INCIDENCE AND ANTIBIOTIC SENSITIVITY OF BACTERIA CAUSING BOVINE AND OVINE CLINICAL MASTITIS IN JORDAN

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ABSTRACT

Twelve dairy herds and 6 Awassi sheep flocks were used in this study. Milk samples were obtained and cultured from 169 reported clinical cases which occurred during the study period and comprised of 108 quarters and 61 udder halves (60 cows and 45 ewes). The incidence of clinical mastitis expressed as the number of clinical cases per 100 cow or ewe-months was 3.35 ± 0.6 and 2.23 ± 0.33 percent, respectively. Of the 169 mastitic milk samples, 15 samples grew no microorganism in culture. Of the total of 170 poitive samples, 40 (29 from cows and 11 from ewes) samples indicated the presence of more than one type of bacteria. *Staphylococcus aureus* was the most common (31.35%) and *Streptococcus agalactiae* was the second common (22.70%) bacterium isolated from all examined milk samples. The sediment technique proved to be superior to pre-culture incubated milk technique. Penicillin was the least effective antibiotic (*in vitro*) against bacteria in cows and ewes. *Streptococcus agalactiae* was found to be highly susceptible to neomycin.

INTRODUCTION

Mastitis, similar to most livestock diseases, is a result of the interaction between the host, pathogen and environment. This inflammation of the mammary gland (mastitis), is known to be a complex and costly disease of both dairy cattle and ewes (Blosser, 1979; Radostitis *et al.*, 1994). The disease is associated with a decrease in milk production, an increase of veterinary services, treatment, labour costs and culling (Dobbins, 1977; Blosser, 1979; Fthenakis, 1994).

The widespread occurrence of mastitis in dairy cattle creates an estimated loss to producers of approximately $ 2 billion in the United States alone. This number included the additional untold losses from altered milk quality and composition and the effects on dairy products that occur once milk has left the farm (Harmon, 1994). Application of hygienic measures determines the rate of infection in lactating cows. Dodd (1983) reported an incidence rate of 50% of udder infection in lactating cows when no hygiene or prevention program was implemented. Where postmilking teat dipping and dry cow therapy are practised as mastitis control measures, environmental pathogens such as coliforms and streptococcus spp. other than *Streptococcus agaloctiae* are the predominant etiologic agents involved in bovine clinical mastitis (Smith *et al.*, 1985). The causative organisms of mastitis are categorized as major or minor pathogens (Hogan and Smith, 1987; Harmon, 1994). The most common major pathogens include *Staphylococcus aureus, streptococcus agalactiae,* coliforms, Streptococci and enterococci, while *Corynebacterium bovis* and Coagulase negative staphylococci are considered to be minor pathogens.

In a field investigation of ovine mastitis in North Greece, clinical mastitis was recorded in 11.4% of ewes examined. Mycoplasma spp. and *Staphylococcus aureus* were the important pathogens as they were isolated from 45.9 and 38.5 percent respectively of mammary secretion samples while coagulase-negative staphylococci, *Pasteurella haemolytica, E. coli,* streptococci, *Corynebacterium pyogenes*, Bacillus spp. and Pseudomonas spp. were isolated at a lower rate (Fthenakis and Jones, 1990).

As Jordan lacks reliable information concerning the appropriate treatment of this disease and due to the unregulated use of veterinary drugs in dairy cows and ewes, present study was conducted to provide a statistically valid information available to the farmers and to be used as a foundation for establishing a mastitis control program in Irbid Governorate.

MATERIALS AND METHODS

Herds and flocks selection

Based on the 1994 farm records register of Jordan Ministry of Agriculture, there were 165 dairy farms in Irbid Governorate, that contained 10 or more adult cows (milking and dry). Of these, 125 farms were

registered in the Irbid Dairy Herd Cooperation (IDHC). At the head quarters of the IDHC a list of all herds were stratified according to their herd-size and location. A list which included all farms was prepared and herds were randomized. Using a table of random numbers, a sample of 12 herds (7 small herds with ≤29 cows in each, 3 medium herds with 30-59 cows and 2 herds with ≥60 cows) with a total of 420 milking cows were selected. All herds maintained individual cows identification. Friesian was the predominant breed with calving occurring throughout the year.

