EPIDEMIOLOGY OF MASTITIS IN DAIRY BUFFALO AND COW IN TEHSIL SAMUNDRI OF DISTRICT FAISALABAD

By

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M.Sc. (Vety. C.M.S.)

A thesis submitted in partial fulfillment of

requirement for the degree of

DOCTOR OF PHILOSOPHY

IN

CLINICAL MEDICINE AND SURGERY
DEPARTMENT OF CLINICAL MEDICINE & SURGERY
FACULTY OF VETERINARY SCIENCES

UNIVERSITY OF AGRICULTURE

FAISALABAD

2009

To

The Controller of Examinations,

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ACKNOWLEDGEMENTS

I consider it my utmost obligation to express my gratitude to **Allah Almighty,** the omnipresent, kind and merciful who gave me the health, thoughts and the opportunity to complete this task. I offer my humble thanks from the core of my heart to the Holy Prophet, **Hazrat Muhammad (Peace Be Upon Him)** who is forever a torch bearer of guidance and knowledge for humanity as a whole.

In the completion of this work, I was fortunate in having the generous advice and encouragement of my learned supervisor, Dr. Ghulam Muhammad Professor, Department of Clinical Medicine and Surgery, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad in selecting the research topic, inspiring guidance, sympathetic and unstinted help at every step right from research synopsis to final manuscript writing. It is my privilege to express deep sense of gratefulness to my kind teacher, Dr. Muhammad Arshad, Associate Professor, Department of Veterinary Microbiology, University of Agriculture, Faisalabad for his valuable suggestions and guidance in planning, execution, analysis and write up of this manuscript.

I must record my special debt and heartiest gratitude to Dr. Ijaz Javed Hasan, Professor, Department of Physiology & Pharmacology, University of Agriculture, Faisalabad who shared his great fund of knowledge during completion of this work.

I am highly obliged and express profound gratitude to Dr. Muhammad Saqib and Dr. Muhammad Nadeem Asi, Lecturers Department of Clinical Medicine & Surgery, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad for assisting me in the execution of research work and thesis defense.

Sincere thanks are in order to Mr. Naeem Hussain Firaz Gill, Principal Noor ul Huda Computer Training Centre, Mamukanjan, Tanveer Ahmad and Waseem Ahmad (Ph.D. Students) for providing computer facilities.

The author expresses with a deep sense of gratitude, his deepest affections for his parents, wife and daughter Nida Mubeen, Sibgha tul Zahra who prayed for my success and encouraged me during this unusually prolonged nerve-wrecking period.

I reserve my final, though no less heartfelt thanks to Veterinary Officers (H) Dr. Muhammad Taqi, Dr. Rana Muhammad Khalid Khan, Dr. Binyamin and Dr. Fazal ur Rehman for helping me at various phases in surveillance of the mastitis in Tehsil Samundri, District Faisalabad, Pakistan.

LIAQUAT ALI

C O N T E N T S

- Acknowledgement
- List of Tables
- List of Figures
- List of Appendices

CHAPTER	TITLE	PAGE
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	5
III	MATERIALS AND METHODS	75
IV	RESULTS	82
V	DISCUSSION	167
VI	SUMMARY	196
	LITERATURE CITED	204

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
4.1	Prevalence of mastitis in buffaloes and cows on the basis of SFMT	99
4.2	Age based prevalence of mastitis in buffaloes	100
4.3	Age based prevalence of mastitis in cows	101
4.4	Lactation based prevalence of mastitis in buffaloes	103
4.5	Lactation based prevalence of mastitis in cows	104
4.6	Stage of lactation based prevalence of mastitis in buffaloes	106
4.7	Stage of lactation based prevalence of mastitis in cows	107
4.8	Breed based distribution of mastitis in cows	109
4.9	Quarter prevalence rates of mastitis in buffaloes and cows	110
4.10	Prevalence of mastitis vis-à-vis position of quarters in buffaloes	111
4.11	Prevalence of mastitis vis-à-vis position of quarters in cows	112
4.12	Prevalence of mastitis in buffaloes vis-à-vis distance between teat tip and ground	114
4.13	Prevalence of mastitis in cows vis-à-vis distance between teat tip and ground	115
4.14	Distribution of udder shape in buffaloes	117
4.15	Distribution of udder shape in cows	118
4.16	Udder shape in relation to prevalence of mastitis in buffaloes	119
4.17	Udder shape in relation to prevalence of mastitis in cows	120
4.18	Shape based distribution of teats in buffaloes	121
4.19	Pattern of teat shape in cows	122
4.20	Teat shape in relation to prevalence of mastitis in buffaloes	123
4.21	Teat shape vis-à-vis prevalence of mastitis in cows	124

4.22	Frequency of dung removal in relation to prevalence of mastitis in buffaloes	125
4.23	Frequency of dung removal in relation to prevalence of mastitis in cows	126
4.24	Drainage quality in relation to prevalence of mastitis in buffaloes	128
4.25	Drainage quality in relation to prevalence of mastitis in cows	129
4.26	Prevalence of mastitis in relation to nature of milk let down stimulus in buffaloes	130
4.27	Prevalence of mastitis in relation to nature of milk let down stimulus in cows	131
4.28	Prevalence of mastitis in buffaloes in relation to number of animals milked by milker	132
4.29	Prevalence of mastitis in cows in relation to the number of animals milked by a milker	133
4.30	General physical condition in relation to prevalence of mastitis in buffaloes	135
4.31	General physical condition in relation to prevalence of mastitis in cows	136
4.32	Education of farmers in relation to prevalence of mastitis in buffaloes	137
4.33	Education for farmers in relation to prevalence of mastitis in cows	138
4.34	Reproductive disorders vis-à-vis mastitis in buffaloes	140
4.35	Reproductive disorders vis-à-vis mastitis in cows	141
4.36	Distribution of reproductive disorders in various breeds of cattle	142
4.37	Association between teat injury and mastitis in buffaloes	143
4.38	Association between teat injury and mastitis in cows	144
4.39	Association between condition of floor in buffaloes	145
4.40	Association between condition of floor and	146

	mastitis in cows	
4.41	Association between ease of milk and mastitis in buffaloes	147
4.42	Association between ease of milk and mastitis in cows	148
4.43	Association between milk technique and mastitis in buffaloes	149
4.44	Association between milk technique and mastitis in cows	150
4.45	Association between udder oedema and mastitis in buffaloes	151
4.46	Association between udder oedema and mastitis in cows	152
4.47	Association between teat oedema and mastitis in buffaloes	153
4.48	Association between teat oedema and mastitis in cows	154
4.49	Association between blood in milk and mastitis in buffaloes	155
4.50	Association between blood in milk and mastitis in cows	156
4.51	Association between wallowing and mastitis in buffaloes	157
4.52	Association between wallowing and mastitis in cows	158
4.53	Association between teat stenosis and mastitis in buffaloes	159
4.54	Association between teat stenosis and mastitis in cows	160
4.55	Association between milk leakage and mastitis in buffaloes	161
4.56	Association between milk leakage and mastitis in cows	162
4.57	Frequency distribution of isolates recovered from buffaloes	163
4.58	Frequency distribution of isolates recovered from cows	164

4.59	Epidemiologic measures of association between factors and mastitis status in buffaloes	165
4.60	Epidemiologic measures of association between factors and mastitis status in cows	166

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
4.1	Age based prevalence of mastitis in buffaloes	102
4.2	Age based prevalence of mastitis in cows	102
4.3	Lactation based prevalence of mastitis in buffaloes	105
4.4	Lactation based prevalence of mastitis in cows	105
4.5	Stage of lactation based prevalence of mastitis in buffaloes	108
4.6	Stage of lactation based prevalence of mastitis in cows	108
4.7	Prevalence of mastitis vis-à-vis position of quarters in buffaloes	113
4.8	Prevalence of mastitis vis-à-vis position of quarters in cows	113
4.9	Prevalence of mastitis in buffaloes vis-à-vis distance between teat tip and ground	116
4.10	Prevalence of mastitis in cows vis-à-vis distance between teat tip and ground	116
4.11	Frequency of dung removal in relation to prevalence of mastitis in buffaloes	127
4.12	Frequency of dung removal in relation to prevalence of mastitis in cows	127
4.13	Prevalence of mastitis in buffaloes in relation to number of animals milked by milker	134
4.14	Prevalence of mastitis in cows in relation to the number of animals milked by a milker	134
4.15	Education of farmers in relation to prevalence of mastitis in buffaloes	139

4.16	Education for farmers in relation to prevalence of mastitis in cows	139

LIST OF APPENDICES

NO.	**TITLE**
I	The Map of Tehsil Samundri of District Faisalabad (Pakistan)
II	Epidemiology of mastitis in buffalo and cow in Tehsil Samundri of District Faisalabad
III	Clinico-Microbiological examination of bubaline and bovine clinical mastitis in Tehsil Samundri of District Faisalabad

CHAPTER 1

INTRODUCTION

The productive efficiency of dairy animals is adversely affected by suboptimal management, poor nutrition and various diseases in particular mastitis, which is one of the most important impediments confronting the economic milk production in Pakistan. It is a multifactor and the most costly disease of the dairy industry throughout the world (De Graves and Fetrow, 1991; Owens *et al.*, 1997) that affects both quality (Seaman *et al.*, 1988; Barbano, 1989) and quantity of milk (Arshad *et al.*, 1995). Field surveys of major livestock diseases in Pakistan have indicated that mastitis is one of the most important diseases of dairy animals in the country (Cady *et al.*, 1983; Ajmal, 1990; Hussain *et al.*, 2005). Owing to transmissibility of such diseases as tuberculosis, brucellosis, leptospirosis etc., through milk to human beings, the disease is also important from zoon tic standpoint.

Mastitis is the outcome of interaction of various factors associated with the host, pathogen(s) and the environment. Infectious agents, in particular various species of bacteria are the most important etiologic agents of mastitis. Association of some host, managerial and housing determinants with mastitis is well-established and was the subject of investigation and review in a recent small study (Bilal, 1999) involving only 200 buffaloes. However, a little or no local information is available on such epidemiological factors of mastitis as the milk-ability, teat and udder edema, teat stenosis, use of oxytocin for milk let down, milking technique etc. in relation to mastitis in cows and dairy buffaloes. The information on these potential risk factors is important for planning a control strategy of this costly disease of dairy industry in Pakistan.

In Pakistan and other developing countries owing to small herd sizes, the animals are predominantly hand-milked. Infectious agents of mastitis may be transmitted from infected to un-infected animals through milker's hand (Philpot, 1975; Oliver, 1975) especially because milk is often used as a lubricant for milking. Studies at National Institute for Research in Dairying, UK revealed that 50% milker's hands were infected before milking compared to 100% during milking (Dodd *et al.*, 1966 cited by Philpot, 1975). Motie *et al.* (1985) reported that mastitis in hand-milked cows was nearly twice as frequent as in machine-milked ones (25.1 VS 14.6 %). A survey of the level of udder infections and mastitis in 12 herds in the Salisbury (South Africa) revealed that cows in the two hand-milked herds investigated were seriously affected with udder infections and mastitis (56 and 61% of the cows in milk) (Milvid *et al.*, 1970 cited by Oliver, 1975).

