Short Communication

Occurrence of mastitis and associated pathogens with antibiogram in animal population of Peshawar, Pakistan

Rafiullah[1] Mirza Ali Khan[2] Muhammad Shafee[3] Ali Akbar[4*] Anwar Ali[1]

Muhammad Shoaib[5] Faiza Ashraf[1] Naimatullah Khan[6]

Abstract

Mastitis is a devastating disease of dairy animals in Pakistan that occurs in clinical and subclinical forms. Various bacterial pathogens are associated with mastitis and the susceptibility of these pathogens remains inconsistent to various antibacterial drugs. This study was aimed to assess the prevalence of mastitis in and around the city of Peshawar, Pakistan.

A total of 2,791 milk samples (Bovine, Ovine and Caprine) were subjected to White side test and Surf field mastitis test. Positive milk samples for mastitis were cultured on different selective culture media for identification of prevalent bacterial pathogens. Antibiogram of these organisms was evaluated against different commonly used antibiotics by disc diffusion method. Out of the 2,791 milk samples, 2,253 (81%) were positive for mastitis using conventional screening tests. From all positive samples different bacterial pathogens were isolated; *Escherichia coli* was 54.5%, *Proteus* spp. 12%, *Staphylococci* spp. 7.5%, *Klebsella* spp. 6%, *Pasteurella* spp. 3%, *Pseudomonas* spp. 2.25%, *Salmonella* spp. 1.49% and *Streptococci* spp. 1.5%. Fungus was 0.75%, whereas mixed infections were 11.2%. In antibiogram studies the sensitivity of antibiotics, enrofloxacin, gentamycin, norfloxacin, ciprofloxacin and streptomycin were found to be 69.4, 60.5, 57.5, 49.3 and 48.5 percent, respectively. It was found that *Escherichia coli* and *Proteus* spp. were the most prevalent pathogens responsible for mastitis in the animals of Peshawar and surrounding areas. Enrofloxacin and gentamicin were found relatively more effective and sensitive against mastitis, providing better outcome in the treatment of mastitis.

Keywords: mastitis, antibiogram, *E. coli, Proteus*, Peshawar

[1]*Veterinary Research Institute, Khyber Pakhtunkhwa, Peshawar, Pakistan*
[2]*Veterinary Research Institute, Khyber Pakhtunkhwa, Peshawar, Pakistan*
[3]*CASVAB, University of Balochistan, Quetta, Pakistan*
[4]*Department of Microbiology, University of Balochistan, Quetta, Pakistan*
[5]*Agriculture University, Khyber Pakhtunkhwa, Peshawar, Pakistan*
[6]*Veterinary officer, Bannu, Khyber Pakhtunkhwa, Pakistan*
[*]***Correspondence:*** *aliakbar.uob@gmail.com, ali.akbar@um.uob.edu.pk*

Introduction

In developing countries such as Pakistan, mastitis is one of the most economically important diseases of dairy herds. Generally, it affects all domesticated animals, affecting the physical, chemical and microbiological properties of milk and at the same time influencing the pathological changes in udder parenchyma cells (Chishty et al., 2007; Beheshti et al., 2010).

Mammary gland infection can alter the nutritional composition of milk secretion. Mastitis affects flavor and shelf life of milk that contains pathogens along with toxins and its consumption may lead to increased risk of milk-borne diseases (Sharif and Muhammad, 2008). The prevalence of subclinical mastitis is 15-40 times higher than clinical forms and is responsible for great economic losses (Jarassaeng et al., 2012). In the subcontinent, the prevalence of subclinical mastitis was reported 17-93% in cows and 4-48% in buffaloes, respectively (Allore, 1993). The risk of global warming and climate change is imitable and several alarming manifestations of destruction have occurred (Ali et al., 2014). Animals may be affected by the climate change in four ways: extreme weather events, heat stress and diseases, animal adaptation to production systems in new environments, and emergence or recurrence of infectious diseases. Vector-borne diseases significantly depend on climatic and environmental conditions (Forman et al., 2008).

