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Characterisation of Clinical Mastitis Occurring in a Dairy Herd of Holstein Friesian Cows

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Abstract

The objective of the present study were to characterize clinical mastitis (CM) occurring in a dairy herd of Holstein Friesian cows located at Agricultural Research and Development Station (ARDS) Simnic-Craiova, Romania (182 m above sea level, 4°19'N and 23°48'E). The severity of CM was classified as grade 1 – when only the milk was abnormal, grade 2 – when abnormal milk was accompanied by swelling or redness of mammary gland and grade 3 – when the cow exhibited systemic signs of illness such as depression, anorexia, dehydration or fever. Only grade 1 and grade 2 of clinical mastitis were analysed in this study. Duplicate quarter milk samples from affected quarters when collected before treatment (Pre-treatment). After the collection, cows were treated using the form protocol. A second set of duplicate quarter milk sample were collected from enrolled quarters at 14 to 21 days after the end of treatment (Post-treatment). Cow level follow-up data was collected for 90 days after the enrolment. Microbiological diagnosis at enrolment included gram-negative, gram-positive and no growth. Data was collected between December 2016 and November 2020, of the 58 cases of CM only 52 cases of grade 1 (n=35) and grade 2 (n=17) were used in analysis. Six cases of CM were of grade 3 (not used in this study). Most causes of CM included in this study were caused by Gram-negative pathogens followed by gram positive pathogens. The common pathogens were E. coli, environmental streptococci Enterobacter spp. and coagulase-negative streptococci. Treatment cure was greater for Gram-negative pathogens.

Identification of pathogens causing CM or severity is important in strategic treatment decisions.

Keywords: Clinical Mastitis; Pathogens Causing Clinical Mastitis; Severity; Grade Of Clinical Mastitis

Introduction

In Holstein dairy cows, selection pressure for increased milk production has led to a higher susceptibility to disease, including mastitis.

Mastitis, inflammation of the mammary gland, is mainly caused by intramammary invasion of pathogens. Mastitis is one of the most frequent diseases of dairy cattle, and it has economic implications for the dairy industry due to costs associated with reduced milk production and milk quality, culling of animals, veterinary treatment, and animal welfare [1-3].

Mastitis is caused by a wide spectrum of pathogens and epidemiologically categorized into contagious and environmental mastitis. A cow is considered to have clinical mastitis (CM) if it presents abnormal milk secretion from one or more quarters, with sings of inflammation of the udder tissues (e.g. heat, swelling or discoloration of the skin; Kelton et al., 1998) [4].

A high proportion of dairy cows have subclinical mastitis (inflammation of the udder) as indicated by an elevated somatic cell count (SCC) but no signs of CM. subclinical mastitis also affects milk production and quality and is characterized by the presence of inflammatory components in the milk [5]. The leukocytes constitute the majority of these inflammatory components in the milk of the affected cows. Subclinical mastitis is more common and has serious impact in older lactating cows than in first lactation heifers.

The incidence of CM is high in many dairy herds around the world. A mean incidence rate of 23% was found among a sample of Canadian herds in 2008 [6].

Despite the fact that more research has been dedicated to mastitis control, it remains a persistent problem. Several studies have been conducted in the past to estimate the incidence rate of clinical mastitis (IRCM) in Europe [7-11], North America, Australia, New Zeeland, and Africa [12-18].

Mastitis is caused by a wide spectrum of pathogens. A wide range of phenotyping and genotyping methods have been developed to study mastitis pathogens in dairy cattle.

In E. coli mastitis; *Escherichia coli* is common cause of intramammary infection in dairy cattle. Infection usually manifests with clinical signs. E. coli is classified as an opportunistic environmental pathogen. The severity of clinical signs, which range from mild to fatal, is largely attributed to host-characteristics [19]. Recurrent cases of clinical E. coli mastitis could be due to repeated episodes of infection and cure, or to persistent infection with alternating subclinical and clinical episodes [20] such repeated episodes could due to chance or to increase host-level or quarter level susceptibility to infection. To be persistent, an intramammary infection would have to be caused by a single strain that was present for a long time, resulting in repeated isolation of the same strain from multiple clinical episodes [20].