According to the Jordanian Department of Statistics, (1994) there were 789 Awassi sheep flocks registered in Irbid Governorate. Sheep farmers usually move their animals from place to place according to grass availability. Of all Awassi sheep flocks, 42 flocks were listed and a random sample of 6 flocks with 493 ewes was selected (due to limited funds) and used in this study.

Each selected farm was visited on a monthly basis. Mastitis animals were milk sampled during the visit. Between visits, all participating farmers were requested to minitor cases of clinical mastitis and to bring their mastitic animals to the Veterinary Diagnostic Laboratory, Veterinary Clinical Health Center, Jordan University of Science and Technology (Irbid) for clinical, bacteriological examination and treatment. Potential benefits of participation to the dairy producers and sheep farmers were the practical application of the laboratory results and the free of charge treatment of their mastitic animals.

Herds and flocks management

Selected dairy herds were housed in brick stanchion barns and milked twice daily using a pipeline milking system. Concentrate feeds were prepared and mixed in the IDHC milk and sold to all these farms. These feeds consisted of barley (55 %), soya bean (48% protein) 24%), wheat bran (18%), NaCl (1.4%) limestone dust (35% Ca) (1.5%) and vitamins and minerals supplementation (0.1%) each lactating cow was fed 5-6 kg of wheat straw per day and the concentrate ration was given according to her current milk pproduction. Wheat straw was replaced by green feed upon availability.

The lambing season in Jordan is between October to mid April. Lambs are usually weaned at the age of 2-3 months and ewes are hand milked until the middle of July. Sheep flocks grazed during spring season until the end of the harvesting season (August) with no to very little feeds supplementation. During fall and winter season sheep are usually housed and group fed on wheat straw and concentrate feeds.

Milk samples and data collection

Participating dairy herds and sheep flocks were monitored for one year and 9 months respectively. Milk samples and data collection began on 1 January, 1994. Clinical mastitis was defined by the presence of abnormalities in the udder (signs of inflammation or by abnormal udder secretions (flakes, clots or abnormalities in color or consistency). Teat of the affected quarter or half was carefully washed, dried and sanitized with Lugol's iodine solution. Finally the teat orifice was scrubbed with 70 % alcohol. Milk sampes from all clinical cases were collected. Approximately 10 ml of milk were collected aseptically after rejecting the first stream of milk. Collected samples were cooled immediately and transferred to the laboratory in an ice box to be immediately examined or refrigerated for not more than 24 hours of sampling. Samples were not collected from animals treated with antibiotics by any route within 96 hours before collection. A minimum of one month had to elapse between two cases of clinical mastitis in order for them to be considered as separate cases. The incidence density of clinical mastitis was calculated every month and was expressed as a rate per 100 cow or ewe-months.

Laboratory examination of milk

Each milk sample was divided and put into two sterile plastic or glass containers. Milk sediment was obtained by revolving one half of each milk sample at 3000 rpm for 10 minutes. The other half of each collected milk sample was incubated at 37°C for overnight. Loopfuls from each of the milk sediment and incubated milk were streaked onto dried plates of the following media: Nutrient agar (Hi-Media Labs.); 5% sheep blood agar (Becton Dickinson); MacConkey's agar (Hi Media Labs) and Sabouraud's agar (Mast Laboratories). All Inoculated plates were incubated at 37°C for 24 hours except for Sabouraud's agar plates which were incubated at 25°C for 3-5 days.

For obtaining pure cultures, suspected colonies on incubated plates were identified and streaked over Nutrient agar slants and incubated at 37°C for 24 hours. Purified cultures were subjected to microscopic examination of Gram stained smears and biochemical reactions. Isolates of staphylococci, streptococci, coliforms, corynebacteria and Pseudomonas were biochemically identified according to Carter et al. (1991). Cultures were considered positive when one or two species of bacteria known to cause mastitis were isolated, or when a contagious mastitis pathogen e.g. *Staphylococcus aureus* or *Streptococcus agalactiae* was recovered even in the mixture of environmental bacteria. Cultures were considered negative when no

bacterial growth was seen on the culture plates. Cultures with 3 or more species or when there was heavy growth of a mixture of environmental origin (environmental streptococci and coliforms) were considered contaminated.

