

Prevalence and Distribution of Mastitis Pathogens and their Resistance Against Antimicrobial Agents in Dairy Cows in Jordan

¹Azmi D. Hawari and ²Fawzi Al-Dabbas

¹Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University, Al-Salt, Jordan

²Al-Salt University College, Al-Balqa Applied University, Alsalt, Jordan

Abstract: The primary objective of this study was to determine the aetiology of bovine mastitis in ten herds of Holstein Friesian cow in Jordan, the prevalence of mastitis pathogens in dairy cows and their resistance to selected antimicrobial agents. Milk samples were collected from 220 lactating cows to determine the clinical and subclinical mastitis by white side test and confirmed by cultural tests. It was found that 138 quarters 15.7% had been diagnosed as clinical mastitis with definite gross pathological lesions and change in udder secretion, in addition to that 276 quarters 31.4% showed subclinical mastitis. The incidence of mastitis was found to be increased in older cows. *Staphylococcus aureus* was considered to be the most common cause of both clinical and subclinical mastitis and followed by *coli* forms, *streptococcus* spp., *corynebacterium* spp., *proteus* spp. and *pseudomonas* spp. Sensitivity tests was applied to different isolated strains using tetracycline, ampicillin, neomycin, erythromycin, penicillin G and sulphamethoxazol trimethoprim.

Key words: Cattle-microbiological diseases, mastitis, Jordan

INTRODUCTION

Mastitis is considered one of the most important dairy cattle diseases and is one of the major causes of antibiotics use in dairy cows^[1,2]. Over 135 different microorganisms have been isolated from bovine intramammary infection, but the majority of infections are caused by *staphylococci*, *streptococci* and gram-negative bacteria^[3]. Where is *staphylococcus aureus* was isolated from 18% of the culture positive quarter^[4].

Mastitis known to be a complex and costly disease of dairy cows that results from the interaction of the cow and the environment including the milking machine and the microorganisms^[5-7], although stress and physical injuries may cause inflammation of mammary glands, infection by invading bacteria or other microorganisms (fungi, yeast and possibly viruses) is the primary cause of mastitis^[8,9].

Jordan has experienced a rapid population growth over the last three decades and an increased demand for milk and dairy products. Mastitis is considered to be the most frequent disease of dairy cattle in Jordan and has received more veterinary attention as a disease of cattle and as a serious economic problem^[10,11].

Mastitis is produced by a variety of pathogenic microorganisms. Cows and herds vary in susceptibility

and extent, type and duration of infection, although some of mammary pathogens can be isolated from the environment of the cow, manure and bedding, water supplies, soil and inadequately cleaned milking machines^[12,13], thus new infection can be reduced by applying rigid herd management practices^[14]. Measures to control mastitis such as improved milking hygiene have reduced *streptococcus agalactia*^[15-17]. The incidence of the disease was influenced with the lactation and age of the animal; higher infection were reported in older cows^[18,19].

The aim of this paper was to determine the prevalence of mastitis pathogens in clinically and subclinically of dairy cows, a further objective was to determine the susceptibility of these bacteria to 6 antimicrobial agents that are or have been commonly used in dairy cows in Jordan.

MATERIALS AND METHODS

Milk samples were collected from 220 Holstein Friesian cows in 10 farms in Thuleil area, full history was reported for cows in all farms. The health status of the udder and the age of the cows were recorded. Prior to sampling the udder was washed and dried and the teat was disinfected. All milk samples were collected

Corresponding Author: Azmi Hawari, Department of Nutrition and Food Processing, Faculty of Agricultural Technology, AL-Balqa Applied University, P.O. Box 7027, AL-Salt, Jordan

all over the year 2006 under aseptic condition. About 15 mL of the foremilk were discarded and the next fifteen mL were collected into screw-capped bottles and transferred in ice boxes to the laboratory. All samples were stored at 4°C from the time of collection until examination within 3-4 h.