The overall picture of mastitis in the literature shows about 40 per cent morbidity amongst dairy cows (and buffaloes) and a quarter infection rate of about 25 per cent. On the average, an affected quarter suffers nearly a 30 per cent reduction in productivity and an affected cow is estimated to lose 15 per cent of its production. The infection originates either from the infected udder or the contaminated environments. The major sources of pathogens and means of transmission include infected quarters and soiled udder, contaminated milking machines, teat cups, milker's hands, washing clothes, flies and surgical instruments. Moreover, the stage of lactation, lactation number, trauma to udder, teat and teat canal, loose teat sphincters, lesions on teat skin, immunological status of each mammary gland, bulk of infection in the environment and managemental conditions are amongst the determinants which dictate the level of mastitis incidence (Radostits *et al.*, 2000).

It is common practice in Punjab (Pakistan) to use a calf for stimulating the let down of milk (Egenolf, 1990). Socci and Redalli (1973) reported that calves are a possible agency of mastitis organism's transmission. Many farmers also inject oxytocin for let down of milk.

However, there is no local information on the association of this hormone and mastitis. The present study was designed with the following objectives in view:

a) to determine frequency distribution of mastitis in dairy buffaloes and cows,

b) to determine association of some potential epidemiologic factors with the disease and,

c) to isolate different types of microorganisms associated with mastitis.

CHAPTER 2

REVIEW OF LITERATURE

A. PREVALENCE AND INCIDENCE OF MASTITIS IN BUFFALOES AND COWS

A sizeable number of epidemiologic studies (mainly of descriptive nature) have been undertaken on the prevalence and incidence rate of mastitis in cows and buffaloes. Allore (1993) reviewed some of these important studies conducted in countries (India, Pakistan. Indonesia, Srilanka and Egypt) which are endowed with both cows and buffaloes. Table 1 and 2 depict the incidence rates of clinical and sub clinical mastitis in cow. Similarly, incidence rates of clinical and sub clinical mastitis in buffaloes have been depicted in Table 3 and 4, respectively. In addition to tabulating the incidence rate of sub clinical and clinical mastitis in cows and buffaloes, Allore (1993) also critically analyzed these studies for their weaknesses. The weaknesses of these and most other studies can be categorized into 4 areas viz., design problems, management factors, genetic differences and scientific accuracy. Each of these areas may be source of variation in the reported incidence rate. Because of the lack of uniform design, management, genetic, and testing method, reports which cite past incidence rates may be of such a different design that they do not form a valid comparison or supportive reference.

Design problems include: (a) grouping of animals of different species, (b) using a subjective animal-side test such as California mastitis test (CMT), Whiteside test (WST), Modified Whiteside Test (MWT), Bromthymol Blue Test (BTB), Sodium Laurylsulphate Test (SLST) and Surf Field Mastitis Test (SFMT) for screening animals for mastitis, (c) not differentiating different breeds, (d) basing results on one test per animal per herd, and (e) reporting only infection rates or quarter infection rates (QIR) which does not give a sense of how many quarters per infected animal on an average were infected as only a small number of animals may be responsible for the majority of infections.

Similarly, lacunae in the past studies related to management include: (a) not stating whether the herd was machine or hand milked, (b) not mentioning whether or not calf suckling was involved, (c) lack of information concerning milking time hygiene practices (e.g. pre and post milking antiseptic teat dipping), and (d) not mentioning whether the herds investigated were research herds or privately owned ones.

Genetic factors include lack of information concerning differences between species and breeds.

Lacunae related to scientific accuracy of the published studies include: (a) the questionable accuracy and repeatability of the indirect tests for different species and across studies, (b) culturing problems (e.g. *Escherichia coli* infections tend to be of short duration and may be spontaneously cured by the time of sampling. Also the number of this organism is generally too small and plating only 0.1mL may miss many *bona fide* infections of this organism), and (c) taxonomy problems (e.g. lack of attempts to classify the organism to species level).

It is worth pointing out that most of the studies reviewed by Allore (1993) dealt with prevalence but she erroneously categorized these as incidence studies. Some important studies not reviewed by Allore (1993) are briefly described below:

According to Kalara and Dhanda (1964), in the rural areas of the North West India, the overall incidence of clinical mastitis was 7.5% in buffaloes and 8.80% in cows. In urban areas, the corresponding value was 10.23% in buffaloes and 11.08% in cows.

Owing to stronger smooth muscles around the teat opening (Uppal, 1994), buffaloes are generally less susceptible to mastitis. Notwithstanding this general conclusion, Said and Malik (1968) reported a staggering figure of 85.07% for prevalence of sub-clinical mastitis in dairy buffaloes. Quarter infection rates of 52.3, 42.4, and 25% were noted in buffaloes kept in modern establishments, those raised in small groups in rural areas and those kept individually under village conditions, respectively.

Hoare and Roberts (1972) reported mastitis incidence of 43.1% for cows and 21.4% for quarters.

Wilson and Richards (1980) reported the results of a national survey of mastitis in England. Five hundred herds were examined to determine the prevalence of sub clinical mastitis in the British dairy herds. The prevalence of the various infections recorded were *Streptococcus agalactiae* 3.4 % of quarters, *Str. dysgalactiae* 1.1%, *Str. uberis* 1.5% and *Staphylococcus pyogenes* 8.1 per cent. There were regional differences in the prevalence of some of these pathogens. The national prevalence of sub clinical mastitis as per the criteria of the International Dairy Federation was 9.6% of all quarters. Udder infections were less prevalent in herds which practiced mastitis control measures. The prevalence of infections reduced as the size of the herds increased. However, as the adoption of mastitis control measures was greater in the larger herds, it seems likely to be widespread use of control measures. The most important factor responsible for the low prevalence of sub clinical mastitis is bigger herds.

Jamaican workers (Zingerser *et al.*, 1991) reported the results of a 17-month national survey of clinical and sub clinical mastitis between April 1985 and August 1986. Eighty nine Jamaican diary herds with 10 or more cows were visited. A total of 645 lactating cows were examined using the CMT and 254 composite milk samples collected for bacteriological examination. Widespread management faults were noted especially related to milking machine usage and maintenance and the abuse of antibiotics. Fifty six per cent of all quarters were found to have CMT scores of one or higher, 0.8% showed clinical mastitis and 3.2% were blind. The most common bacterial pathogen, *Staphylococcus aureus* was recovered from 31% of sampled cows. The resultant milk loss from clinical and sub clinical mastitis was estimated to be 20% of the potential national production.

A research study conducted in 1994 by Saini *et al.* involving 123 crossbred cows and 241 buffaloes indicated the presence of sub clinical mastitis in 4.87 and 2.59% quarters, and 17.33 and 9.57% animals of respective dairy species. Seventy two, 5 and 2 respectively of the affected animals had 1, 2 and 3 quarters affected, with incidence being greater in hind quarters in both species. Incidence of sub clinical mastitis tended to increase with lactation number and was higher in early and late lactation.

Arshad *et al.* (1995) investigated some epidemiological aspects of mastitis in District Gujrat (Pakistan). A total of 4500 smallholder herds were surveyed. Quarter infection rate was significantly higher in hind than in fore quarters in buffaloes. Age and lactation based distribution of mastitis cases varied in different age and lactation groups, being highest in 6-8 year age group. Mastitis prevalence was higher during early stage of lactation than in middle and late stages. The disease was estimated to cause 20.63% decrease in milk production in affected animals.

Bansal *et al.* (1995) investigated milk samples obtained from 154 cows in different herds, and 117 buffaloes in 5 herds in the Indian Punjab province. Sub clinical mastitis was found in 48% of cows and 27.05% cow udder quarters, and 23.93% of buffaloes and 11.32% of buffalo's udder quarters.

Prabhakar *et al.* (1995) studied the incidence of clinical mastitis and its etiologic agents at five farms. The overall monthly incidence was found to be 4.06 per cent. Of the total of 421 buffaloes, 40 were affected with clinical mastitis in 76 quarters. Staphylococci were the major causative organisms (34.21% *S. aureus* and 13.16% coagulase-negative Staphylococci), followed by *Str. agalactiae* (14.74%), *E. coli* (10.53%), *Pseudomonas* spp (7.89%), *Str. pyogenes* (3.95%), *Klebsiella* spp. (3.95%), *Str. dysgalactiae* (2.63%), *Proteus* spp. (2.63%), *Str. uberis*, Diptheroids and mixed infections (1.31% each). No organism could be isolated from 2.63% quarters.

To investigate the prevalence of clinical and sub clinical mastitis in buffalo in Hyderabad (Pakistan), Soomro *et al.* (1997) conducted a study on 785 milk samples collected from 200 buffaloes during 1994-95. The physical examination of the udder of these animals revealed 6% clinical (chronic) and 1.0% congenital abnormalities of udder. Three chemical tests viz., Bromothymol blue test, chloride test and Whiteside test were applied on milk, which respectively revealed 19.7%, 15.9% and 13.85% quarters affected with sub clinical mastitis. In general, 33.3 percent animals had sub clinical mastitis. More recently, Khan *et al.* (2004) using Surf field mastitis test and bacteriological examination of quarter milk samples of 50 buffaloes documented 27, 4, 10 per cent prevalence for sub clinical mastitis, clinical mastitis and blind quarters, respectively.

Qazi *et al.* (1999) surveyed 45 different small livestock units/herds in Lahore (Pakistan) for epidemiologic data on mastitis. Analysis of data showed a prevalence of 8.8% in herds. The prevalence in lactating animals was 8.3%. Of 1000 quarters milk samples, 14.3% were positive for sub clinical mastitis. Highest prevalence of mastitis was recorded in 6-8 year old cows and buffaloes. Out of positive cases the prevalence was highest (53.63%) during early lactation followed by middle (21.97%) and late lactation (24.4%).The prevalence was higher in high yielding animals. Surgically manipulated animals were more prone to disease (4%).

Using a modification of California mastitis test, Lalrinthuanga *et al.* (2003) screened 987 quarters of 248 cows in various dairy pockets and villages of Aizawl area of Mizoram province (India). The results indicated that 37.5 per cent of the animals and 11.65 per cent of quarters were positive for mastitis. Among them, only 2.6 percent of quarters were positive for clinical mastitis, the rest (9.05%) had sub clinical mastitis.

Recently, Hussain *et al.* (2005) reported the results of a farmer's participatory surveillance study of livestock diseases in Islamabad capital territory. The technique of proportional piling was used to estimate the relative prevalence of livestock diseases in the area. For this purpose, 100 beans (or pebbles at some places) were given to the farmers and they were asked to make piles according the relative incidence of 5 most prevalent disease. The results indicated that hemorrhagic septicemia, foot-and-mouth disease and mastitis were the 3 most common disease problems. Gender stratification of the incidence data revealed that 15.8 and 16.3% of the male and female livestock keepers respectively had noticed occurrence of mastitis in their cows and buffaloes.