All isolates were subjected to *in vitro* antibiotic sensitivity using discs of ampicillin, chloramphenicol, erythromycin, gentamycin, neomycin, penicillin, tetracycline and sulfamethoxazole. The diameter of the inhibition zone of each antibiotic used was recorded and compared with the standards published by the manufacturing company (Arcomex; Arab Comppany for Medical Diagnostics, Amman, Jordan).

RESULTS

Twelve dairy herds and 6 Awassi sheep flocks were used in this study and subjected to the analysis. Milk samples were obtained and cultured from 169 reported clinical cases occuring during the study period; 108 quarters and 61 udder halves (60 cows and 45 ewes). The incidence of clinical mastitis expressed as the number of clinical cases per 100 cows or ewe-months revealed an average incidence rate (mean$\pm$SD) of 3.35 ± 0.6 and 2.23 ± 0.33 per 100 cow and ewes-months, respectively.

The numbers and percentages of cows and ewes (clinical mastitis cases) with the different microbial isolates are shown in Table 1. Of the 169 mastitis milk samples (29 from cows and 11 from ewes indicated the presence of more than one type of bacteria. *Staphylococcus aureus* was the most common (31.35%) and *Streptococcus agalactiae* was the second common (22.70%) bacterium isolated from all examined milk samples.

The summary of comparison between the sediment and the incubated milk method in their ability for recovery of pathogens in the examined milk samples is shown in Table 2. About 67% of the isolated bacteria were recovered from the sediment method.

Sensitivity pattern of each isolate against the commonly available antibiotics for cows and Awassi ewes is presented in Table 3 and Table 4, respectively. Penicillin was the least effective antibiotic against bacteria recovered from cows and ewes.

DISCUSSION

Incidence of bovine clinical mastitis

Different methods have been used in measuring the incidence of clinical mastitis. Dohoo et al. (1983) used the lactational incidence rate (LIR) which is defined as the percentage of lactations with one or more occurrences of clinical mastitis. Others measured the incidence rate as a number of clinical cases per 100-cow quarters at risk (Kirk and Bartlett, 1988) or as an annual incidence rate per 100 heads at risk (Weigler *et al.*, 1990). Incidence also has been expressed as cases per 100 cow-months at risk (Bartlett *et al.*, 1992; Lafi *et al.*, 1994). Schukken *et al.* (1989) found an annual incidence rate of 1.5 cases per 100 cow-months among herds with low somatic cell count. Wilesmith *et al.* (1986) reported an overall incidence rate of 3.43 case per 100 cow-months. Weigler *et al.* (1990) reported an annual incidence rate of clinical mastitis of 36.8 cases per 100 heads at risk. This represent a rate of 3.1 cases per 100 cow-months. Bartlett *et al.* (1992) found an incidence of 3.17 cases per 100 cow-months. Lafi *et al.* (1994) found an overall incidence rate of clinical mastitis of 5.6 cases per 100 cow-months in 1991-92. The incidence found in present study is within the range reported in these studies.

In the literature, a great variation exists in type and percentage of bacterial agents isolated from clinical

Table 1: Microbial isolates recovered from 185 clinical mastitis cases (60 cows and 45 Awassi ewes) in Irbid Governorate, Jordan, 1994-95

Bacterial Isolates	Dairy Cows (n=60)[1]	%	Awassi Ewes (N=45)	%	Total	%
Staphylococcus aureus	37	31.36	21	31.34	58	31.35
Streptococcus agalactiae	25	21.19	17	25.38	42	22.70
Escherichia coli	24	20.34	13	19.40	37	20.00
Corynebacterium bovis	12	10.17	--	--	12	6.49
Corynebacterium pyogenes			5	7.46	5	2.70
Klebsiella spp.	10	8.47	--	--	10	5.41
Pseudomonas aeruginsa	--	--	6	8.96	6	3.24
No growth	10	9.32	5	7.46	15	8.11
Total number of isolates	118[2]	100	67[2]	100	185	100

[1]n = Number of animals; [2] some milk samples grew 2 microorganisms

Table 2: Comparison between the sediment and incubated milk method for the isolation of pathogens from mastitis milk samples in Irbid Governorate, Jordan, 1994-1995.