All samples were centrifuged for 15 min at 3000 rpm and a loopfull was taken from sediment and inoculated separately on each of the following media, 5% sheep blood agar, Edwards and Mac Conkey's agar. The inoculated plates were then incubated at 37°C for 24, 48 and 72 h. From growing colonies smears were made and stained with Grams stain. In case of *Streptococcus* strains it was identified by the morphological character on blood agar and specific Edwards media. For identification of isolated *Staphylococcus* strains by colonial appearance on blood agar, pigments, haemolysis and for pathogenicity by using tube method of coagulase test. *Coliforms*, *Proteus* spp., *Corynebacterium* spp., *pseudomonas* spp. and the other Gram negative bacilli were identified by morphological character on sheep blood agar and by biochemical reaction when necessary.

Sensitivity tests were carried out on the isolates by using sensitest agar (oxid) and susceptibility discs (oxid). The discs used contained Neomycin 30 µg, Ampicillin 10 µg, Tetracycline 30 µg, Erythromycin 15 µg, Penicillin G 10 IU, Sulthamethoxazole Trimethoprim (SXT) 25 µg.

RESULTS AND DISCUSSION

The incidence of clinical mastitis in udder quarters of Holstein Friesian cows in ten farms was found to be 15.7% Table 1. This is a little higher than that reported before in Holstein Friesian cows in Egypt 12.08%^[20]. The incidence of subclinical mastitis, however, averaged 31.4% in this investigation as based on udder quarters (Table 2).

It was evident in this study that mastitis was increased with advanced age. It is noticed in some herds the incidence of clinical and subclinical mastitis was increased with the sixth years old Table 3, Fig. 1.

The relative frequency of different types of microorganisms in this study revealed that *staphylococcus aureus*, *Coliforms* and *streptococcus* spp. were the chief aetiological agents responsible for both clinical and subclinical mastitis Table 4. These results agree with those presented by others^[3,10,21].

In clinical mastitis the incidence of *Coliform* 26.1%, *Streptococcus* spp 8.7%, *Staphylococcus aureus* 40.6%, *Proteus* spp. 1.4%, *Corynebacterium* spp. 5.8% *Pseudomonas* spp. 4.3% , mixed (*coryne*, *strept*, *staph*

Table 1: Incidence of clinical mastitis quarters

No. of animal examined	No. of animal examined	Clinically mastitis quarters (%)	Apparently normal quarter
220	880	138	642

Table 2: Incidence of sub clinical mastitis

No. of examined quarters	Scores of white side test				Normal quarters
	+	++	+++	Total (%)	
880	72	94	110	276	31.4

Table 3: Incidence of clinical and sub clinical mastitis in relation to the age

Age of the cow (years)	3	4	5	6	7	Total
Diseased quarters	61	70	73	106	104	414
%	14.7	16.9	17.7	25.6	25.1	100