Dogan et al. (2006)[21], reported that half of the recurrent cases occurred in the same mammary quarter as the initial case, and half of the recurrent cases within a mammary quarter were due to the same strain as the initial case. This shows that repeated infections and persistent infections do indeed occur. The high incidence of clinical E. coli mastitis in early lactation has been attributed to increased host susceptibility at that time [19]. Bradley and Green (2000) [22] using ERIC-typing showed that many clinical episodes of E. coli mastitis in early lactation could be traced back to infections that originated in the non-lactating period rather lactating period. This ERIC-typing method led to evaluation of antimicrobial products with a gram-negative spectrum for treatment and prevention of mastitis during the non-lactating period. Use of such a product reduced the incidence of E. coli mastitis during non-lactating period as well as the first 100 days of the following lactation [23].

In *Klebsiella mastitis*, the most common Klebsiella species causing bovine mastitis *are K-pneumoniae* and *K. oxytoca*. The heterogeneity of Klebsiella strains in the environment is reflected by strains heterogeneity among infected cows within a herd [24,25]. Molecular typing showed that apparent transmission was caused by contamination of the milking machine with different strains of Klebsiella by different cows.

Mastitis caused by K. pneumonia respond poorly to antibiotic treatment, and as a consequence, infections tend to be severe and long lasting [25].

Non-coliform gram-negative species may occasionally cause severe mastitis problems. *Pseudomonas aeruginosa* has been associated with mono-or polymicrobial abscesses and septic mastitis in women. Outbreaks of *P. aeruginosa* mastitis in dairy cattle have been reported from Australia [26], Ireland, Israel and Netherlands often with a high fatality rate [27-29].

In an outbreak of Serratia mastitis [30] a common risk factor was identified across herds, i.e. use of a chlorhexidine based teat dip. The suspect product had been contaminated on the individual farms. Within farm, animals were usually infected with a single strain of Serratia marcescens and the same strain was found in teat dip on some farms.

In dairy cattle, mastitis is the only disease associated with *Strep. agalactiae* infections. Transmission within herds is thought to be strictly contagious, i.e. from cow to cow, due to insufficient hygiene in the milking parlour, allowing multiple animals to come into contact with equipment, hands or towels that are contaminated by milk from an infected cow. This made of transmission results in the presence of a single strain in multiple cows in a herd [31-33].

Streptococcus uberis is strictly an animal pathogen. In one study [34], as many as 330 strains were detected among 343 isolated. An aseptically collected milk sample from an individual udder quarter usually contains a single strain of *Strep. uberis*.

Infections of multiple cows within a herd with a single strain have been described and have been attributed to cow to cow transmission. The cow factors rather than strains determine the duration of infections [35].

Streptococcus dysgalactiae has been described as a contagious pathogen. To date, all yielded results that fit with a mixed contagious-environmental epidemiology.

Wang, et al. [36] showed that in each of 3 herds they investigated, most or all of the infections were caused by the same strain. For *Strep. agalactiae, Strep. uberis* and for *Strep. dysgalactiae* mobile genetic elements may act as a vehicle for L.G.T. (lateral gene transfer) between streptococcal strains and species, including transfer of virulence genes and antimicrobial resistance genes [37,38]. The lactose-operon that is shared by *strep. agalactiae* and *strep. dysgalactiae subsp*. Dysgalactiae could constitute a major survival advantage in the bovine mammary gland [39]. *Strep. dysgalactiae subsp. dysgalactiae* also shares genes with *strep. pyogenes* and *strep. equi subsp. zooepidemicus, strep. uberis* and *strep. suis* [38,40].

Other streptococci that are occasionally associated with bovine mastitis included *strep. equi subsp. zooepidemicus* and *strep. canis*. In bovine mastitis diagnostics streptococci are often grouped with other genera such as enterococci and lactococci.

Staphylococcus aureus is a commensal and pathogen of humans and several animal species, including cattle.