Bacterial isolates	Sediment		Incubated	
	n	%	n	%
Staphylococcus aureus (58)[1]	40	68.97	18	31.03
Streptococcus agalactiae (42)	23	54.76	19	45.24
Escherichia coli (37)	28	75.67	9	24.32
Corynebacterium bovis (12)	8	66.66	4	33.33
Corynebacterium pyogenes (5)	3	60.00	2	40.00
Klebsiella spp. (10)	7	70.00	3	30.00
Pseudomonas aeruginosa (6)	3	50.00	3	50.00
No growth (15)	5	33.34	10	66.66
Total	117	63.24	68	36.76

[1]Number in parenthesis denotes the total number of isolates.

mastitis cases. *Streptococcus agalactiae and Staphylococcus aureus* were the predominant isolates from clinical cases in Australia (Daniel *et al.*, 1982). *E. coli*, caogulase negative staphylococci and *Staphylococcus aureus* were the most common isolates in Netherlands (Schukken *et al.*, 1989). In british herds, Wilesmith *et al.* (1986) found that *E. coli* was the predominant organism isolated during their three years cohort study. In Sudan, Mohamed *et al.* (1993) found *Staphylococcus aureus* to be the most important cause of clinical mastitis in Friesian dairy cattle. In the present study, the most common bacterial isolates from bovine clinical mastitis was *Staphylococcus aureus* (31.36%) followed by *Streptococcus agalactiae* (21.19%). The variation in the importance of the different type and percentage of organisms observed in these studies is probably the results of the variations in farm husbandry and management factors.

Widespread antibiotic resistance were noted to Penicillin as 51.35% (19.37) of *Staphlococcus aureus* isolates were resistant to penicillin, as were 60 per cent of *Streptococcus agalactiae*, 66.66 per cent of *E. coli*, 50 per cent of *Corynebacterium bovis* and 60 per cent of Klebsiella spp. The low levels of *Staphylococcus aureus* strains sensitive to penicillin is well known phenomenon and may be due to the production of ß-lactamase (Penicillinase). Similar observation regarding penicillin resistance were reported in dairy herds raised in a comparable management conditions (Zingeser *et al.*, 1992). *Streptococcus agalactiae* was found to be highly susceptible to neomycin. This finding is in congruent with the data published by Orlova (1982). Sensitivity to chloramphenicol existed for 62.96 per cent (68/108) for all isolated bacteria.

Incidence of ovine clinical mastitis

Ovine clinical mastitis cases were diagnosed during the veterinary visit to the flock and between consecutive visits, the diagnosis of clinical case were dependent on the shepherds and/or farmers'experience and knowledge of clinical mastitis. Some mild case of clinical mastitis might not have been reported or overlooked by shepherds. The average incidence rate of our study was 2.23 cases per 100-ewe months. Torres-Hernandez and Hohenboken (1980) found an incidence of 11.5 per cent in their 5-month prospective study (equal to 2.3 cases per 100-ewe month). Previously Gross *et al.* (1978) reported an overall incidence rate of 100 clinical cases per 100-ewes during 2 consecutive spring seasons (equal to 3.33 cases per 100 cow-ewe). Our results are within the range reported in these studies.

Staphylococcus aureus, *Streptococcus agalactiae* and *E. coli* were the main aetiological agents of mastitis in ewes of the present study. Similar results had been reported by Fthenakis and Jones (1990) and Tola and Arbi (1990).

It is worth mentioning that Yeasts could not be detected in all examined samples. *Pseudomonas aeruginosa* failed to be isolated from the milk of mastitis cows, while *Corynebacterium bovis* and *klebsiella* spp. were not recovered from the ewe's milk.