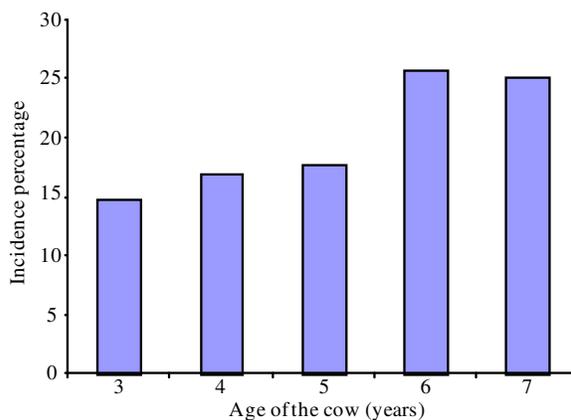


Fig. 1: Incidence of clinical and subclinical mastitis in relation to the age

and *Coliforms*) 7.3% and others 5.8%. While in subclinical mastitis *Coliforms* were 31.9%, *Streptococcus* spp. 7.2%, *Staphylococcus aureus* 42.7%, *Proteus* spp. 2.2%, *Corynebacterium* spp. 2.9%, mixed and others 13.1%. National cross-sectional study of mastitis in dairy cattle in Jordan^[22], it was reported that the most estimates of prevalence of bacterial pathogens intramammary infections were: coagulase negative *Staphylococci* 16.04%, *Staph. aureus* 9.41%, *Klebsiella* spp. 6.17%, *Corynebacterium bovis* 5.35% and *Brucella melitensis* 4.52%. In U.K. the distribution of the 1457 clinical cases according to the result of the culture was *E-coli* 32%, *strept uberis* 23% *staph* 7%, *Corypyogens* 1% mixed 10% and others 24%^[23] while In Germany the mastitis pathogens were isolated from 26.4% of the milk samples and the major pathogens, *staph. aureus* 5.7% and *streptococcus uberis* 1% had the highest prevalence, at the same time streptococcus

Table 4: Incidence of various organisms in clinical and sub clinical mastitis

Mastitis	No. of quarters infected by each type of bacteria								
	<i>Coliforms</i>	<i>Strept.</i>	<i>Staphylococcus aureus</i>	<i>Proteus</i> spp.	<i>Corynebacterium</i> spp.	<i>Pseudomonas</i> spp.	Mixed ₁	Others ₂	Total
Clinical No	36.0	12.0	56.0	2.0	8.0	6.0	10.0	8.0	138
	26.1%	8.7	40.6	1.4	5.8	4.3	7.3	5.8	
Subclinical No	88.0	20.0	118.0	6.0	8.0	00	30.0	6.0	276
	31.9%	7.2	42.7	2.2	2.9	00	10.9	2.2	

Mixed = *Corynebacterium*, *strept*, *Staph.* and *coliform*

Others = Sterile and unsatisfactory samples

Table 5: Percentage for the sensitivity of bacteria that isolated from milking cows to different antibiotics

Antibiotics	Disc potency	<i>Coliforms</i>	<i>Strept</i> spp.	<i>Staph aureus</i>	<i>Proteus</i> spp.	<i>Corynebacterium</i> spp.	<i>Pseudomonas</i> spp.	Total (%)
Tetracycline	30 ug	34.5	75	71.8	75	87.5	100	64
Ampicillin	10 ug	9.7	62.5	80.5	0	87.5	0	52.8
Neomycin	30 ug	17.7	12.5	27.6	12.8	12.5	0	21.4
Erythromycin	15 ug	24.2	87.5	35.6	50	62.5	0	36
Penicillin G	10 lu	23.3	56.2	69	0	81.25	0	54
Sulphamethoxazo	125 ug	74.2	62.5	74.7	100	75	100	75
Trimethoprim (SXT).								

Table 6: Analysis of variance for six antibiotics and six bacteria

Source of variation	DF	SS	MSS	F-ratio
Antibiotics	5	10675.945	2135.189	4.635
Bacteria	5	3465.065	693.013	1.500
Error	25	11514.307	460.572	
Total	35	25655.317		

agalactia was isolated in samples from 29% of the herds^[19]. Bacteriological data from herds surveys by the Massachusetts Mastitis Laboratory were analyzed and showed that *Staph. aureus* and *coliforms* were the predominant organisms isolated from Holstein Friesian cow mastitis a result which is similar to our result^[24].

It would be of interest to note that in Sudan^[25] the organisms isolated in clinical and subclinical mastitis in Zebu cattle were *staph* spp.50 and 68.7%. *Strept* spp. 15.3 and 12.6%, *Coryn.* spp. 11 and 00% *coliforms* 11 and 4.8% and mixed 10.2 and 13%. In Jordan it was reported that the most common organisms isolated from clinical and subclinical cases were *staph.* spp. 30%^[11].