Based on epidemiological studies and mastitis control efforts, *Staph. aureus* has been classified as a contagious pathogen [41], and this is supported by molecular data, which show that in most herds with *staph. aureus* mastitis, a single strain affects multiple cows and is often the most prevalent strain [42]. Molecular typing also supports a role of flies in transmission of *staph. aureus* between cows [43]. Many staphylococcal enterotoxin genes can be present in bovine staph. aureus, including staphylococcal enterotoxin A through D, G through O and U, toxic shock syndrome toxin and exfoliative toxins A and B [44]. LGT may contribute to the emergence of animal pathogenic strains from humans strains and vice versa [45,46].

Coagulase negative staphylococci (CoNS) are heterogeneous group of microorganisms with limited but non-negligible impact on udder health and productivity [47]. The molecular epidemiology of some of the most common CoNS species has been explored (Table 1).

Species identification method	Strain typing method	Target species (number of isolates)	Epidemiological comparison	Reference
API	PFGE	S. chromogenes (66)	Within – herd heterogeneity of CoNS populations.	Gillespie, et al. [48]
		S. epidermidis (37)		
		S. hyicus (38)		
		S. simulans (10)		
		S. warneri (7)		
VITEK	PFGE	S. chromogenes (27)	Within – cow: Persistence over dry period	Rajala –Schutz et al, [49]
		S. warneri (2)		
		S. xylosus (5)		
API Staph. system	PFGE	S. epidermidis (36)	Within – herd: Clonality of strains with antimicrobial resistance	Sawant, et al. [50]
API Staph. ribotyping	PFGE	S. chromogenes (46)	Within – herd heterogeneity in milk, bovine body sites and humans	Taponen, et al. [51]
		S. epidermidis (4)		
		S. simulans (21)		
Conventional	PFGE	S. epidermidis (200)	Between host comparison of human and bovine strain	Therberge, et al.

Table 1: Strain level molecular epidemiology studies of coagulase negative staphylococci from bovine milk and extra sources [20].

Staph. epidermidis from human skin is more common than isolation from bovine, antimicrobial resistance may contribute to clonal dissemination of *Staph. epidermidis* strains.

Staph. chromogenes and *staph. hyicus* infections may or may not persist over dry period. *Staph. hyicus* infection can last up to 10 months in lactation period [48].

More strains typing will be essential for detailed studies of transmission, persistence and cure of CoNS infections in dairy cattle.

Molecular methods for species-level identification have been developed for other genera of mastitis pathogens, including Prototheca and Mycoplasma. Prototheca have described as a cause of mastitis in Japan [52], Europe [53-54], and South and North America [55-56]. Molecular analyses were used to identify species and subspecies genotypes of Prototheca. P. zopfii genotype 1 and genotype 2, and *P. blaschkae* were identified. Mycoplasma spp. may affect multiple organ systems.

Asymptomatic carriage in the ears, and respiratory tract, otitis, pneumonia, and arthritis in calves, and mastitis in heifers and adult cows [57-58] were described using molecular epidemiology of mastitis-associated. *Mycoplasma spp., M. bovis*, *M. californicum* and Mycoplasma sp. bovine group 7 were identified. *M. bovis* or *M. californicum* were isolated from milk, udder parenchyma and supramammary lymph nodes. Mycoplasma is more heterogeneous in the respiratory tract.

Over 135 different microorganisms have been isolated from bovine intramammary infections (IMI), and majority of infections are caused by staphylococci streptococci, and gram-negative bacteria [59].

Diagnosis of mastitis needs to be early, rapid and accurate for management or therapeutic purposes. This envisages application of conventional or advanced diagnostic tests. The conventional diagnostic tests are relatively cheap rapidly available and field applicable, but usually non-specific. The advances tests are costly, requiring technical skill, but usually accurate and specific for different forms of mastitis [60,61].