In Khyber Pakhtunkhwa province, the prevalence of clinical and subclinical mastitis has been recorded at 8.95% and 34.95% in cattle and buffaloes, respectively, causing estimated loss of 15 billion rupees per year (PARC, 2009). Different organisms such as *Escherichia coli*, *Staphylococci*, *Streptococci*, *Pseudomonas*, *Proteus* spp., *Pasteurella*, *Salmonella* and *Bacillus* spp. are involved in causing mastitis (Iqbal et al., 2004; Jarassaeng et al., 2012). The risk of transmission of zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human being is also associated with mastitis (Radostits et al., 2000). This study was designed to investigate the common microorganisms responsible for mastitis and their susceptibility pattern to various antibiotics.

Materials and Methods

Study area and population: The study was conducted in the diagnostic laboratory in Peshawar, Khyber Pakhtunkhwa (KP), Pakistan. The city is the provincial capital, located at 4.00°N 71.32°E. Animal samples were submitted to the laboratory, referred by veterinarians from periphery or even from remote areas (http://www.pbs.gov.pk). Lactating cattle, buffaloes, sheep and goats were investigated during the study.

Sampling and screening for mastitis: A total of 2,791 freshly obtained milk samples were collected during 2010-2013 from suspected mastitis infected animals and were initially checked by Surf field mastitis test (SFMT) for subclinical mastitis. SFMT-positive samples were submitted to the mastitis section of Veterinary Research Institute Laboratory, Peshawar, KP. The collected milk samples were mostly from milch animals, while a few milk samples were from goats and sheep. SFMT was used as screening test following Muhammad et al. (1995).

Culturing milk samples: The milk samples positive for SFMT were further subjected to pathogen isolation and identification. A portion (1 mL) of the selected positive sample was inoculated on Tryptone soy agar (Merck, Germany), MacConkey's agar (Himedia, India) and Blood agar plates (Himedia, India) and incubated at 37 ^{0}C for 24-48 h. Isolated organisms were identified on the basis of morphology, culture characteristics and biochemical profiling as proposed by Soomro et al. (2002).

Antibiogram profiling: Microbial isolates were tested for their antibiotic sensitivity and resistance pattern, using disk diffusion assay following Akbar and Anal (2015), to 17 different antibiotic discs (Oxoid), i.e. enrofloxacin, gentamicin, norfloxacin, ciprofloxacin, streptomycin, kanamycin, chloramphenicol, oxytetracycline, flumaquine, amoxicillin, cephradine, tobramycin, ampicillin, cloxacillin, neomycin, lincomycin and penicillin.

Results and Discussion

The total of 2,791 milk samples from four different animal species were tested in the laboratory for mastitis. Amongst them, 2,253 were found positive. The overall occurrence of mastitis was 81% as shown in Figure 1. This shows a very high degree of infection that ranged from 75% to 85% in all three years. The findings are different from an earlier report of 23-50% mastitis cases in cows, buffalos and sheep/goat (Iqbal et al., 2004). Our findings are also in contrast to those of PARC (2009), in which 9% and 35% overall prevalence of clinical mastitis and subclinical mastitis was recorded in Peshawar, respectively. The high prevalence of mastitis cases in our study could be due to the referral of suspected clinical and subclinical cases by veterinarians.

Mastitis is an economically important disease and is a great challenge for the dairy industry. It affects the industry in terms of decreased milk production, treatment and labor costs, risk of culling, mortality and reduced milk production and quality. The presence of microbial load turns milk more risky for human consumption (Indurr et al., 2008).