There results of sensitivity tests of the organisms isolated to antibiotics Table 5 shown that 75% of strains were sensitive to Sulphamethaxazol Trimethoprim, 64% to Tetracycline, 54% to Penicillin G, 52.8% to Ampicillin, 36% to Erythromycin and 21.4% to Neomycin.

Statistical analysis: The analysis of variance for antibiotics sensitivity showed that there are differences between antibiotics treatment at 1% level of significant (Table 6). At the same time there are no significant differences between isolated organisms.

CONCLUSION

In conclusion, more efforts are needed to improve the general udder health in dairy herds as approximately 47.1% of the cows had at least one infected quarter (clinically or sub-clinically), so the recommended preventive and control measures against mastitis should include application of good sanitary and hygienic measures. In addition to that regular monitoring of the prevalence and the distribution of mastitis-causing pathogens provides, however, valuable knowledge about the effectiveness of proposed control strategies and helps in prioritizing specific mastitis control efforts.

ACKNOWLEDGEMENT

We thank Dhia Hassawi for his help in the statistical analysis of the results.

REFERENCES

1. Mitchell, J.M., M.W. Griffiths, S.A. McEwen, W.B. Menab and A.J. Yee, 1998. Antimicrobial drug residues in milk and meat: Causes, concerns, prevalence, regulations, tests and test performance. *J. Food Protect.* 61: 742-756. <http://www.ingentaconnect.com/content/iafp/jfp/1998/00000061/00000006/art00020>.
2. DANMAP (Danish Integrated Antimicrobial Resistance monitoring and Research Programme.)2003. Use of antimicrobial agents and occurrence and antimicrobial resistance in bacteria from food animals, foods and humans in Denmark 2003. DANMAP, Soborg, Denmark. http://www.danmap.org/pdfFiles/Danmap_2003.pdf.