The conventional tests aid in the confirmation of diagnosis when is used in combination with advanced tests, and are helpful in preliminary screening when is used alone. The various diagnostic tests of mastitis have been divided into general or phenotypic and specific or genotype tests. General mastitis indicators/markers are phenotypic mastitis diagnostic tests and indicate the general change that may be visible or non-visible and which are not specific to any pathogen but are diagnostic to mastitis. They include physico-chemico-biological diagnostics (Ph, electric conductivity, enzymes, biochemical molecules, SCC, CMT, digital mastitis detection teste, intramammary thermography, biosecresor or proteomics approaches). Specific mastitis diagnostic tests include the genotypic type of mastitis diagnostic tests that specifically detect the pathogen that cause the clinical, subclinical mastitis or their genetic materials. Also estimate the biomarkers relates to the pathogen [62]. These tests are: specific culture, PCR and its version, sequencing/molecular typing methods, advanced specific mastitis diagnostics (MALDI-TOF, specific, immunoassay, mastitis specific biomarkers).

Due to the strict milk quality regulations within the EU, there was adoption of individual cow SCC measurements at regular intervals. These measurements are carried out on milk collected for official determination of milk production, fat and protein levels as part of Romanian Dairy herd improvement program. At Agricultural Research and Development Station (ARDS) Simnic, Romania, 100% of cows are tested at 28 days intervals. Also, cow level SCC measurements are used to identify cows with infections subsequent collection of selected milk samples for bacteriological culturing.

In normal, healthy cow, SCC is around 70.000 cells/ml of milk but when a cow has an IMI, it increases sharply. Since SCC increases with the severity of mastitis it is used to indicate the IMI status at the time of sampling. Once SCC exceeds the selected cut-off (Australian cut-off ≥250.000 cells/ml, European Union cut-off ≥200.000 cells/ml, and New Zeeland cut-off ≥150.000 cells/ml), a cow is considered to have an IMI.

A simple test-day SCC may not be stable enough to accurately monitor subclinical mastitis, as SCC widely fluctuates between test days [63].

Finding additional predictors (such as electrical conductivity of milk), could increase the robustness and early predictive power of subclinical mastitis.

The objectives of the present study were to characterize clinical mastitis occurring in a herd of Holstein Friesian cows.

Materials and Methods

Data was collected between December 2016 and November 2020 from a research dairy herd with Holstein Friesian cows milked twice daily. The farm is located at Agricultural Research and Development Station (ARDS) Simnic – Craiova Romania (182 m above sea level, 4°19'N, 23°48'E). The herd size is 120 lactating cows and all are tested at 28 days interval for Romanian dairy herd improvement (DHI) program, use a milking routine that includes fore-stripping quarters for detection of mastitis, and use antimicrobials to treat affected cows. The research personnel were trained to classify severity of clinical mastitis using a previously defined system (Pinzón-Sánchez and Ruegg, 2011) [64]: grade 1 – when only the milk was abnormal; grade 2 – when abnormal milk was accompanied by swelling or redness of mammary of gland; grade 3 – when the cow exhibited systemic signs of illness such as depression, anorexia, dehydration, or fever.

Sampling and data collection were conducted by research personnel, using a previously procedure [65]. Mastitis cases were detected by research personnel who collected

duplicate quarter milk samples from only the chemically affected quarter(s) before treatment (PRE–treatment). After collection, cows were treated using the farm protocol. A second set of duplicate quarter milk samples were collected from the enrolled quarter(s) at 14 to 21 days after the end of treatment (POST–treatment). Milk samples were sent to ARDS Simnic; milk quality laboratory.

The research personnel also, recorded data for each case including the following information about cow characteristics: the date the clinical mastitis was detected, affected quarter, severity grade, drug and doses used, the date when milk returned to normal appearance (clinical cure). For repeated cases that occurred within 90 days after enrolment research personnel collected the same data as described above. Research personnel recorded additional information such as: death or culling of an enrolled cows, reason and date, date of the end of lactation, last of a quarter or any disease. Milk production and SCC for each cow were obtained from DHI test-day occurring 3 to 34 days before occurrence of the enrolled clinical mastitis case and from the DHI test-day occurring 14 to 52 days after treatment ended.