In an attempt to increase the rate of recovery of pathogens, cultivation was carried out from milk sediment and pre-culture incubated milk. The

Table 3: Bacterial isolates from clinically mastitic cases of 60 cows in Irbid Governorate, Jordan, 1994-95 and their in vitro antibiotic sensitivity

	Dose/ disc	*Staphylococcus aureus* (n=37)[1]			*Streptococcus agalactiae* (n=25)			*Escherichia coli* (n = 24)			*Corynebacterium bovis* (12)			*Klebsiella* spp. (n = 10)		
Antibiotics		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	20 μg	5	9	23	15	7	4	13	6	5	7	2	3	8	2	0
Chloramphenicol	30 μg	3	8	26	3	8	14	1	6	17	2	7	3	1	1	8
Gentamycin	10 μg	2	9	26	9	1	8	3	5	12	2	6	4	6	2	2
Amoxicillin	25 μg	3	7	27	7	6	12	11	6	7	3	3	6	3	2	5
Neomycin	30 IU	2	10	25	2	7	16	3	7	14	2	6	4	4	2	4
Penicillin	10 IU	19	10	8	15	6	4	16	6	2	6	4	2	6	3	1
Tetracycline	30 μg	5	6	26	13	5	7	10	3	11	w	6	4	2	3	5
Sulfamethoxazole	25 μg	2	7	28	13	5	6	6	6	12	2	7	5	5	3	2

[1]n = Number of isolates, R = Resistant, 1 = Intermediate, S = Sensitive.

Table 4: Bacterial isolates from clinically mastitic cases of 45 Awassi ewes in Irbid Governorte, Jordan, 1994-1995 and their in vitro antibiotic sensitivity.

	Dose/ disc	*Staphylococcus aureus* (n=21)[1]			*Streptococcus agalactiae* (n=17)			*Escherichia coli* (n = 13)			*Corynebacterium bovis* (5)			*Pseudomonas aeruginosa* (n = 10)		
Antibiotics		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	20 μg	4	6	11	8	2	7	6	3	4	0	1	4	8	2	0
Chloramphenicol	30 μg	3	5	13	3	8	10	1	1	11	3	1	1	7	2	1
Gentamycin	10 μg	5	5	11	8	1	8	3	5	12	0	0	5	1	1	8
Amoxicillin	25 μg	3	7	11	7	6	4	3	1	9	1	2	2	3	2	5
Neomycin	30 IU	2	10	9	3	3	11	3	7	3	3	1	1	1	2	7
Penicillin	10 IU	14	5	2	11	4	2	3	2	8	1	1	3	6	3	1
Tetracycline	30 μg	5	6	11	7	3	7	3	1	9	1	1	3	5	3	2
Sulfamethoxazole	25 μg	0	7	14	8	6	3	6	4	3	3	1	1	6	2	2

[1]n = Number of isolates, R = Resistant, 1 = Intermediate, S = Sensitive.

superiority of the sediment technique over the pre-culture incubated milk technique in the ability to recover the aetiological agents of mastitis was proved, where 63.24 per cent of the isolated organisms could be recovered by the former and 36.76 per cent of the later (Table 2). These results substantiate what has been recommended by Dinsmore *et al.* (1992).

Our results showed that *Staphylococcus aureus* is the predominant cause of mastitis in cows and ewes, this may be due to the mechanism of virulence in Staphylococcal infections and the ability of the organism to invade tissues rather than to excrete substances.

The contamination of milker's hands, wash cloths, milking machine cups and bedding grounds may increase the incidence of both *Staphylococcus aureus* and *Streptococcus agalactiae*. The predominant position of *Streptococcus agalactiae* as a cause of clinical mastitis has been usurped by *Staphylococcus aureus* especially, in areas such as Jordan where the treatment of mastitis with penicillin has been practiced intensively and mechine milking has replaced hand milking (Farah, 1992). Although *Staphylococcus aureus* is still pre-eminent as a cuase of clinical mastitis, its prevalence has been significantly curbed by modern control programs based on teat dipping and dry period treatment. These programs have also led to an increase in infections by *E. coli*, Pseudomonas spp. and Klebsiella spp. The change in balance away from Gram-positive cocci to Gram-negative bacteria has been significant because they are resistant to hygienic control measures (Radostitis *et al.*, 1994).

The results of this study will be useful in the establishment of reference values for the incidence of clinical mastitis, bacterial isolation and their sensitivity to antibiotics which can be used as a foundation for establishing a mastitis control program in Irbid Governorate, Jordan.

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