Karimuribo *et al.,* (2006) conducted a cross-sectional study of 400 randomly selected stallholder dairy farms in the Tanga and Iringa regions of Tanzania. Fourteen percent (confidence interval; CI = 11.6-17.3) of cows had developed clinical mastitis during the pervious year. The point prevalence of sub clinical mastitis, defined as a quarter positive by the California mastitis Test (CMT) or by bacteriological culture was 46.2% (95% CI=43.6-48.8) and 24.3% (95% CI= 22.2-26-6,) respectively. In a longitudinal disease study in Iringa, the incidence of clinical mastitis was 31.7 cases per 100 cows-years. A randomized intervention trail indicated that intramammary antibiotics significantly reduced the proportion of bacteriologic ally positive quarter in the short-term (14 days post-infusion) but teat dipping has no detectable effect on bacteriological infection and CMT positive quarters. Other risk and protective factors identified from both the cross-sectional and longitudinal studies included animals with Boran breeding (odds ratio (OR) = 3.40, 95% CI = 1.00-11-57, P<0.05 for clinical mastitis, and OR= 3.51, 95% CI = 1.29-9.55,P<0.01 for a CMT positive quarter), while the practice of residual calf suckling was protective for a bacteriologic ally positive quarter (OR = 0.63, 95% CI = 0.48-0.81, P < 0.001) and for a CMT positive

quarter (OR = 0.69, 95% CI = 0.63-0.75, P <0.001). A mastitis training course for farmers and extension officers was held and the knowledge gained and use of different methods of dissemination was assessed over time. In a subsequent randomized controlled trial, there were associations between knowledge gained and both the individual questions asked and the combination of dissemination methods (village meeting, video and handout) used. This study demonstrated that both clinical and sub clinical mastitis is common in small holder dairying in Tanzania, and that some of the risk and protective factors for mastitis can be addressed by practical management of dairy cows following effective knowledge transfer.

B) ASSOCIATION OF HOST, MANAGERIAL, ENVIRONMENTAL AND OTHER DETERMINANTS WITH BOVINE (COW) AND BUBALINE (BUFFALO) MASTITIS

Mastitis is associated with three basic interrelated factors: environment, microorganisms and animal individual features, such as structures of the udder and teat (Saratis and Grunert, 1993)

B.1 Association of host related determinants with mastitis

Oliver *et al.* (1956) investigated the variation in incidence of udder infection and mastitis with the stage of lactation and season of the year and was recorded the highest incidence during the 4th lactation.

Ewbank (1966) reported in housed herd, tied up cows, higher the incidence of sub clinical mastitis was occurred in side the udder more frequently in contact with the floor. The more time a cow spent lying on one particular side, the greater the difference in mastitis status between lain-on and un lain-on sides of the udder. There was, however, no difference in clinical signs or in the rate of infection with pathogenic bacteria between the two sides of the udder. The Little bedding was provided, and the general level of mastitis in the herd was found high.

Certain morphological patterns of udder form are known to predispose the dairy animals to mastitis. Khamis and Saleh (1969) examined

500 lactating buffaloes to classify the different morphological patterns of their udder form. Only 82 animals (16.4%) had morphologically ideal udder. The predominant buffalo udder form was the hanging type (54.8%). The problem of the high percentage of the deformed udder was discussed and it was related to the milking.

According to Smith and Schultze (1970), cows are known to differ in their susceptibility to udder infection. The difference may be an expression of variability in ease with which bacteria traverse the teat canal and the ability of these bacteria to multiply within the udder. These workers developed a simple index to measure the resistance of cows to mastitis. This index presupposes that more susceptible an individual is to a disease, the sooner the symptoms can be observed after the initiation of a standardized exposure period. Thus this index measures resistance by the percentage of a lactation that elapses before mastitis is detected. These workers considered the following 4 possibilities:

$$I_1 = \frac{D_1}{DL}$$

$$I_2 = \frac{D_1 + D_2}{2(DL)}$$

$$I_3 = \frac{D_1 + D_2 + D_3}{3(DL)}$$

$$I_4 = \frac{D_1 + D_2 + D_3 + D_4}{4(DL)}$$

Where DL = days in lactation

D_1 = days in lactation until mastitis was reported in the 1st quarter to show symptoms
D_2 = days in lactation until mastitis was reported in the second quarter to show symptoms
D_3 = days in lactation until mastitis was reported in the third quarter
D_4 = days in lactation until mastitis was reported in the fourth quarter

Using these indices of mastitis resistance, if a cow has mastitis in one or more quarters when she freshens she may receive a 0 for resistance score and if she passes the complete lactation without showing mastitis she receives a score of 1. Mastitis was determined by the strip cup test used at each milking. Other measures, such as days of discovery of an infection when cows are cultured routinely or days until cell count rises above some maximum normal value may also be useful as measures of mastitis in the indices.

Ronie and Munsterhjelm (1974) analyzed 178 cases of acute mastitis and concluded that the incidence was highest in 5 years old animals. The disease occurred most frequently in the 2nd to 3rd month (23.6% of the cases), or 1st week (22.5%) of lactation; 66.1% of infections were in rear udder quarters, and infections were more frequent in left (56%) than right quarters. Fifty four per cent of staphylococcal and 15% of coliform infections were diagnosed within one week after parturition and 6 of 7 coryneform infections occurred during the dry period. Staphylococcal and streptococcal infections occurred most frequently (71.4% and 73%, respectively) in the rear quarters.

Madsen *et al.* (1974) investigated the udder infections in a total of 7000 cows in 400 Danish dairy herds, representative of the whole country on the basis of age of cows and herd size. A quarter infection rates of 19.1% were recorded. *Staphylococcus aureus* accounted for 33.1% of the infections, Streptococci for 33.1%, Micrococci for 23.5% and mixed other infections for 10.1%. The number of infected quarters was not affected by stage of lactation, but increased with lactation number upto the 6^{th} lactation.

According to Harrop *et al.* (1974), the incidence of mastitis in cows varied from 24.10 to 32.80% during first four lactations. The rate of mastitis increased with the increase of lactation number subsequent to second lactation. As per Miller *et al.* (1976), the incidence of mastitis increased at a decreasing rate as the total milk yield in the affected lactation increased.

Rathore (1976) grouped a total of 584 randomly selected the Friesian cows from six dairy farms in New South Wales according to their teat shape categories. The results showed that cows with cylindrical shaped teats produced 10.9% less milk than cows with funnel shaped teats. On the basis of somatic cell count (SCC = 440000±394000), cows with cylindrical shaped teats had a significantly higher incidence of mastitis as compared to cows with funnel shaped teats (207000±123000) possibly due to a higher incidence of teat cup crawl in the former teat shape category.

Smith and Coetzee (1978) reported that of the 888 milking cows, 151 (17%) were infected in the right and left halves of the udder. Nearly 57% of all infections were in the hind quarters and 43.4% in forequarters. As for the parity, 34.7% of udders were infected at the end of first lactation whereas at the end of the 4^{th} or later lactations, 77.1% of the udders were infected.

Pearson and Machie (1979) investigated the occurrence of clinical mastitis in three dairy herds over a 3 years period. Hind quarters were affected twice as often as front quarters and cows were more susceptible during first two months of lactation.

Gonzalez *et al.* (1980) reported the lowest frequency of sub clinical mastitis in the first lactation cows.

Sharma (1983) reported that clinical mastitis reduced milk production in cows and buffaloes to the tune of 26% and 19% respectively while sub clinical mastitis affected reductions of 19 and 12%, respectively. Most of the *Staphylococcal* and *E. coli* infections occurred within one week of calving. New quarter infection was predominantly associated with Staphylococcal and Streptococcal infections. Whereas in dry period corynebacterial infections predominated.

The rate of occurrence of mastitis in buffaloes and cows as reported by Khalaf (1983) increased with the increase of lactation number and age, and was high in early lactation.

The clinical mastitis was reported by Lucey and Rowladim (1984) in 24% of lactations. No recovery of milk was observed in subsequent lactations in quarters that were free from mastitis, indicating that once a cow had contracted mastitis it might not achieve its full milk yield potential in the subsequent lactations.

A close correlation between susceptibility or resistance to mastitis and the shape, size of udder, teats, rate of milking and immunological status of cows was observed by Henskh (1985).

Slee and Orist (1985) reported that the mastitis in cows caused by pyogenic bacteria occurred during peak lactation at or soon after calving in winter or early spring.

Golodetz (1985) reported that mastitis caused by *E. coli* was higher in early lactation than in middle and late lactation.

Rasool *et al.* (1985) reported that the incidence of mastitis increased with the increase of lactation number, age and in early lactation. It was maximum in 7th and 6th lactations in cows and buffaloes, respectively. Above investigators documented that the difference in the incidence of disease in the left and right quarters was non-significant. Moreover in buffaloes the incidence of mastitis was higher in hind quarters vs front .

Okuneva and Bairak (1985) reported that the mammary infection in cows was associated with the poor lysozyme activity in milk.

Smith and Hagstad (1985) reported that the incidence of opportunistic staphylococcal infections e.g. *S. epidermidis* decreased with the advancing age while that of *S. aureus* increased with the increase in age. The prevalence of *S. aureus* infections increased throughout lactation. Above workers reported that *S. epidermidis* increased upto the 10th month then decreased, and infection with coagulase negative staphylococci decreased with advancing month of lactation upto 10th month. Henceforth there was an increase followed by a decline.

The occurrence of mastitis in buffaloes between the fourth and sixth lactation was reported by Didonet *et al.* (1986).And was noted that the disease had tendency to affect only one quarter without predilection.

According to Sheikh (1987), 6.9% of 4523 milk samples of buffaloes and 8.18% of 8981 cows examined at Veterinary Research Institute, Lahore (Pakistan) and found positive for mastitis in various tests.

Al-Shawabkeh and Abdul Aziz (1987) founded the incidence of mastitis increased with the number of lactations. Moreover the incidence of mastitis in hind quarters was higher than in the fore quarters and in right than left ones. It was further observed that the sub clinical mastitis was more likely to occur in older animals had shown previously mastitis.

According to Andersen (1987), approximately 25 per cent acute clinical mastitis occurred in the first 10 days of calving.

Breeds of dairy cow differ in their susceptibility to mastitis. Morese *et al.* (1987) documented that among Holstein – Friesian cows, least squares mean occurrence of clinical mastitis increased from 44 to 212% between 1st and 5th parity compared with an increase from 50 to 89 percent for Jersey. Mastitis incidence was highest in May-June calving and lowest after calving in September-October.

Prabhakar (1988) examined the milk samples and uterine contents of 9 cows and 5 buffaloes that developed mastitis concurrently with metritis for the presence of *S. aureus*, *S. epidermidis*, *Str. agalactiae* and *E. coli*. The same organisms were found in both samples for 12 (85.71%) of the animals.

According to Sastry *et al.* (1988), a higher incidence of mastitis was observed in hind udder quarters vs fore quarters (42.4 vs 37.6%) in buffaloes.

In a study focusing on factors responsible for clinical mastitis, Heescheu (1988) founded that any thing that resulted in teat damage, (for example trampled teats, barbed wire injury or milking machine injury) increased the chances of mastitis.

Pluvinage *et al.* (1988) reported that the incidence of mastitis was 8.5 percent in the 1st lactation. The risk factors for mastitis also included the hind quarters being more developed than front quarters beneath the hock line.

According to Dutta *et al.* (1988), the lactational incidence of mastitis ranged from 16.1 to 52.2% for Jersey cows and 16.5 to 33.3 per cent for crossbred cows. The risk ratios for the development of mastitis were 1.21 to 1.98 times greater in Jersey cows than in crossbred cows.