Microbiological Analysis

At the laboratory the frozen samples were thawed at room temperature and the microbiologic procedures were conducted according to Oliveira et al., [65], to Pinzón-Sánchez [64], and National Mastitis Council (NMC) guidelines [66]. On short: 100 μL of milk from each duplicate sample were plated onto each half of a blood agar and 10 μL were plated onto a quarter of a MacConkey agar. Plates were incubated at 37°C for 24 to 48 hours. Mannitol and tube coagulase reactions were used to diferentiate staphylococcus aureus from other staphylococci. Suspected Streptococcus spp. were identified as catalase negative, gram-positive cocci by the Christie, Atkins, Munch-Petersen test and esculin reaction. Gram-negative bacteria were identified using MacConkey agar, Gram strain, motility, indole, ornithine reactions, oxidase, and growth on triple sugar iron slant. Infection status was defined at the quarter level. An IMI was defined as the presence of 300 cfu/ml of identical colonies. Mixed infection was defined as the recovery of atleast 300 cfu/ml of 2 different types of bacteria from a sample. Milk samples were considered contaminated if 3 or more different colony types were found in the same milk samples. Data from quarters with non-significant growth (<300 cfu/ml) were combined with no growth for analysis. Results of each duplicate quarter milk sample were compared with a final case diagnosis (Table 1).

Days until clinical cure was defined as the number of days until the milk returned to normal appearance.

Microbiological out comes of PRE-treatment milk sample were categorized as Gram-positive, Gram-negative or no growth. Bacteriological cure was defined as absence of pathogens in the POST-treatment milk sample, regardless of recovery of a combative pathogen isolated in the PRE-treatment milk sample. When a pathogen was recovered in the PRE-treatment milk sample but POST-treatment milk sample was culture negative, the outcome was defined as treatment cure, and when no pathogens were recovered from either the PRE- or POST-treatment milk samples, the outcome was defined as spontaneous cure. Quarters with either treatment cure or spontaneous cure were classified as experiencing bacteriological cure. An enrolled quarter was classified as not experiencing bacteriological cure when any pathogen (or mixed infection) was present in the POST-treatment milk sample. A new infection was defined when a different pathogen (as compared with the PRE-treatment milk sample) was obtained in the POST-treatment milk sample, or when no pathogen was recovered in the PRE-treatment milk sample but a pathogen was recovered in the POST-treatment milk sample. Treatment failure was defined when the same pathogen was presented in both, the PRE- and POST-treatment milk samples.

Enrolled quarters with either new infection or treatment failure were classified as not experiencing bacteriological cure. Recurrence of clinical mastitis during follow-up period was defined as the occurrence of a case of clinical mastitis in any quarter of the same cow after the end of the milk-withholding period for the enrolled case. Somatic cell count (SCC) reduction after infection was defined at the cow level as a SCC below 200.000 cells/mL at the DHI test-day occurring between 14 to 52 post-treatment [64-65].

Milk production deviation was defined at the cow level as the difference between milk production at DHI test-day occurring between 3 to 34 d before occurrence of the clinical mastitis case and milk production at the DHI test day occurring between 14-21 to 52-55 d post treatment. Culling was defined as cows leaving the herd during 90 days follow-up period because of sale or death, as opposed to remaining in the herd as lactating or dry cows.

Statistical Analysis

It was performed only for cows treated solely in the clinically affected quarter using a commercially marketed intramammary (IMM) product containing 125 mg of ceftiofur and with a microbiologic diagnosis of Gram-positive, Gram-negative, or no growth.

The data were entered into Microsoft Excel computer program 2007. STATA version 14 was used to summarize the data and descriptive statistic was used to express the results.

Microbiological diagnosis of sample A	Microbiological diagnosis of sample 2	Diagnosis of case
Identical to B[1]	Identical A[1]	As identified
Pathogen[2]	No growth	Pathogen
Pathogen[2]	Contaminated[3]	Pathogen
No growth	Contaminated[3]	No growth
Pathogen[2]	Missing	Pathogen
No sample	No sample	Missing
TOTAL	-	-

Table 2: Criteria used to define diagnosis of cases based on microbiological results duplicate milk samples (A and B)[64].