The prevalence of mastitis in the years 2010-11, 2011-12 and 2012-13 in cows was 75%, 85% and 84%; in buffaloes 75.7%, 85.9% and 75.3%; in goats 36.4%, 81.2% and 100%; and in sheep 50%, 100% and 100%, respectively. The overall prevalence of mastitis was recorded at 81%, 79%, 66 % and 75% in cows, buffaloes, goats and sheep, respectively (Table 1). Out of the 2,253 positive milk samples, only 213 (9.5%) were cultured (considering the history of medication and farmers' consent), in which 134/213 (62.9%) showed bacterial growth. Khan et al. (2015) reported 20.95% clinical mastitis cases in Peshawar. This contradiction might be due to random evaluation of flocks (diseased and healthy), while in our study only apparently diseased and physically altered milk samples were included.

In the present study, the SFMT-positive milk samples from cattle, buffaloes, goats and sheep; 94/149 (63%), 37/60 (62%), 2/3 (97%) and 1/1 (100%), respectively; produced microbial growth on the culture media. The relative occurrence of microbial isolates in the milk samples from cattle, buffaloes, goats and sheep is indicated in Table 2. A total of 94 (63%) microorganisms were isolated from the cattle comprising *Escherichia coli* 51/94 (54%), *Proteus* spp. 9/94 (9%), *Staphylococci* spp. 8/94 (9%), *Klebsiella* spp. 6/94 (6%), *Pasteurella* spp. 3/94 (3%), *Pseudomonas* spp. 3/94 (3%), *Salmonella* spp. 2/94 (2%), *Streptococci* spp. 2/94 (2%) and mixed growth infection 10/94 (11%).

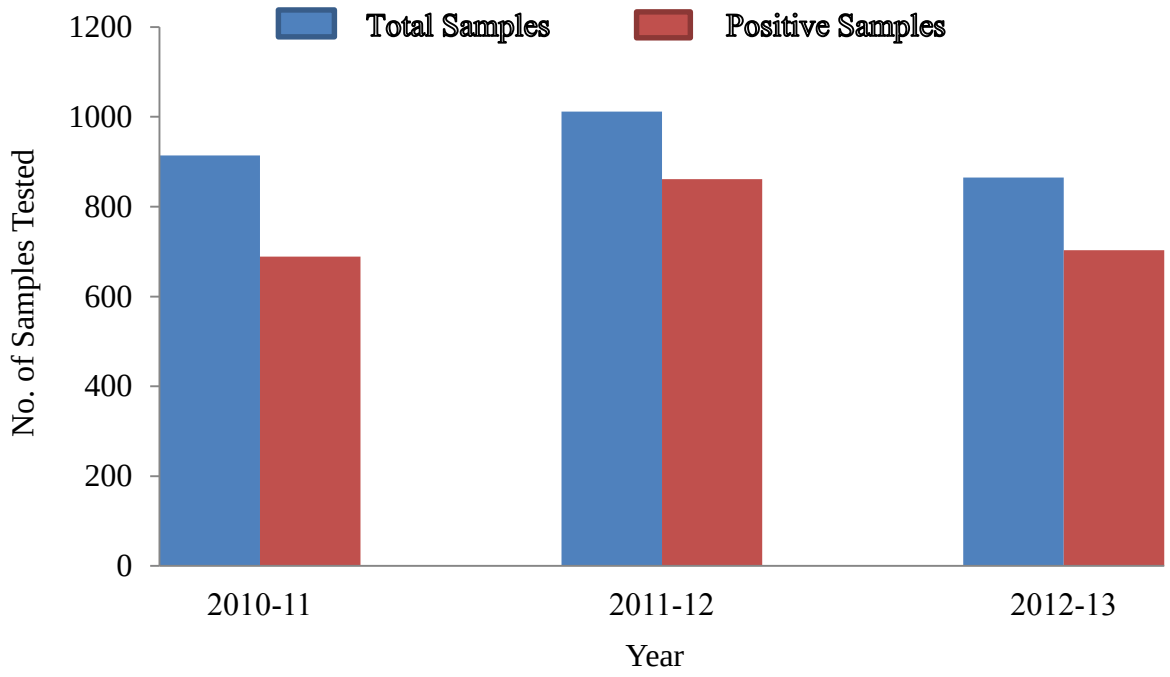

Figure 1 Year wise occurrence of mastitis in tested animals of Peshawar, Pakistan