Marlos *et al.* (1988) reported that 83% of cows with endometritis developed acute mastitis following parturition.

Regression analysis by Geer *et al.* (1988) indicated the increased risk of mastitis due to the leakage of milk, teat lesions and abnormal teat shape while low milk ability decreased the risk of mastitis.Avove investigators reported that 74 percent of affected cows had mastitis in hind quarters, and 50 percent of cases was occurred in the first 30 days of lactation.

According to Badran (1989), the rate of mastitis was higher during the middle and the late stages of lactation than during the early stage. The rate of mastitis was higher during periods of high milk yield vs of low milk yield. Mastitis score was higher during winter, spring and autumn than during summer.

According to Hogan *et al.* (1989), 305 days milk yield from healthy cows averaged 6600 kg. Cows with clinical and sub clinical mastitis produced 275 and 485 kg less milk, respectively. The age and lactation-wise distribution of mastitis cases varied in different age groups, being highest in 6-8 years of age group (40.55%).

The relationship between the retained placenta and mastitis was investigated by Schukken *et al.* (1989). Cows with retained placenta (n = 62) were three times more likely to develop mastitis during hospitalization than animals without retained placenta (n = 134).

Mastitis has been implicated in decreasing reproductive performance of dairy cows. Moor *et al.* (1991) reported a negative correlation between clinical mastitis and reproduction due to altered interest us intervals and decreased luteal phase length in cows with clinical mastitis caused by Gram negative pathogens. Gram negative mastitis pathogens may stimulate production of prostaglandin (F2α) which subsequently would cause luteal regression.

Risk factors for clinical mastitis were investigated by Schukken *et al.* (1991).The factors included cleaning procedures, cow and cubicle cleanliness, feeds and feeding, dry cow management, milking procedures, milking machine, milk production and disease prevention. The milk production, drinking water source, amount of bedding and ventilation were other important factors in the *S. aureus* model. Teat disinfection was important risk factor in the *E. coli* model but was less important in *S. aureus*.

According to Feroze (1992), age and lactation-wise distribution of mastitis varied in different age and lactation groups, being highest in 6-8 years age group (40.55%). Number of case was higher (53.63%) during early stage of lactation than in middle and late stage. The disease was reported to cause 20.63% decrease in milk production of affected animals.

Summer mastitis was observed by Pyroala *et al.* (1992) in 20 cows and in 3 heifers. Most cases of mastitis occurred in housed animals and had often been preceded by teat injury. They also found higher number of cases (53.63%) during early stage of lactation than in middle and late stages. Affected animals showed 20.63% decrease in milk yield.

Adkinson *et al.* (1993) investigated the clinical mastitis episodes occurring from 1962 through 1991 in Louisiana State University Dairy Research Herd (USA) to determine the distribution of clinical mastitis among quarters of the udder. Data were derived from detailed records of all mastitis episodes that occurred during 1630 Holstein lactations. Incidence of episodes (categorized according to which quarters within a cow were clinical for a given case) were compared with mathematical expectations based upon the assumption that quarters were independent. Results indicated that quarters within a cow were more alike with respect to clinical mastitis than would be expected if quarters were independent. More episodes occurred in which either no quarter or all four quarters were clinical. Deviation of observed frequencies from expectation could have resulted from generalized cow differences, such as cow milk yield, immune competency, mammary type characteristics, and general health. Front quarters had less clinical mastitis than rear quarters while all episodes were considered. No difference was observed in the incidence between front and rear quarters in first episodes and in first lactations. The investigator did not observe any difference between the incidence in left and right quarters. Diagonal pairs occurred less often

Indian worker (Roy *et al.*, 1993) investigated the influence in the size of the teat and lactation number on the incidence of sub clinical mastitis in crossbred cows. Animals were divided into 3 groups on the basis of the size (length) of teats. Animals having teat length less than 5.5 cm were included in Group A and those with teat length from 5.6 to 7.5 cm and above 7.5 in Group B and C, respectively. California mastitis test was used to detect mastitis in quarter milk samples. Data were subjected to Chi-square test. Duncan's Multiple Range Test modified by Kramer (1957) was used to examine the significance of difference between the means. The results indicated that the incidence of sub clinical mastitis increased with the advancement of the lactation number. The values also revealed that the prevalence of sub clinical mastitis was the lowest in animals in Group A having teat length below 5.5 cm and the highest in animals of Group C with teats longer than 7.5 cm. The values between the groups within the lactation in all cases differed significantly. The percentage of reactors differed significantly between first and second lactation and between second and third lactation in Group C while no difference was noticed in Group A and B. The study pointed to the possibility of using selection on the basis of teat size as a means of mastitis control.

There is relationship between zinc and udder health. Zinc is important in the maintenance of health and integrity of epithelial tissue such as skin (teats) and mammary tissue due to its role in cell division a protein synthesis occurred. An additional mode of action for zinc in reducing somatic cell count which is related to zinc's role in keratin formation. Approximately 40% of keratin lining in teat canals of Holstein dairy cattle is removed during the milking process, thus requiring continuous regeneration. Gapuco *et al.* (1993) estimated that approximately 1.3 mg of keratin must be regenerated during the inter-milking period.

Hamana *et al.* (1994) reported that the teat canal diameter of the positive cases in bacterial examination was significantly larger than that of the negative group. It was suggested that the teat with the larger teat gradient and/or teat canal diameter more susceptible to mastitis.

According to Premchand *et al.* (1995), the season of calving had a significant effect in the incidence of mastitis in buffaloes. The animals calved in rainy season had the highest incidence of mastitis. The Incidence of mastitis increased as the lactation number increased. Furthermore, above workers reported that the incidence of mastitis was higher in the hind quarters than in the fore quarters in all breeds of buffalo.

The number of mastitis cases within lactation was modeled by Lescourret *et al.* (1995) through over dispersed Poisson regression with individual and herd co-variants. The results emphasized the role of the herd variables. Increased production potential increased the number of cases per lactation at a rate of 1.4/10 kg. The Calving month also played an important role in production potential. The scientist reported that the incidence of mastitis was greater when calving took place in early autumn or winter, which led to an expanded housing period. The interval from calving to the first case of mastitis and the intervals between successive cases were modeled for cases occurring during lactation through random selections from fitted gamma distributions, these distributions being truncated to consider the lactation length. The results of both steps can be used to stimulate mastitis occurrence in different conditions.

Mitra *et al.* (1995) studied the prevalence of sub clinical mastitis (SCM) in an organized buffalo farm. Five hundred and twenty eight milk samples of 132 lactating buffaloes were screened for the prevalence of SCM. Quarter wise and teat wise prevalence in relation to lactation period were recorded. The Positive milk samples were processed for isolation of etiological agents. Out of 528 milk samples, 116 (21.96%) were found to be positive. Quarter wise testing revealed highest incidence of 56 (45.58%) in the right hind quarters and lowest incidence of 16 (13.79%) in the left fore quarters. The incidence of SCM was highest, 64 (55.17%) during early lactation (1-3 months) and during 1-3rd lactation. Microorganisms could be isolated from 116 (21.96%) milk samples. Out of 116 isolates, 48 (41.73%), 32 (27.58%), 28 (24.13%) and 8 (6.89%) isolates were *Staphylococci*, *Streptococci*, *E. coli* and *Corynebacterium*, respectively.

Joshi and Shrestha (1995) reported that the prevalence of bovine clinical mastitis in the Western Hills of Nepal was the highest (17.6%) during 1st lactation, declining in successive lactations. The prevalence of clinical mastitis was higher in younger animals and found more than 88.8%during the 1st month of lactation.

Lactation failure and purulent uterine discharge relation (assumed to be due to metritis) in 127 dairy cows from 3 dairy farms to mastitis were investigated by Esmat and Badr (1996). They observed that 87 cows (68.5%) had acute mastitis, 23 (18.11%) sub clinical mastitis (SCM) and 17 (13.4%) lactational failure without mammary abnormalities.

Indian workers (Shukla *et al.*, 1997) attempted to determine the relationship of teat type, teat length and quarters affected with the occurrence of mastitis in a prevalence study. The study was conducted on 154 animals (597 quarters) representing 78 crossbred (302 quarters), 36

Sahiwal cows (145 quarters) and 40 Murrah buffaloes (155 quarters). The teat tips were categorized into four types viz., funnel, round, flat and plate shaped. Teat length was categorized into small (<5.5 cm), medium (5.6-7.5 cm) and large (> 7.5 cm). Quarter milk samples were examined by performing California mastitis test, somatic cell counts and microbiological examination of milk. Maximum prevalence of mastitis was recorded in case of animals with funnel shaped teat tip (Table 5) and was attributed to the retention of some milk which facilitates the microbial growth to establish mastitis. However, the results of the total somatic cell counts showed the round shaped teat tips to be equally susceptible to infection as they are more frequently exposed to environmental contamination and are also more likely to be injured. The investigators opined that plate type teat tips should be preferred over funnel and round shaped teat tips in breeding programmed to decrease the incidence of mastitis. Smaller teats were more prone to mastitis (53.66%) than medium (35.29%), larger (18.33%) teats. It may be due to the shorter teat canal enabling the microbes to move upward without much hindrance in comparison to large teat canal. The fore quarters were affected more frequently (49.05%) than the hind quarters (35.91%) in case of cows. In buffaloes, hind quarters had higher incidence of mastitis than fore quarters. Similarly, left quarters had higher prevalence (44.04%) than right quarters (33.33%). The investigators could not proffer any explanation advance any reason to these observations.

Lancelot *et al.* (1997) studied 844 mastitis cases affecting 597 lactations of 500 French Friesian cows from 44 herds. They documented certain aspects of lactation, udder conformation and management practices. Distribution was modeled using a hierarchical logistic regression. Rear quarters were affected in 61.9% of cases. The only significant risk factor was the cow's parity; rear quarter clinical mastitis was more frequent in primiparous than in multiparous cows. Udder conformation did not seem to play a significant role in mastitis distribution.

Indian workers (Thirunvukkarasu and Prabaharan, 1997) conducted the incidence of mastitis on five organized institutions and 25 private dairy farms. Data pertaining to 301 mastitis cases (261 cows and 40 buffaloes) were analyzed. Of the 2006 cows studied, 261 (13.01%) were affected with mastitis, while only 40 out of 543 buffaloes (7.37%) developed mastitis during the two year study period. Incidence was found to be significantly associated with the chosen factors like breed, milk yield, lactation order and season only in cow (Table 6). There was a higher incidence of single quarter involvement (53.49%) than two quarters (31.57%), three quarters (11.96%) and four quarters (2.99%) involvement of the 261 cows and 40 buffaloes affected with mastitis, one quarter was seen to be affected in 138 cows (52.87%) and 23 buffaloes (57.5%) and two quarters in 83 cows (31.8%) and 12 buffaloes (30.0%). Of the total of 434 quarters affected in cows, hind quarters showed higher involvement (58.76%) than fore quarters (41.24%). Furthermore among quarters, left hind quarters were found to be more susceptible to udder infection with 144 (33.18%) out of 434 clinical quarters. Buffaloes exhibited a very similar trend. The reasons for higher hind quarters involvement might be due to more frequent exposure to dung and urine, larger capacity and mass, greater vulnerability to direct trauma and relatively more closeness to the floor as compared to fore quarters. The present study indicates a close resemblens with the finding of previous Indian study (Singh *et al.*, 1991).