Results

Herd characteristic

The herd size was 120 lactating Holstein Friesian cows, and mean daily milk production per cow was 305 kg. This study was made from December 2016 to November 2020 (48 months). The average bulk tank SCC was 250.000cells/ml all cows were milked in a herring-bone parlor (2x5, DeLaval). All cows were milked using a complete milking routine consisting of stripping of fore milk, pre and past dipping disinfection. At drying off the farm used an external sealant.

Characteristics of clinical mastitis

All cases of CM (58 cases occurring in 58 cows), that occurred during the sample period were recorded. The 3 symptoms were 60%, 29% and 11% respectively (Table 3).

Variable	n	%
Number of milking cows	120	-
Milk production (kg/cow period)	30.5	-
Bulk tank SCC (x 1000 cells/ml)	250.2	-
Duration of sampling period (d)	90	-
Al cases of CM:	58	-
Grade 1	35	60
Grade 2	17	29
Grade 3	6	11
Cases eligible for enrolment	58	-
Cases treated with IMM ceftiofur	52	-
Cases used in statistical analysis:	52	-
grade 1	35	67
grade 2	17	33
PRE-treatment diagnosis:		
Gram-positive	14	26.9
Gram-negative	18	34.6
No growth	20	38.5
Parity:		
First	4	7.7
Second	20	38.5
Third	13	25
> Third	15	28.8

Table 3: Characteristics of herd, cows and cases of clinical mastitis.

Of initial CM cases 52 cows were treated with IMM ceftiofur for treatment (Table 3).

Of cases included in statistical analysis (n=52) most occurred in multiparous cows (92.3%), compared with primiparous cows (7.7%) and greater proportion exhibited grade 1 as compared with grade 2 symptoms (Table 3).

Microbiological results

Microbiological diagnosis of the PRE-treatment samples was distributed as gram-positive (26.9%), gram-negative (34.6%) no growth (38.5%; Table 4).

Microbiological diagnosis	Pre-treatment		Post-treatment	
	n	%	n	%
Total gram-negative	18	34.6	3	5.7
Escherichia coli	10	19.2	1	1.9
Enterobacter spp.	5	9.6	1	1.9
Other gram negative	3	5.8	1	1.9
Total Gram-positive[1]	14	26.9	5	9.6
Environmental streptococci	8	15.4	2	3.8
Coagulase-negative staphylococci	4	7.7	1	1.9
Enterococcus spp.	1	1.9	1	1.9
Gram-positive *Bacillus* spp.	1	1.9	1	1.9
No growth	20	38.5	44	84.6
TOTAL	52	100	52	100

Table 4: Microbiological diagnosis of milk samples from clinical mastitis cases collected at enrolment (Pre-treatment) and 14-21 d after the end of treatment (POST-treatment).
1 = *Citrobacter* spp., *Pasteurella* spp. and *Pseudomonas* spp.

Most of the POST-treatment milk samples resulted in no bacterial growth. The most prevalent pathogens POST-treatment were environmental streptococci.

Variable	Cows		Grade of CM				
			Grade 1		Grade 2		P. value
	n	Mean	n	Mean	n	Mean	
Days in milk (DIM)	58	119.4	35	117.2	17	112.8	0.404
Individual SCC (x 1000 cell/ml)	58	250.2	35	258.2	17	259.1	0.128
Milk yield (kg/d)	58	30.5	35	29.8	17	29.4	0.222
Duration of treatment (d)	58	4.8	35	4.2	17	4.5	0.063
Days to clinical cure	58	5.2	35	5.2	17	5.4	0.448
Days of milk discard	58	7.1	35	6.6	17	7.8	0.212

Table 5: Characteristic of cows and treatment of grade 1 and grade 2 cases of clinical mastitis (CM), treated with IMM ceftiofur.

The average DIM at enrolment was 119.4 and was not associated with grade severity of CM (P = 0.404; table 5).

