sometimes, reddish discoloration of milk may be due to feeding of fodders belonging to different plant families such as *Rubiaceae* Atyabi et al., 2006). However, toxins of some plant present in conifers, ranunculi, poplars and even the alders may cause capillary damage and that could leads to abnormal coloration especially the reddish discoloration of milk. Moreover, feeding of moldy sweet clover may also cause bloody milk (Muhammad et al., 1997). Some green plants such as sedge, spurge or euphorbia and shave grass contain a red dye which might appear in milk leading to the reddish discoloration (Raval et al., 1998). In addition, decrease platelets count of animals affected with diseases characterized by thrombocytopenia may also show red color or pinkish discoloration of milk due to seeping of blood into milk (Smith, 2009).

Gangrenous mastitis is developed by severe inflammation with specific clinical manifestations as the diseased animal will show high fever, beside redness, swelling and pain of the affected udder which might progress to necrosis with coldness of the affected area. However, the characteristic blue black coloration with fluid exudation may lead to crepitation. However, the histopathological features indicated a lesions characterized by progressive swelling, degeneration of vessels and focal ulcerations occur throughout the mammary ducts (Constable et al., 2017; Islam et al., 2008).

In the current report, diseased buffalo show different clinical signs which were also mentioned by others (Reddy et al., 2016; Fagiolo and Lai, 2007; Pal et al., 2011; Smith, 2009). Nevertheless, results of this report indicated that the causative agent for gangrenous mastitis in the local buffalo breed is *Proteus mirabilis.* Since, *Proteus spp.* are uncommon environmental mastitis pathogens that have been known to cause outbreaks (Różalski et al., 2012; Raval et al., 1998).

Proteus spp. is a gram-negative and similar in structure to other coliform mastitis pathogens. There is little information related to *Proteus spp.* as to how the infection of the mammary gland will occur. However, procedures effective in controlling coliform mastitis pathogens should apply to *Proteus spp.* as well. When grown on blood agar, *Proteus spp.* have been found to swarm on the plate and spread, same result were indicated in this report. Moreover, It is believed that the spreading of *Proteus spp.* can occur via environmental contact, but is not limited to bedding, dirty stables or animal houses and muddy grounds due to standing water (Fernández-Delgado et al., 2007). Furthermore, George *et al* (2008) and Atyabi *et al.* (2006) added that, Proteus spp. Must be indicated as environmental coliform mastitis-causing pathogens since it could be found in bedding, feed, and water. Although, little is known about how *Proteus* spp. infect the mammary gland, but control measures effective against other coliforms appear to be satisfactory. The question which comes here is, when are *Proteus spp.* of mastitis infections more likely to occur, as the new infections can occur at any time during lactation. Animals at early stage of lactation will be considered at risk for different new infections due to the increased stress and an exhausted immune system associated with the postpartum period. *Proteus spp.* have been found to cause herd outbreaks and infections tend to be acute and chronic, therefore making prevention key (Constable et al., 2017; Islam et al., 2008; Emmanuel, 2006).

CONCLUSION

It has been concluded that the microorganism *Proteus mirabilis* could cause gangrenous mastitis in the local buffalo breed. Therefore, It needs more attention for prophylactic measure.

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