Ramachandraiah *et al.* (1998) investigated the sub clinical mastitis in 85 Murrah buffaloes with the help of CMT and microbiological examination of quarter milk samples. Occurrence of mastitis increased with the increase in lactation number. The prevalence of mastitis was highest in the left fore quarters (29.3%) followed by left hind (28.0%), right hind (22.0%) and the right fore (20.7%) quarters. The higher prevalence of left side quarters was ascribed due to the common practice of milkmen milking the animals, while sitting on the left side of the animals; while they exert pressure on the left side of quarters.

Clinical mastitis as reported by Barker (1998) in the first 150 days of lactation had a highly negative effect on service period. Florida researchers reported 2.7 time higher risk of abortion in cows with clinical mastitis during the first 45 days of lactation.

Thirunvukkarasu and Prabaharan (1998) documented 1.757 times greater incidence of mastitis in cow than in diary buffalo. The milk yield, stage of lactation, lactation number, udder abnormalities, season, stall hygiene and milking hygiene were found to be significantly associated with mastitis ($P<0.05$).

According to Bilal (1999) the relative risk for clinical mastitis increased with the advancing age and was maximum (1.1) in buffaloes 10-11 years.

A case-control study was conducted by Waage *et al.* (2000) evaluated the risk factors for clinical mastitis in dairy heifers between 1 and 14 days of calving. The Case- control heifers were matched in herds; the control was the heifer that calved closest to time before or after the particular problem. Data were analyzed by conditional logistic regression. The final multivariate model included 339 case-control pairs. Blood in the milk, udder oedema, teat oedema and milk leakage (all recorded at the time of parturition) were the significant risk factors. The purchased heifers and heifers with the skin lesions between udder and thigh were not at increased risk of clinical mastitis. Separate analysis of a subgroup of case-control pairs identified teat edema, blood in the milk, and milk leakage at calving as risk factors for clinical mastitis caused by *S. aureus*.

According to Lalrintluanga *et al.* (2003) screening of 987 quarters of 248 cows by modified California mastitis test revealed that cows of 4-6 years age group (51.1%) were most frequently affected. The incidence of mastitis was found to be higher in early stage of third lactation (30.6%). Single quarter infection (63.44%) was recorded more frequently than any other combination of quarters. Left hind quarters (30.25%) were more frequently affected as compared to the other three quarters.

Some selected physiological (stage of lactation and lactation number) and manage mental (source of milk let down, method of milking, and floor condition) factors were studied by Bilal *et al.* (2004) to determine the effect of clinical mastitis in buffalo. The study was undertaken in peri-urban and rural areas of Faisalabad (Pakistan). The data indicated that the prevalence of clinical mastitis was higher in peri-urban (25.21%) than rural (19.74%) areas. The highest incidence was observed during 4 to 6 months after calving both in peri-urban (45.67%) and rural (45.08%) areas.The maximum cases of mastitis were found during third lactation both in peri-urban (19.00%) and rural (22.98%) areas. Prevalence was higher in animals milked with folded-thumb pressure and in those in which milk let down was induced through suckling calves. Cemented and brick floors contributed more towards mastitis in comparison to Kasha floors. The incidence was higher in hind quarters (73.3 and 63.1%) than in fore quarters (26.6 and 36.8%) in peri-urban and rural areas, respectively.

B.2 Association of Manage mental and Environmental Determinants with Mastitis

Neave *et al.* (1969) reported that the rate of mastitis infection in commercial herds could be reduced by 45% by using hygienic measures like udder washing with disinfectants and drying with a clean towel. In the process of machine milking, disinfection of teat cups before and after milking and the post milking dipping of teats with disinfectant solution was effective in controlling mastitis The scientist further observed that combination of simple hygienic measures and effective antibiotic therapy resulted in 75% reduction in the incidence of mastitis.

Oxytocin injection is used quite commonly by the farmers in Pakistan for let downofmilk particularly in buffaloes because of buffalo cows have small udder cistern and almost 95% of the milk stored in the alveolar compartment. As a result, pre-milking stimulation is of extreme importance for optimal milk ejection in buffaloes different from cows, the buffaloes' cisternal compartment are more prominent in the teats as with in the gland (Thomas *et al.,* 2003). The buffaloes' stimulation for milk let down requires more time as compared to cows, in average 2 minutes. For this purpose, at the time of milking by hand the calf is used, in most of the animals. However, the practice of using calves is not adopted in some herds where buffalo cows are machine milked in parlors (Svennersten-Sjauiya, 2000).

As far as could be ascertained there is no report on the epidemiologic association between the use of oxytocin and mastitis. Findlay and Grosvenor (1969) cited by Newbould (1970) hypothesized that during active suckling, mechanical and oxytocin stimulation could act together to dilate ducts and contract alveoli. Newbould (1970) cited Espe and Cannon (1942) who had earlier observed that at let down there was an increased tonicity of smooth muscles in the wall of the teat and the teat cistern tended to balloon. Newbould (1970) suggested that the stimulus for these phenomenon's also affects the smooth muscles around the proximal part of the teat duct near Furstenberg' Rosette resulting in its dilation. Thus instead of keeping the duct closed, the smooth muscles around the duct under stimulation, open the proximal end, and allow direct access for microorganisms to the cistern. In theory at least, animals milked by exogenous administration of oxytocin are at a greater risk to develop mastitis than animals not receiving this milk let down hormone. Oxytocin reportedly increases N:K ratio and somatic cell count in the milk (Allen, 1990).

Hoare and Roberts (1972) reported that herds udders were washed with running water and soap had significantly indicate less mastitis than washing was carried out with the cloth and bucket of disinfectant. This effect was independent of the level of management practiced. There was highly significant effect of milking management on the incidence of mastitis with all udder washing methods. The incidence of mastitis in herds where post milking teat dipping was carried out did not significantly differ from other herds using the same udder washing methods and similar standards of management.

Socci and Redaelli (1973) observed that the environmental factors like malfunctioning of milking machine, feeding and transmission via other species or calves were causally associated with mastitis.

In developing countries like Pakistan dairy animals are predominantly hand milked is often used as a lubricant during milking and milker’s hands are often heavily soiled with milk during this process. Standard mastitis control practices like post milking antiseptic teat dipping, prompt detection and treatment of clinical cases and dry-cow therapy were shown to be in sufficient by themselves to affect a desired reduction in intramammary infections in hand milked herds (Oliver, 1975). Owing to a relevance to the present study, a fairly detailed description of South African study (mostly based on verbatim reproduction) is in order. In 1970, the owner of one of the hand milked herds mentioned above asked for advice on the introduction of a system for the control of infection and mastitis in his herd. Because of the high incidence of infection (67% of cows and 29% of quarters at the original herd test in Nov. 1969), the farmer agreed to have all quarters treated simultaneously (100000 i.u. procain penicillin and 100 mg dihydrostreptomycin in archis oil at 24 hours intervals on three occasions). Teat dipping after milking was continued and an iodophor was used at a strength recommended by the manufacturer. Dry cow therapy was also adopted as a standard practice. Udder washing was not satisfactory because all that could be achieved at the time was the use of a fresh pailful of tap water for each cow. The same cloth was used to wash all udders although it was rinsed in iodophor solution at udder wash strength after each udder had been washed. Before milking began each milker greased his hands with a salve which contained poly-brominated salicyl analide in a petrolatum base.

The result of the control scheme was disappointing. Although, infection by streptococci was almost eliminated, the level of infection by staphylococci was apparently unchanged (±12% of quarters tested). Analysis of data revealed that in fact 46.5% staphylococcal infections had been eliminated by therapy but that a high rate of new infection took place in the 10 days which followed the blitz therapy. This occurred primarily in quarters which had not been infected at the time of the blitz but also in quarters from which streptococci had been eliminated by therapy. Seventy days after blitz, infection with streptococci had returned to its former level.

Routine herd testing was discontinued because the farmer introduced machine milking. Before he did so, an attempt to discover the reasons for the high new infection rate was made. A series of swabs were taken and plated on aesculin nutrient blood agar (ABA), salt blood agar and Edwards's medium (CVT). Instead of dabbing the swab on each plate in turn 0.1 mL of the litmus milk in which it was contained was spread on the plates. The main findings were that milker's hands before and after milking and the milking salve was heavily contaminated with all of the common mastitis pathogens. The investigation was transferred to another hand milked herd where the same salve was in use. The herd had been under observation for 4 years. Immediately before a cow was milked, a jet of tap water was directed onto the udder and teats while they were rubbed vigorously by hand. They were then damp dried with a paper towel and milking begun. Teats were dipped after milking in Hibitane (Chlorhexidine) at the strength recommended by the manufacturer. Cows were infused with an antibiotic in each quarter after the last milking of lactation. In spite of this routine, the incidence of udder infection and mastitis in the herd remained high and similar to that of the herd previously described. Tests showed those milkers' hands before and after milking, teat orifices and individual tins of salve were heavily contaminated with mastitis staphylococci and streptococci. An attempt to dispense uncontaminated salve from large sterile syringe did not result in a significant reduction in the contamination of teat orifices in a trial which lasted for two months.

Laboratory comparisons were made with another salve which contained 0.2% hexachlorophene in a vanishing cream base. The new salve had an inhibitory effect on both staphylococci and streptococci whereas little or no inhibition was shown by the petrolatum base salve. In consequence a change was made to the vanishing cream-base salve and a foot-operated dispenser constructed. Within two months, the proportion of teat orifices contaminated with staphylococci fell from over 50% to below 7%, *Str. agalactiae* from 30 to under 2%. *Streptococcus dysgalactiae* was unchanged at 2 to 3% but *Str. uberis* like organism appeared to increase i.e. from 80 to 90%.

At the end of one year, the number of the various pathogens recovered from teat orifice swabs was still low except *Strep. uberis* like organism. Concomitantly with the decline in the number of teat orifice infections, there was a decline in the incidence of clinical mastitis. The rise in clinical cases in November and December coincided with the advent of summer rains and such rise is commonly encountered in dairy herds. The decline in mastitis was associated with an overall decline in herd milk bulk cell counts. It was not possible to judge the effect on milk yield because this had been rising over the period of investigation as a result of improved feeding.

Carroll (1977) documented the poor management practices were the basic factors in the development of mastitis. These included leaving wet materials like fasces and bedding in yards, which was seldom changed and cows allowed to calve in dirty environment. The author further reported that the incidence of mastitis was higher during the time when animals were housed than during the pasture time. Loose housed cows with soft bedding in resting area had a lower incidence of mastitis than those kept at hard bedding and tied-in cows.

Prost (1984) showed by improving the conditions of buildings, milking equipment and general animal health, the incidence of clinical mastitis fell from 1.4 to 0.2 per cent, sub clinical from 34 to 15 per cent and high milk cell count from 23 to 43 per cent. According to him mastitis occurred in 82 per cent of 835 cows having a dry period less than 6 weeks, as compared with 34 per cent of cows having a dry period of more than 8 weeks.