The average SCC at the DHI test previous to the cases of CM was 250.2 (x 1000) cells/ml. The SCC at previous DHI test from cows that experienced grade 2 CM was greater than those that experienced grade 1 cases of CM (259.1 vs. 258.2 (x 1000) cell/ml). Milk yield at enrolment was 30.5 kg/d at the DHI test previous to the cases of CM. Duration of treatment was 4.8 days for all cows enrolment in this study and was greater than that of grade 1 of CM (4.2 days) or grade 2 of CM (4.5 days; Table 5). The number of days to clinical cure

was 5.2 days for all cows enrolment in this study. The milk was discarded for 7.1 days for all cows (n = 58) enrolled in this study.

Bacteriological cure

The total proportion of bacteriological cure of enrolled quarters was 84.6% (44/52; Table 6).

PRE-treatment diagnosis	Bacteriological cure		Total
	n	%	n
Gram negative	15	83.3	18
Escherichia coli	9	90	10
Enterobacter spp.	4	80	5
Other gram negative[1]	2	66.6	3
Total Gram-positive	9	64.3	14
Environmental streptococci	6	75	8
Coagulase-negative staphylococci	3	75	4
Enterococcus spp.	0	0	1
Gram-positive *Bacillus* spp.	0	0	1
No growth	20	100	20
TOTAL	44	84.6	52

Table 6: Bacteriological cure for 52 cases of grade 1 and grade 2 clinical mastitis.
1 = *Pasteurella* spp. and *Pseudomonas* spp.

Bacteriological cure by microbiological diagnosis, at PRE-treatment was 83.3% for Gram-negative, 64.3 for Gram-positive. The proportion of bacteriological cure was 90% for Escherichia coli and for Enterobacter spp. 80%, as compared with 75% for environmental Streptococcus spp. and for coagulase-negative staphylococci (Table 6).

A single antimicrobial (ceftiofur) was used for treatment of cases presenting grade 1 and grade 2 symptoms. The proportions of cows that experienced SCC reduction at the DHI test date 14 to 52 d after the case was detected recurrence of clinical mastitis, and removal of the cows from the herd within 90 days follow-up period are not presented in this study.

Discussion

Most cases of clinical mastitis included in this study were caused by Gram-negative pathogens followed by Gram-positive pathogens. The common pathogens were E. coli, environmental streptococci. *Enterobacter* spp. and coagulase-negative streptococci. In this study *Staph. aureus* and *Strep. agalactiae* were not recovered from any cases. *E. coli* are considered an opportunistic pathogen and some risk factors associated with IMI include high milk yield, leaking milk, teat lesions, reduced capacity of the immune system exposure in an environmental source as bedding national and dirt.

Environmental streptococci were the most common gram-positive pathogen responsible for clinical mastitis in this study. They were frequently isolated in cases of CM in other studies [6,67].

The aim of this research was on the short-term outcomes after the treatment of grade 1 and grade 2 cases of CM occurring in a single quarter of affected cows. The farmers often evaluate treatments over the short-term rather than determine the effect over the entire lactation.

Although all treatments were recorded, only cases treated with IMM ceftiofur were able to be used for analysis.

The number of treatments using other compounds was not sufficient for analysis. Ceftiofur is a bread-spectrum third-generation cephalosporin antimicrobial that inhibits bacterial cell wall synthesis.

Bacteriological cure is the traditional method used to evaluate treatment efficiency. It is more objective then observation of clinical cure [65], but is not practical to evaluate this outcome in most form. Farmers usually do not have microbiological diagnosis before initiating treatment and microbiologically negative cases are treated without regard to etiology.

Researchers have reported a wide range of bacteriological cure (38-100%) for clinical mastitis caused by Gram-negative pathogens [64,65,68]. This can be explained because E. coli are more likely to respond explained because E. coli are more likely to respond favourably to treatments. Most cases of CM were grade 1 and grade 2 in severity.

Conclusions

In this dairy herd, environmental pathogens are the major cause of CM.

Characteristics and outcomes of CM cases depend on the pathogen causing CM.

Bacteriological cure was greatest for CM caused by Gram-negative pathogens.

Identification of pathogens causing CM, or severity, is important in strategic treatment decisions.

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