3. Bradley, A.J., 2002. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clin. Pharm. Toxicol.*, 96: 71-281. DOI: 10.1111/j.1742-7843.2005.pto960401.x.
4. Sofie Piepers, Luc De Meulemeester, Aart de Kruif, Greet Opsomer, Herman w Barkema and Sarne De Vlieghe, 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. *J. Dairy Res.*, 74: 478-483. DOI: 10.1017/S0022029907002840.
5. Brown, R.W., R.J. Eberhart, J.S. Mc Donald, R.P. Natzke, D.S. Postle and O.W. Schalm, 1972. Supplement to Current Concepts of Bovine Mastitis. National Mastitis Council, Washington, DC.
6. Blossor, T.H. 1979. Economic losses from and the national research program on mastitis in the United States. *J. Dairy Sci.*, 62: 119-127. <http://jds.fass.org/cgi/content/abstract/62/1/119>.
7. Radostitis, O.M., D.C. Blood and C.C. Gay, 1994. *Veterinary Medicine*. 8th Edn. Billiere Tindall, London, pp: 563-614.
8. Wattiaux, M.A., 1999. Mastitis: The Disease and its Transmission. In: *Dairy Essentials*. Chapter 23. Babcock Institute for International Dairy Research and Development. University of Wisconsin-Madison, pp: 89-92.
9. Ruegg, P.L., 2001. Mastitis Control. In: *Dairy Updates: Milking and Milk Quality No. 405*. The Babcock Institute, University of Wisconsin, pp: 10.
10. Lafi, S.Q. and N.Q. Hailat, 1998. Incidence and antibiotic sensitivity of bacteria causing bovine and ovine clinical mastitis in Jordan. *Pak. Vet. J.*, 18: 88-94. <http://www.parc.gov.pk/data/CatPak/cataction.asp?NAV=16&Title=aus&Author=&corporateAuthor=&Publisher=&PublicationDate=&subjectheadings=>
11. Lafi, S.Q., O.F. Al-Rawashdeh, K.L. Ereifej and N.Q. Hailat, 1994. Incidence of clinical mastitis and prevalence of subclinical udder infection in Jordan. *Dairy Cattle Prev. Vet. Med.*, 18: 89-98. <http://www.fao.org/agris/search/display.do?f=.1994/v2011/NL9401714.xml;NL9401714>.
12. Jain, N.C., 1979. Common pathogens and factors in infection and mastitis. *J. Dairy Sci.*, 62: 128-134. <http://jds.fass.org/cgi/content/abstract/62/1/128>.
13. Philpot, W.N., 1979. Control of mastitis by hygiene and therapy. *J. Dairy Sci.*, 62 : 168-176. <http://www.ncbi.nlm.nih.gov/pubmed/379060>.
14. Schalm, O.W., E.J. Carroll and N.C. Jain, 1971. *Bovine Mastitis*, Lea and Febiger. Philadelphia.
15. Myllyly, V., K. Asplund, E. Brofeld, V. Hirvela-Koski, T. Homkanen-Buzalski, J. Junttila, L. Kulkas, O. Myllykan, M. Niskanen, H. Saloniemi, M. Sandholm and T. Saranpaa, 1998. Bovine mastitis in Finland in 1988 and 1995-change in prevalence and antimicrobial resistance. *Acta Vet. Scand.*, 39: 119-126. <http://www.ncbi.nlm.nih.gov/pubmed/9592952>.
16. Makovec, J.A. and P.L. Ruegg, 2003a. Results of milk samples submitted for microbiological examination in Wisconsin from 1994-2001. *J. Dairy Sci.*, 86: 3466-3472. <http://jds.fass.org/cgi/content/full/86/11/3466>.
17. Pitkala, A., M. Haveri, S. Pyorala, V. Myllyly and T. Honkanen-Buzalski, 2004. Bovine mastitis in Finland 2001-prevalence, distribution of bacteria and antimicrobial resistance. *J. Dairy Sci.*, 87: 2433-2441. <http://jds.fass.org/cgi/content/abstract/87/8/2433>.
18. Sharma, R.M. and R.A. Pacher, 1980. Occurrence and ecologic features of *Streptococcus uberis* in the dairy cow. *Am. J. Vet.*, 31: 1197. <http://www.ncbi.nlm.nih.gov/pubmed/4915060>.
19. Tenhagen, B.A., G. Koster, J. Wallman and W. Heuwieser, 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *J. Dairy Sci.*, 89: 2542-2551. <http://www.dairy-science.org/cgi/reprint/89/7/2542>.
20. Abd El-Moneim, M., 1979. Incidence of mastitis in a village of Sharkia province and its treatment. M.V. Sc. Thesis, Cairo University.
21. Smith, M.C., 1990. Exclusion of infectious diseases from sheep and goat farms. *Vet. Clin. North Am. Food Anim. Pract. (USA)*, 6: 705-720. <http://www.ncbi.nlm.nih.gov/sites/entrez>.
22. Lafi, S., O. Al-Rawashdeh, T. Na'Was and N. Hailat, 1994. National cross-sectional study of mastitis in dairy cattle in Jordan. *Trop. Anim. Health Prod.* 26: 168-174. DOI: 10.1007/BF02241077.
23. Fauli, W.B., J.R. Walton, A.J. Bramly and J.W. Huges, 1983. Mastitis in a large Zero-grazed dairy herd. *Vet. Rec.*, 29: 415-420. <http://veterinaryrecord.bvapublications.com/cgi/content/abstract/113/18/415>.
24. Oliver, S.P. and A.B. Mitchell, 1984. Prevalence of mastitis pathogens in herds. Participating in a mastitis control program. *J. Dairy Sci.*, 67: 2436-2440. <http://jds.fass.org/cgi/content/abstract/67/10/2436?ck=nck>.
25. Bagadi, H.O., 1970. The aetiology of bovine mastitis in three areas in Sudan. *Trop. Anim. Health Prod.*, 2: 28-34. DOI: 10.1007/BF02359326.