Dodd and Phipps (1985) founded if udders, milking pails and hands were kept clean, the milk of good microbial quality could be obtained through hand milking. Immersion between milking in 3% caustic soda was suggested as a simple method for disinfecting bucket. Teat dipping and cleanliness of bedding were measures to be minimizing the mastitis.

Hogan *et al.* (1989) reported that tied housing, stall length, milking operation and unhygienic measures were the risk factors for mastitis in cows. Above workers founded that organic materials used as bedding for lactating cows had significantly higher moisture contents with higher Gram negative bacterial (coliform, Klebsiella) and Streptococcal counts than did inorganic materials. Streptococcal and Klebsiella spp. counts were higher in sawdust than in chopped straw. Bacterial counts did not differ between sand and crushed limestone. Gram negative bacterial and coliform counts were higher during summer and autumn vs winter and spring months. Streptococcal count did not differ among seasons of the year. Linear relationships were significant between total rates of clinical mastitis during lactation in both Gram negative bacteria and Klebsiella spp. Counts. It was concluded that rates of clinical mastitis were related to bacterial counts during bedding in lactating cows.

Gonzalez *et al.* (1990) reported that the milkers were the reservoir of source of infection and its transmission to susceptible cows may have been by direct contact with the milkers or by mechanical transfer from cow to cow via the teat cups.

According to the Oltenacu *et al.* (1990), the incidence rates of trampled teats, udder injuries and clinical mastitis in Swedish Red and White tied cows as well as inter relationship between the 4 disorders depend upon the stall length, manure system, type of bedding and calving disorders. According to him cows in herds with liquid manure system were at higher risk of udder injuries and mastitis vs in herds with solid manure system. Lower risk of both udder injuries and mastitis was found for cows in herds with short stall size (180 cm) as compared to herds with large stall size (205-219 cm) length. It was suggested that the factors such as slipperiness of the stall floor and presence of type of feeding barrier may leads to trampled teats. The farms with solid manure systems cut straw or sawdust bedding increased the risk of trampled teats and mastitis. Trampled teats and udder injuries were the most serious risk factors for clinical mastitis.

Mastitis causing organisms were isolated by Prabhakar *et al* (1990) from various body sites of the animals, milk, environment (cowshed and milking parlor floors), udder washing water in hand milked cows and calf's pharynx.

It was observed by Enevoldsea and Sorensen (1992) that a dry period length of seven weeks appears to be associated with the lowest risk of clinical mastitis but factors like milk yield at drying off and previous mastitis status are considered to be much more important predisposing factors.

Dutch workers (Schukken *et al.*, 1991) investigated the incidence rate of clinical mastitis due to *E. coli* and *S. aureus* in 125 herds with low annual bulk milk somatic cell count (<150,000 cells/mL). Risk factors that were offered to a multivariate Poisson regression model included general management, housing, cleaning procedures, cow and cubicle cleanliness, feeds and feeding, dry cow management, milking procedures, machine milking, disease prevention and milk production. Some differences in epidemiology between *E. coli* and *S. aureus* were observed. In the *S. aureus* model, more milking procedure and milking machine variables were present. The milk production, drinking water source, amount of bedding and ventilation were other important factors in the *S. aureus* model. Teat disinfection was an important risk factor in the *E. coli* model but was much less important in the *S. aureus* model. Cleaning procedures were more important in *E. coli* model. The main breed on the farm and percentage of cows leaking milk were other important factors in *E. coli* model.

A stratified random sample of Ohio (USA) dairy herds was studied by Bartlett *et al.* (1993) to relate herd management and environmental conditions to intramammary infection with coagulase-positive staphylococci. The management and environmental conditions were assessed through interview with the farmers. Separate analyses for each of 70 management and environmental independent variables were identified to many potential disease determinants. A logistic regression model used 5 models degrees of freedom to predict the prevalence of coagulase-positive staphylococci more than 1% of quarters. Increased risk of infection with coagulase-positive staphylococci was associated with dirty udders, high-line milking systems and less crowded housing conditions. Decreased risk of infection was associated with a herd size of 50-100 cows.

Ruffo and Zecconi (1994) stated that the environmental mastitis was linked with the lack of efficient control, external factors like climate, geography and penology, and internal factors e.g. housing, litter, diet, management and immunological status of the udder.

Motie *et al.* (1985) reported the prevalence of mastitis through machine milked and hand milked cows was 14.6 and 25.1% respectively. Some milking machine relatations to mastitis (e.g. teat end vacuum, collapse differential, condition and frequency of change of teat liner etc.) were not investigated in the study.

According to Vekatasubramanian and Fulzele (1997) education and illiteracy of the farmers significantly affects the control of mammary gland infection. Illiterate farmers are generally blasé about unhygienic conditions prevailing on farms. Farmer's attitudes to keep udders clean, inadequate and high costs of medicines and improper milking methods all relate to the level of mastitis on the farms.

French workers, Lancelot *et al.* (1997) investigated the distribution of clinical bovine mastitis between rear vs front quarters using data from 4 year survey of commercial dairy herds in western France. The study involved 844 mastitis cases from 500 French Friesian cows from 44 herds. The Risk factor hypotheses were related to certain aspects of lactation, udder conformation and management practices. Distribution was modeled using a hierarchical logistic regression. The Rear quarters were affected 61.9% of cases. The only significant risk factor was the cow’s parity; rear quarter clinical mastitis was more frequent in primiparous than in multiparous cows. In this retrospective study, udder conformation did not seem to play a significant role in mastitis distribution. More over dispersion parameter was observed, indicating that each mastitis case could be considered as an independent event.

Thirunvukkarasu *et al.* (1998) developed a discriminate model by discriminating 301 mastitic and 148 non-mastitic bovines from 5 Government/University and 25 private dairy farms with11 attributes considered, breed, milk yield, stage of lactation, stall hygiene, season, udder hygiene and udder morphology were able to significantly discriminate mastitic animals from the non-mastitic group in Tamil Nadu (India). The Mahalanobis D2 Statistic and F ratio of 2.3498 and 20.7204, respectively of the function showed a significant discriminating power of the model. Average daily milk yield, udder hygiene, stage of lactation, season and udder morphology was the important factors which collectively contributed 93.05% of the total distance measured between mastitic and non-mastitic animals.

Pakistani workers (Raza *et al.*, 1998) investigated the effects of type of bedding on udder and hoof health and behavior in Nili-Ravi buffaloes for a period of 6 weeks. Twelve lactating Nili-Ravi buffaloes were randomly allotted to three buildings, viz., (A) concrete floor, (B) concrete floor + paddy straw, and (C) concrete floor + sand. Data on hoof growth, wear, udder health, somatic cell counts (SCC) and their behavior was analyzed. Results revealed that the bedding had a significant effect on hoof wear and tear, somatic cell count and animal behavior. Animals on sand bedding showed significantly less wear and tear in hooves, minimum SCC and exhibited better behavior. The highest mean value for SCC (1000/mL) was calculated in treatment A (424848±19412) whereas the lowest mean value was found in animals kept on sand (210909±11463). Statistical analysis of the data on SCC in milk produced under different treatments showed significant differences in SCC among the treatments. Mean values for SCC were significantly ($P<0.05$) lower in treatments B than in treatments A and C whilst the difference between treatments A and C was non significant. The SCC ranged from 210909±11463 to 424848±19412/mL in milk samples. According to the workers, although a higher number of somatic cells were observed in treatments with a normal range of 50,000 to 100,000/mL, still milk appeared to be normal and no signs of mastitis, oedema, teat injury or inflammation of udder were observed in any treatment.

Hand-milked dairy animals have been shown to have higher prevalence of mastitis than machine milked-animals (Lafi *et al.*, 1994; Motie *et al.*, 1985). In hand-milked animals, the technique of milking is also important in relation to prevalence of mastitis. Thus the recent study focusing on the factors affecting the prevalence of clinical mastitis in buffaloes around Faisalabad (Pakistan), Bilal *et al.* (2004) encountered 4.2 magnitudes higher prevalence (39.2%) in buffaloes milked by folded-thumb method (thumb-knuckle and finger method) than those milked by full-hand method (9.04%). These workers also reported higher mastitis prevalence in peri-urban areas (25.12%) as compared to rural areas (19.74%). The relatively higher prevalence was ascribed to relatively larger herd size, use of hired laborers for milking (who can not be desirably careful in milking) and more common housing of animals on brick floor.

According to Oliver (1975), the hand milking was in its heyday in Europe and elsewhere; milkers were highly skilled in the practice of full hand milking. This art is generally believed by farmers and research workers, to cause the minimum damage of the delicate membranes which line the teat.

Barrett *et al.* (2005) studied the factors relating to the occurrence of mastitis on 12 Irish dairy herds with histories of elevated somatic cell count (SCC) and/or increased incidence of clinical mastitis. Milk recording data were analyzed, housing conditions and calving areas were examined; dry cow therapy, clinical mastitis records, milking techniques and aspects of milking machine function were assessed. Herds with less than 110 cubicles per 100 cows were more likely to be experience environmental mastitis. Herds with inadequate calving facilities, where cows spent prolonged periods on straw bedding, were likely to acquire environmental mastitis. In the majority of the herds, the selection of dry cow therapy lacked adequate planning. The majority of farmers took no action to reduce pain experienced by cows suffering from mastitis. Deficiencies in parlor hygiene were evident in all herds experiencing elevation in SCC.

In a recent Swedish study (Ekman *et al.*, 2004), the probability of isolating *Str. uberis* from cases of acute clinical mastitis (987 quarters of 829 cows) was half as big in warm loose housing systems as in cold ones. The use of straw doubled the probability of isolating *Str. uberis* as compared to sawdust. Use of wood shavings of any kind reduced the risk of finding *S. aureus*, but increased the probability of Klebsiella spp, four times as compared to straw or peat. The correlation between sawdust and acute cases of clinical mastitis caused by Klebsiella spp was well known (Oz *et al.*, 1985). The study identified no differences between loose housing and tie stall systems in general. The probability of retrieving *S. aureus* or *Str. uberis* was almost twice as high in Swedish Holstein as in Swedish Red. Incidence of mastitis caused by *S. aureus* was higher during November – April months as compared to May – October period.