Table 1 Bacterial species detected in healthy and periodontitis dogs

Year	Cattle		Buffalo		Goat		Sheep	
	Samples tested	Positive (%)	Samples tested	Positive (%)	Samples tested	Positive (%)	Samples tested	Positive (%)
2010-11	701	524 (75)	189	143 (76)	22	08 (36)	02	01 (50)
2011-12	678	578 (85)	311	267 (86)	22	18 (82)	01	01 (100)
2012-13	568	477 (84)	287	216 (75)	09	09 (100)	01	01 (100)
Total	1947	1579 (81)	787	626 (79)	53	35 (66)	4	03 (75)

Table 2 Relative prevalence of microbial agents isolated from total mastitic milk samples

Microbes	Cattle		Buffalo		Goat		Sheep		Overall	
	No.	%	No.	%	No.	%	No.	%	Total	%
Escherichia coli	51	54	22	59	0	0	0	0	73	54
Proteus spp.	9	10	6	16	1	50	0	0	16	12
Mixed growth	10	11	4	10	1	50	0	0	15	11
Staphylococci spp.	8	9	2	5	0	0	0	0	10	7
Klebsiella spp.	6	6	2	5	0	0	0	0	8	5
Pasteurella spp.	3	3	0	0	0	0	1	100	4	3
Pseudomonas spp.	3	3	0	0	0	0	0	0	3	2
Salmonella spp.	2	2	0	0	0	0	0	0	2	1
Streptococci spp.	2	2	0	0	0	0	0	0	2	1
Fungus	0	0	1	2.70	0	0	0	0	1	0.75
Total	94	100	37	100	2	100	1	100	134	100

Note: % = percentage

The buffalo milk samples showed the growth of *E. coli* 22/37 (59%), *Proteus* spp. 6/37 (16%), *Staphylococci* spp. 2/37 (5%), *Klebsiella* spp. 2/37 (5%), fungus 1/37 (3%) and mixed growth 4/37 (11%). Similarly, in the goat milk samples, only *Proteus* spp. and mix growth were found, while there was only one *Pasteurella* spp. growth in the sheep milk samples in the ratio of 1/37 (3%). The overall relative percentage of microorganisms was recorded as *E. coli* 73/134 (54%), *Proteus* spp. 16/134 (12%), *Staphylococci* spp. 10/134 (7%), *Klebsiella* spp. 8/134 (6%), *Pasteurella* spp. 4/134 (3%), *Pseudomonas* spp. 3/134 (2%), *Salmonella* spp. 2/134 (1.5%), *Streptococci* spp. 2/134 (1.5%), fungus 01/134 (0.75%) and mixed growth 15/134 (11.19%). Our results correspond with that of Iqbal et al. (2004), which showed the highest incidence of *E. coli* (40.7 %). The high prevalence of *E. coli* in mastitis milk samples has also been reported in some previous studies (Deborah et al., 1991; Balakrishnan et al., 2004; Soomro et al., 2002). However, the results of Behishti et al. (2011) differ; they recorded 48.6% prevalence of *Staphylococci* spp. This might be due to good management practices at their farms, as *E. coli* is an environmental pathogen resulting from poor management practices and general unhygienic conditions in farms (PARC, 2009).

Although a small number of milk samples, 213 (9.5%), were subjected to culture for bacterial growth, 134 (63%) yielded growth. A total of 93 (63%) microorganisms were isolated from the cattle, mostly contaminated by *E. coli* followed by *Proteus* spp., *Staphylococci* spp., *Klebsiella* spp., *Pasteurella* spp., *Pseudomonas* spp., *Salmonella* spp., *Streptococci* spp. and mixed growth infections.