In an epidemiologic study of bovine mastitis in Kashmir valley of occupied Kashmir, Biffa *et al.* (2005) a cross-sectional study to elucidate magnitude of mastitis, its distribution, and associated risk factors in lactating dairy cows in Southern Ethiopia from February 2001 to March 2002 in a total of 974 milking cows using California Mastitis Test and clinical inspection of udder. Of the total animals examined, 34.9% (340) had mastitis, 11.9% (116) clinical and 23.0% (224) sub clinical. Prevalence of mastitis varied significantly (χ^2 = 54.5, P<0.001) between the study sites. It was higher in Areka (54.7%; odds ration [OR], 7.5; 95% confidence interval [CI], 5.5-10.0) and Arbegona (55.0% OR, 7.5; 95% CI, 5.0-10.9) districts and lower (13.7% OR, 1.0) in the Awassa district. Cows managed under semi-intensive husbandry practice were more affected (43.8%; OR, 2.0; 95% CI, 1.6-2.5) then those managed under extensive (25.8%; OR 1.2; 95% CI, 1.0-1.4) and intensive (28.9%; OR, 1.0) systems. Prevalence of mastitis was significantly influenced by season (χ^2 = 28.7, P<0.001). During the long rain season, cows were at greater risk (OR, 2.6; 95% CL, 2.0-3.4) of acquiring udder infection than during the long dry season (OR, 1.0). Significant difference (χ^2 = 47.5, P<0.001) in prevalence of mastitis was reported between breeds. Holstein-Friesian cows were affected at a higher rate (56.5%; OR, 3.3; 95% CI, 2.5-4.4) compared with local zebu (30.90%; OR, 1.2; 95% CI, 1.0-1.5) and Jersey cows (28.9%; OR, 1.0). Udder/teat injuries caused mainly by ticks were the major predisposing factors of mastitis in Southern Ethiopia. Cows with repeated episodes of mammary glands infections were about 5 times (57.0%; OR, 4.5; 95% CI, 3.7-5.5) at higher risk of re-injection than previously uninfected ones (22%; OR, 1.0; χ^2 = 21.8, P<0.001). Inadequate sanitation of dairy environment, poor animal health service, and lack of proper attention to health of the mammary glands were important factors contributing to high prevalence of mastitis. Some recommendations were forwarded for improved control of mastitis in the region. Wani and Bhatt (2003) found the highest incidence of mastitis in summer and the lowest in winter months.

B.3 Association of some other determinants with mastitis

The occurrence of mastitis in the wake of diseases affect the teat skin is fairly well known. The several such diseases, foot-and-mouth disease and pox are particularly known to predispose mastitis in cattle and buffaloes.

Muhammad *et al.* (1998) investigated on outbreak of pox in 6 small holder dairy farm (buffalo n = 185; cattle n = 7) in Faisalabad metropolis (Pakistan). A cumulative incidence rate of 55.3% was recorded over a 1 month period. Milk from nearly 47% of the affected teats and 23% of the adjacent unaffected ones reacted positive to California mastitis test. The prevalence of clinical mastitis in affected and unaffected teats was 19 and 6%, respectively. According to Radostits *et al.* (2000), observation in a foot-and-mouth disease, vesicles may appear on the teat as with the involvement of teat orifice the severe mastitis often follows.

Prabhakar *et al.* (1988) examined the milk samples and uterine content of 9 cows and 5 buffaloes (that developed mastitis concurrently with metritis) for the presence of *S. aureus*, *S. epidermidis*, *Str. agalactiae* and *E.coli*. The same organisms were found in both samples for 12 (85.71%) of the animals.

Schukken *et al.* (1989) investigated the relationship between the retained placenta and mastitis. Cows with the retained placenta (n = 62) were three times more likely to develop mastitis during hospitalization than animals without retained placenta (n = 134).

Egenolf (1990) reported that the people of Pakistan Punjab province commonly use for example brown sugar, buffer fat, pepper or black salt, and cardamom to treat the diseased animals. Additionally, the ‘peer’ (faith healer) is requested to use his magical powers in putting verses from the Quran in an amulet as a necklace for the sick buffalo.

Esmat and Badr (1996) investigated lactation failure and purulent uterine discharge (assumed to be metritis) in 127 dairy cows from 3 dairy farms in relation to mastitis. Eighty seven cows (68.5%) had acute mastitis and 23(18.1%) sub clinical one. Bacteriological examination of the udder and uterine secretion of cows showed mastitis-metritis-agalactia syndrome.

Bilal (1999) Investigated the risk factors from 100 clinically mastitic dairy buffaloes 14.3, 11.4, 9.5 and 4.8% had suffered from retained placenta, metritis, vaginal prolepses and dystokia at calving.

The use of bovine somatotrophin (BST) to increase milk production is the common practice particularly in peri-urban dairying in Pakistan. Willeberg (1993) applying general epidemiological methods reevaluated the published data on clinical mastitis in bovine somatotropin trials. Results of pooled analyses were used to estimate relative risks and population attributable fractions. The estimates suggested that BST treatment is associated with 15-45% excess incidence of clinical mastitis, which is probably due to an indirect causal effect through increased milk yield. The increased risk is of concern to acceptability and usage of BST under common management conditions, and it should be taken into account in evaluating BST preparations for marketing authorization in countries those have not yet be registered.

C. IMPORTANT PATHOGENS ASSOCIATED WITH MASTITIS IN COWS AND BUFFALOES

Ghuman (1967) investigated the organisms association between mastitis in buffaloes in Faisalabad (formally Lyallpur) district of Pakistan. One hundred and twenty five samples were examined. *Staphylococcus aureus* was isolated from 114, *E. coli* from 2 and *Pseudomonas aureginosa* and *Str. agalactiae*, each from one sample of milk. No organism could be detected in 7 milk samples.

Chander and Baxi (1975) studied 304 quarter samples of 78 apparently healthy cows. Thirty four percent of quarters and 56.4 percent of cows were culturally positive. The principal causative organisms included staphylococci (68.8%) and streptococci (16.2%). Leukocyte count showed the highest percent agreement (82.8%) with bacteriological examination.

Hashmi *et al*. (1980) examined 250 animals (198 buffaloes and 52 cows) for sub clinical mastitis. The prevalence of sub clinical mastitis in buffaloes was 45.45 percent and in cows 50 percent. Quarter infection rate in buffaloes and cows was 23.38 and 25.95%, respectively. The organisms isolated were streptococci (36.82%), staphylococci (43.09%), coliforms (12.15%), pseudomonas (2.92%), corynebacterium (4.18%) and yeast cells (0.44%).

Anwar and Chaudary (1983) reported the prevalence rate of sub clinical mastitis 47.50 percent in buffaloes around the Lahore city (Pakistan). The common isolates staphylococci (40%), streptococci (45.06%), *E. coli* (25(%) and pseudomonas (5%) were observed.

Hodges *et al.* (1984) conducted a taxonomic study on 900 isolates of staphylococci or micrococcus from bovine milk. Of these, 831 were coagulase positive staphylococci (810 *S. aureus* and *S. intermedius*). Of 65 coagulase negative staphylococci, 19 could not be identified by the identification system used. The remainders were identified as *S. hyicus* (29), *S. haemolyticus* (17), *S. hominis* (3), *S. epidermidis* (4), *S. capitis* (1), *S. hominis* or *S. warneri* (1). Four other isolates could not be assigned to the genus *Staphylococci or Micrococci* and were designated as “irregular strains”. No micrococci were identified.

Chanda *et al.* (1989) isolated 57.72 percent staphylococci, 35.40 percent streptococci, 5.3 percent corynebacterium and 1.77 percent *E. coli* from cases positive for sub clinical mastitis.

Iqbal (1992) applied Whiteside test and pH – indicator paper technique to 3980 quarter milk samples. A total of 486 (12.21%) samples were positive by Whiteside test as compared to 10.93 percent by pH – indicator paper technique. In most of the animals, only two quarter were affected, while four quarter prevalence was minimum. The prevalence was higher in hind quarters as compared to the fore quarters and slightly higher in right quarters than left one. A total of 190 pooled samples were positive for bacterial microorganisms. Different pathogenic micro-organisms isolated were *S. aureus* (32.60%), coagulase negative staphylococci (12.06%), *Str. agalactiae* (16.58%), *Str. uberis* (3.01%), *Str. dysgalactiae* (3.01%), *E. coli* (16.08%), *Pseudomonas aureginosa* (7.50%), *Corynebacteium pyogenes* (3.51%), Bacillus spp. (3.01%) and Klebsiella (2.01%).

Lafi *et al.* (1994) reported the findings of a National cross-sectional study of mastitis in Jordan. Between July 1991 and August 1992, 63 Jordanian dairy farms selected by stratified random sample were visited to identify the major causes and prevalence of intramammary infections in dairy cows. Of 773 cows examined 60% of all sampled quarters had

>283000 cells/mL. The mean value of somatic cell count (SCC) was positively associated with age in lactations and negatively with herd’s size. Cows milked by bucket milking machines or in fully automatic parlors had a lower mean SCC than those milked by hand. The most common isolate from clinical cases was *S. aureus* (37.5%). Estimates of prevalence of bacterial pathogens in non-clinical intramammary infections were: coagulase – negative staphylococci (16.04%), *S. aureus* (9.41%), *Klebsiella spp*. (6.17%), *Corynebacterium bovis* (5.35%), and *Brucella melitensis* (4.52%).

The study designed to evaluate the efficiency of a new animal side mastitis test (surf field mastitis test; SFMT) and to compare its efficiency with CMT, WST, and SCC (in terms of sensitivity, specificity, accuracy, positive and negative predictive values and kappa values), Fazal-ur-Rehman (1995) examined 400 quarters of 50 buffaloes and 50 cows for isolation of microorganisms. Of the 200 buffalo quarters, 63 and 70 reacted positive to SFMT and CMT, respectively. Similarly, of the 200 cow quarters, 77 and 83 reacted positive to SFMT and CMT. Sixty one and 90 quarters of buffaloes and cows respectively yielded growths of different microorganisms. Staphylococci isolates were speciated using API STAPH-Trae system. *Staphylococcus aureus* was the most frequently encountered pathogen (39.1% in buffaloes and 47.3% in cows). Other organisms isolated were

Staphylococcus hyicus (4.7% in buffaloes and 17.0% in cows), *Staphylococcus epidermidis* (3.1% in buffaloes and 6.4% in cows), *Staphylococcus capitis* (1.6/% in buffaloes), unidentified staphylococcus (1.6% in buffaloes and 3.2% in cows, *Micrococcus varians* (3.2% in cows), *Streptococcus dysgalactiae* (10.9% in buffaloes and 7.4% in cows), *Streptococcus agalactiae* (3.1% in buffaloes and 1.1% in cows), *Streptococcus lactis* (1.1% in cows), *Streptococcus pyogenese* (1.6% in buffaloes, *Corynebacterium bovis* (7.8% in buffaloes and 1.1% in cows), *C. pyogenese* (3.1% in buffaloes and 2.1% in cows), other diphtheroids (4.7% in buffaloes), *E. coli* (6.2% in buffaloes and 1.1% in cows), non-coliform Gram –ve bacteria in buffaloes and 1.1% in cows), *Bacillus spp*. (7.8% in buffaloes and 4.3% in cows), yeast (3.1% in buffaloes and prototheca (1.6% in buffaloes). Mixed infection of 2 organisms was encountered in 1.5 and 2 percent quarters of buffaloes and cows, respectively.

According to Gonalez *et al.* (1980), microorganisms isolated from sub clinical cases of mastitis in cows included *S. aureus* (43% of samples) *S. epidermidis* (21%), *Str. uberis* (19%), *Str. agalactiae* (13%), *Str. dysgalactiae* (9%), *Corynebacterium pyogenes* (1.3%), *Corynebacterium bovis* (7%) and coliform (1.7%). Mixed streptococcal and staphylococcus infections also occurred.

The pathogenic microorganisms were isolated by Slee and McOrist (1985) from cows affected with summer mastitis. Of the 31 isolates, *Actinomyces pyogenes* was present in 25, *Fusobacterium necrophorum* in 23, *Microaerophilic coccus* in 16, *Peptostreptococcus indolicus* in 15 and *Bacerioides* spp. in 7.

A study conducted on bovine acute mastitis by Pyorala and Syvajarvi (1987) reported that 26.7% of quarters were infected by *S. aureus*, 19.4% by *Streptococci*, 17% by coliform and 3.8% by *Actinomyces pyogenes*.