In this study, seventeen common antibiotics in use were evaluated against the isolates, in which enrofloxacin, gentamicin, norfloxacin, ciprofloxacin and streptomycin were the most effective antibiotics showing efficacy of 69.4, 60.5, 57.5, 49.3 and 48.5 percent respectively. The antibiogram profile of the isolated microorganism is depicted in Table 3. Our findings substantiate a previous study conducted in Lahore, Pakistan reporting ciprofloxacin and gentamicin being the most sensitive drugs in mastitis (Mustafa, 2011).

Table 3 Antibacterial susceptibility of individual isolates in percentage

Isolates	*E. coli*	*Proteus* spp.	*Staphylococcus* spp.	*Klebsiella* spp.	*Pasteurella* spp.	*Pseudomonas* spp.	*Salmonella* spp.	*Streptococcus* spp.	*Fungus*	*Mixed growth*	*Total*
No. of isolates	**73**	**16**	**10**	**8**	**4**	**3**	**2**	**2**	**1**	**15**	**134**
Antibiotics applied											
Enrofloxacin	68	62	70	87.5	25	100	50	50	0	80	69.40
Gentamicin	63	69	30	50	50	100	50	50	0	60	60.45
Norfloxacin	56	50	40	75	50	66	100	0	0	73	57
Ciprofloxacin	47	62	40	50	0	66	0	50	0	66	49
Streptomycin	56	31	60	37	75	33	50	0	0	33	48
Kanamycin	45	50	50	50	25	33	100	0	0	33	45
Chloramphenicol	36	37	40	37	25	0	50	100	0	20	34
Oxytetracycline	23	37	50	25	0	33	50	100	0	33	30
Flumequine	32	31	10	13	25	0	50	0	0	20	27
Amoxicillin	14	6	40	13	0	0	50	0	0	27	16
Cephradine	11	6	40	0	0	0	0	0	0	26	13
Tobramycin	10	31	0	13	0	33	0	0	0	0	10
Ampicillin	4	13	20	0	25	0	50	0	0	7	8
Cloxacillin	3	0	30	13	0	0	0	50	0	7	6
Neomycin	7	0	0	0	0	0	0	0	0	7	4
Lincomycin	0	12	20	0	25	0	0	50	0	0	4
Penicillin	0	6	10	13	0	0	0	0	0	0	3

Mastitis is a global problem in dairy herds, especially in low resource settings. It has also been reported in Iran with 42.5% prevalence (Hashemi et al., 2011). The result of the present study clearly indicates that the microbial quality of raw milk supplied to cities is unhygienic and unsatisfactory. Mastitis in dairy farms ultimately causes financial burden on dairy owners, leading to severe economic losses (Samiullah et al., 2000).

This study identifies the need for early diagnosis and prevention of subclinical mastitis performed by dairy holders in order to acquire good quality milk and prevent economical losses. The control and prevention measure for mastitis is

necessary and can be achieved through continuous monitoring of animals' health.

References

Akbar, A., and Anal AK. 2015. Isolation of *Salmonella* from ready-to-eat poultry meat and evaluation of its survival at low temperature, microwaving and simulated gastric fluids. J. Food Sci. Tech, 52(5): 3051-3057.

Ali I., Akbar A., Punnapayak H., Prasongsuk S and Yanwisetpakdee B. 2014. "Seven big challenges for Pakistan—and the lessons they could teach," *The Futurist*, 48(5): 22-26.

Allore HG. 1993. A review of the incidence of the mastitis in buffaloes and cows. Pak.Vet. J. 13: 1-7.

Balakrishnan G, Unny M, Dorairajan N and Subramaniah M. 2004. Indian Vet J. 81:1166-1167.

Beheshti R, Behra E, Jalal S, Ghalehkandi JG and Valiei K. 2011. Prevalence and etiology of subclinical mastitis in Buffalo of the Tabriz region. Iran J. American Sci. 7: 642-645.

Chishty MA, Arshad M, Avais M and Ijaz M. 2007. Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of tehsil Gojra Pakistan. Buff. Bull. 26(2): 50-55.

Deborah AT, Smith KL, Hogan JS and Schoenberger PS. 1991. Epidemiological survey of mastitis and evaluation of economic losses due to clinical and sub-clinical mastitis in NWFP. 2009, Final Completion Report. PARC. Am. J. Vet. Res. 52: 184-188.

Forman S, Hungerford N, Yamakawa M, Yanase T, Tsai HJ, Joo YS, Yang DK,,Nha JJ. 2008. Climate change impacts and risks for animal health in Asia. Rev. Sci. Tech. 27(2): 581-97.

Hashemi M, Kafi M, Safdarian M. 2011. The prevalence of clinical and sub clinical mastitis in dairy cows in the central region of Fars province, South of Iran. Intl. J. Vet. Res. 12(3): 236-241.

http://www.pbs.gov.pk/sites/default/files/aco/publicati ons/pakistan-livestock cencus2006/pak-11a.pdf (Accessed on. 20-12.2015).

InDürr JW, Cue RI, Monardes HG, Moro-Méndez J, Wade KM. 2008. Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. Livestock Sci. 117(2-3): 225-232.

Iqbal M, Khan MA, Daraz B and Siddique U. 2004. Bacteriology of mastitic milk and in vitro antibiogram of the isolates. Pak. Vet. J. 24(4): 161-164.

Jarassaeng CS, Aiumlamai C, Wachirapakorn M, Techakumphu Jos PTM, Noordhuizen AC, Beynen and Suadsong S, 2012. Risk factors of subclinical mastitis in small holder dairy cows in Kohn Kaen Province, Thailand. Thai J. Vet. Med. 42(2): 143-151.

Khan A, Mushtaq MH, Ahmad M, Chaudhary M and khan AW. 2015. Prevalence of clinical mastitis in bovines in different climatic conditions in KPK, Pakistan. Sci. Intl. 27(3): 2289- 2293.

Muhammad G, Athar M, Shakoor A, Khan MZ, Rehman F and Ahmed MT. 1995. Surf field mastitis test: An expensive new tool for evaluation of wholesomeness of fresh milk. Pak. J Food Sci. 5: 91-93.

Mustafa YS, Awan FN, Zaman T, Chaudhary SR and Zoyfro Y. 2011. Prevalence and antibacterial susceptibility in mastitis in buffalo and cow in and around Lahore, Pakistan. Pak. J. Pharm. 24: 29-33.

PARC. 2009. Epidemiological survey of mastitis and evaluation of economic losses due to clinical and sub clinical mastitis in NWFP. pp 28-29.

Radostits OM. Blood DC. Gay CC. Hinchiff KW and Handerson JA. 2000. Veterinary Medicine. 9th Ed., W. B. Saunders Company, London, U.K.

Samiullah M, Syed UD, Arif M and Khan M. 2000. Frequency and causes of culling and mortality in Holstein and Friesien cattle in NWFP. J. Anim. Health Prod. 20: 22-24.

Sharif A. and Muhammad G. 2008. Somatic cell count as an indicator of udder health status under modern dairy production: a review. Pak. Vet. J. 28(4): 194-200.

Soomro AH, Arain MA, Khaskheli M and Bhutto B. 2002. Isolation of E coli from raw milk and milk products in relation to public health sold under market conditions at Tandojam, Pakistan. Pak. J. Nutri. 1(3): 151-152.

บทคัดย่อ

อุบัติการณ์ของเต้านมอักเสบและความไวต่อยาต้านจุลชีพของเชื้อก่อโรคในสัตว์ ในเขต เพชาวาร์ ประเทศปากีสถาน

ราฟิลาห์[1] มิซ่า อาลี ข่าน[2] มูฮัมหมัด ชารีฟ[3] อาลี อักกา[4] อันวา อาลี[1]*
มูฮัมหมัด โซอิบ[5] ไฟซา อัชรอฟ[1] นีมาชูลา ข่าน[6]

โรคเต้านมอักเสบแบบแสดงอาการ และไม่แสดงอาการ เป็นโรคที่มีความสำคัญในโคนมในประเทศปากีสถาน โดยมีเชื้อแบคทีเรียหลายชนิดเป็นสาเหตุของการเกิดโรคเต้านมอักเสบ และมีความไวของเชื้อต่อยาต้านจุลชีพที่หลากหลาย การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาอุบัติการณ์ของโรคเต้านมอักเสบ ในเขตเพชาวาร์ ประเทศปากีสถาน โดยเก็บตัวอย่างน้ำนมจำนวน 2,791 ตัวอย่าง จาก โค แกะ และแพะ และนำมาตรวจด้วยวิธี White side test และ Surf field mastitis test จากนั้นนำตัวอย่างน้ำนมที่ให้ผลบวกมาเพาะแยกเชื้อในอาหารเลี้ยงเชื้อจำเพาะ เพื่อตรวจพิสูจน์ชนิดของเชื้อแบคทีเรีย และตรวจหาความไวต่อยาต้านจุลชีพของเชื้อแบคทีเรีย ด้วยวิธี disc diffusion method ผลการศึกษาพบว่า น้ำนมจำนวน 2,253 ตัวอย่างคิดเป็น 81% ให้ผลบวกต่อการตรวจคัดกรองโรคเต้านมอักเสบ และสามารถเพาะแยกเชื้อแบคทีเรียได้หลายชนิด ได้แก่ *Escherichia coli* 54.5%, *Proteus* spp. 12%, *Staphylococci* spp. 7.5%, *Klebsella* spp. 6%, *Pasteurella* spp. 3%, *Pseudomonas* spp. 2.25%, *Salmonella* spp. 1.49%, *Streptococci* spp. 1.5% และพบเชื้อรา Fungus 0.75% และมีการติดเชื้อร่วมระหว่างแบคทีเรียและเชื้อรา 11.2% ผลการศึกษาความไวต่อยาต้านจุลชีพพบว่า เชื้อแบคทีเรียไวต่อยา enrofloxacin, gentamycin, norfloxacin, ciprofloxacin และ streptomycin คิดเป็น 69.4, 60.5, 57.5, 49.3 และ 48.5 เปอร์เซ็นต์ตามลำดับ โดยพบว่าเชื้อ *Escherichia coli* และ *Proteus* spp เป็นเชื้อแบคทีเรียที่เป็นสาเหตุของการเกิดโรคเต้านมอักเสบสูง ในเขตเพชาวาร์ ประเทศปากีสถาน *และพบว่ายา* enrofloxacin และ gentamicin เป็นยาที่ให้ผลในการต้านเชื้อแบคทีเรียและเหมาะสำหรับการรักษาโรคเต้านมอักเสบ

คำสำคัญ: เต้านมอักเสบ ความไวต่อยาต้านจุลชีพ *E. coli Proteus* เพชาวาร์

[1]สถาบันวิจัยทางสัตวแพทย์ ไคเบอร์ปัคตูนควา เพชาวาร์ ประเทศปากีสถาน
[2]สถาบันวิจัยทางสัตวแพทย์ ไคเบอร์ปัคตูนควา เพชาวาร์ ประเทศปากีสถาน
[3]มหาวิทยาลัยบาโลชิสถาน คิวตา ประเทศปากีสถาน
[4]ภาควิชาจุลชีววิทยา มหาวิทยาลัยบาโลชิสถาน คิวตา ประเทศปากีสถาน
[5]มหาวิทยาลัยเกษตรศาสตร์ ไคเบอร์ปัคตูนควา เพชาวาร์ ประเทศปากีสถาน
[6]สำนักงานสัตวแพทย์ บานนู ไคเบอร์ปัคตูนควา เพชาวาร์ ประเทศปากีสถาน
***ผู้รับผิดชอบบทความ E-mail:** aliakbar.uob@gmail.com, ali.akbar@um.uob.edu.pk*