Al-Sha Wabkeh and Abdul Aziz (1987) reported *S. aureus* as the most common cause of both clinical and sub clinical mastitis in cows, followed by coliform, *Corynebacterium* spp., *Proteus* spp., *Streptococcus* spp., and *Pseudomonas* spp.

El-Bayomi and Mahmoud (1987) isolated *Str. agalactiae* (55.26%), S. aureus (28.95%), *Str. dysgalactiae* (10.35%) and *E. coli* (5.26%) from cases of bovine mastitis. Similarly, Dutch workers (Schukken *et al.*, 1989) isolated *E. coli* (16.2%), coagulase negative staphylococci (13%), *S. aureus* (96%) and *Str. uberis* (8.0%) in cases of bovine clinical mastitis.

The bacteriological group like *Staphylococcus* spp. was most frequently isolated by Hogan *et al.* (1989) from infected quarters of cows at calving and at drying off, while coliform and bacteriologically negative and environmental Streptococci accounted for 82.2% of clinical cases.

According to the Trinidad *et al*. (1990), the most common isolates from teat canal keratin and mammary secretion samples of heifers were *S. chromogenes*, *S. hyicus* and *S. aureus*. *Staphylococcus aureus* was isolated from teat canal of 31% of heifers and 12.3% of quarters while from mammary secretions of 37.1% of heifers and 14.7% of quarters with clinical symptoms.

A total of 4620 lactating animals comprising of 2216 Sahiwal cows, 917 crossbred cows and 1483 buffaloes were investigated by Ahmad *et al.* (1991) for the prevalence of sub clinical mastitis. Overall prevalence was 6.95%. Physical examination of 4620 udders revealed 332 (7.19%) blind teats. Somatic cell count appeared close to culturally positive results. *Staphylococci* were isolated from most (72.32%) of the affected udders of the 3 types of dairy animals, followed by *Streptococcus* (20.96%) and other microorganisms (6.75%).

According to Waage and Aursiq (1992), of the 20905 milk samples analyzed in Norwegian mastitis laboratories, 50% did not yield any microbial growth on cultural examination. *Staphylococcus aureus*, *Str. dysgalactiae*, and *E. coli* were recovered from 24.5, 5 and 0.5% per cent of the milk samples, respectively. The incapability to isolate microorganisms from 50% of the milk samples may be due to Gram-negative infections which characteristically have few bacterial cells per milliliter of milk.

Studies conducted upto 1993 on incidence of bovine and bubaline mastitis in countries endowed with both cattle and buffaloes were reviewed by the US worker Allore (1993). According to her, the investigators cultured a number of organisms, mostly founded *S. aureus* with the highest frequency followed by streptococci. Most of the studies did not differentiate between *S. aureus* and staphylococci or the contagious *Str. agalactiae* from the environmental streptococci but such types of studies give the impression that contagious organisms (*S. aureus* and *Str. agalactiae*) are the most frequently recovered organisms from both clinical and sub clinical mastitis in cow and buffalo.

Ramachandraiah *et al.* (1998) used California mastitis test (CMT) and cultural examination to determine the incidence of sub clinical mastitis in an organized buffalo dairy farm. Of the 85 Murrah buffaloes tested with these procedures, 53% tested positive for mastitis. Streptococcus spp. had the highest (46.34%) frequency among the isolates of positive milk samples followed by Staphylococcus spp. (34.15%). Mastitis due to isolates of a single species accounted for 98.5% and mixed infections 1.5%.

Indian workers (Larintluanga *et al.*, 2003) screened 987 quarters of 248 cows with the help of a modified California mastitis test (MCMT). Quarters (n = 115) reacting positive in this test were examined for mastitis pathogens. Eighty nine (77.39%) of 115 quarter milk samples reacting positive in MCMT yielded growth on bacteriological examination. A total of 98 isolates were recovered. Coagulase-negative *Staphylococci* (55.1%) were the most predominant isolates followed by *Streptococci* (22.45%), *S. aureus* (7.14%), *Corynebacteria* (6.12%), *E. coli* (3.06%), *Proteus* spp. (20.04%), *Klebsiella* (2.04%) and *Citrobacter* spp (2.04%).

Khan *et al*. (2004) examined the milk samples from 50 buffaloes with the help of Surf field mastitis test (a CMT-like test) and microbiological examination of milk. Twenty seven, four and ten of the quarters were found to be affected with sub clinical and clinical mastitis and non-functionality (blind) of teats, respectively. *Staphylococcus aureus* (48%) was the most frequently recovered organism among the bacterial isolates.

Streptococcal diseases are primarily diseases of the zones with a cold continental climate. The staphylococci prefer to the warm zones, chiefly the damp tropics. However, the morbidity rate for staphylococcal infections in the cool temperate zones has registered an increase and this shift has coincided with the rise in temperature of the world climate that has set in since the end of the last century (Mayr, 1992).

In conclusion, it appears that in developing countries where mastitis control is not in place, contagious organism *like S. aureus, Str. agalactiae* and corynebacterium are the most predominant mastitis pathogens. Before the advent of era of mastitis control (period before 1960's), these organisms were the most prevalent etiologic agents of mastitis in the currently developed countries (North America, Europe, Australia etc.). However, currently the coagulase negative staphylococci (CNS) are considered to be the most prevalent mastitis pathogens in developed countries.

D. ASSOCIATIONS BETWEEN METABOLIC DISEASES AND MASTITIS

Several epidemiological studies have demonstrated that there is an association between the development of metabolic disease and subsequent development of mastitis. In a study of NY dairies (2, 190 cows) there was a very strong association between parturient hypocalcaemia or milk fever and mastitis. The odds ratio (multiplicative increased in occurrence) suggested that a milk fever cows was 8.1 times more likely to develop mastitis than a cow that had not milk fever. The odds ratio for development of coliform mastitis was even greater (odds ratio, 9.0) (Curtis *et al.,* 1993). In a Swedish study, ketosis increased the risk of mastitis 2 fold (Oltenacu and Ekesbo, 1994). A second larger study, (18, 110 Swedish Red and White cows in 924 herds and 14,940 Swedish Friesian cows in 772 herds) found that the risk of mastitis was increased in cows that had suffered a retained placenta (Emanuelson *et al.,* 1993.). Yeining, dystocia, retained fetal membranes and lameness before first breeding service increased the risk of mastitis before first service in a 10 herd study from England (Peelaer *et al.,* 1994).

Table 1: Incidence of Clinical Mastitis (CM) in Cow

Study	Method of detection	Total Cows (No.)	Cow with CM		Organism	
			No.	%	Frequency	%
Chanda *et al.*, (1989)	BTB, WST, CMT	94	50 QIR	13.4 QIR	1	56
					2	32
					3	4
					4	8
Dhanda & Sethi (1962)						
A		171	104	60.8	2	42.7
					6	26
					14	30.7
B		367	135	36.8	2	34
					4	0.8
					6	48.9
					12	0.8
					14	14.0
					15	1.5
C		86	32	37.2	2	49.9
					6	12.5
					14	6.3
D				35.4	2	52.5
					6	30
					14	17.5
E				19.2	2 & 6	30
					14	10
F				68.2	2	19.9
					6	44.2
G		100	21	21	2	48.1
					6	62
H				33	2	40
					6	50
I				30.1	2	28
					6	52
					14	20
El-Shabiny *et al.* (1989)	MC	180	102	56.6	16	36.1
		Infected			17	20.5
Hirpurkar *et al.* (1987)	CMT, MC	52	15	28.8		
Hussain *et al.* (1984)	WST, MC	84 to 110 over 6 months	21 to 47	23.5 to 43.5	2 spp	27.66
					2 ag	25.9
					3	5.06
					4	17.33
					5	0.26

Table 1 ---- continued

					6	16.53
Ismail *et al.* (1988)	CMT, MC	56 Healthy	0	0	*Compylobacter fetus jejuni*	
		100 infected	1	1	Fetus jejuni	
Kapur & Singh (1978)	MC	63	36.84 QIR	36.8	1	7.37
					2 spp	20.99
					2 ag	16.84
					4	6.31
					5	1
					6	41
					7	5.26
Naipospos-Hutabarat (1986)	CMT	252	8	3.2	1	
Prasad *et al.* (1983)	MC	73	63	86.7	1	71.84
			CM & SCH		2	1.72
					10	10.34
					11	2.3
					12	
Singh *et al.* (1988)	CMT, SLST, Mastaid, SCC, MC	19	9	47.0		
Singh *et al.* (1989)	MC	270	23	8.52	19	8.52 only org.
Siniussi *et al.* (1975)	MC	170	25	14.4	2 ag	10.25
	Negretti field test		39 QIR	5.7 QIR	2 spp	15.37
					3	5.12
					5	2.56
					6	38.46
					14	15.37
Verma (1988)	SLST, MC	197	55	42.1	2	6.56
					3	11.48
					4	9.84
					6	55.7
					7	3.28
					8	3.28
Wanasingh (1985)	CMT, MC	240	272, QIR	30.2	2 spp	23.0
					6	7.0

(Adopted from Allore, 1993)

Method of Detection

BTB: Bromothymol Blue Card Test
CMT: California Mastitis Test
SLST: Sodium Lauryl Sufate Test
MWT: Modified Whiteside Test
WST: Whiteside Test
MC: Microbial Culture
SCC: Somatic Cell Count

Organisms

1 Staphlsococcus spp.
2 Streptococcus
3 Escherichia coli
4 Corynebacterium spp
5 Pseudomonas spp.
6 Staphylococcus aureus
7 Klebsiella spp.
8 Proteus mirabilis
9 Fungi
10 Gram negative rods
11 Gram positive
12 Yeast
13 Mycoplasma tuberculosis
14 Mixed infections
15 Diptheroids
16 Mycoplasma bovis
17 Mycoplasma bovigenitalium
18 Anthracoid
19 Mycotic

Study

A Government Dairy Farm, Haringhata, Bengal (India)
B Government Farm, Karnal (India)
C Indian Veterinary Research Institute, Izantnagar (India)
D Military Dairy Farm, Jabalpur (India)
E Military Dairy Farm, Secunderabad (India)
F Indian Veterinary Research Institute, Mukteswar (India)
G Military Farm, Kirkee (India)
H Military Farm, Pimpri (India)
I Military Farm, Bangalore (India)

Other Abbreviation: QIR = Quarter Infection Rate

Table 2: Incidence of Subclinical Mastitis in Cattle

Study	Method of detection	Total Cows (No.)	Cow with SCM		Organism	
			No.	%	Frequency	%
Ali *et al.* (1989)	CMT	290	212	73.1		
				44.8 QIR		
Chanda *et al.* (1989)	BTB, WST, CMT, MC	94	63 QIR	16.9 QIR	1	58.73
					2	38.09
					4	3.17
El-Kholy *et al.* (1988)	CMT, MC	125	100	80	2	78
					3	12
					6	12
Hirpurkar *et al.* (1987)	CMT, MC	52	17	32.7		
Hutabarat *et al.* (1986)						
A	CMT	56	35	62.8		
		68 QIR	30.3 QIR			
B		894	599	67		
Mahmoud (1988)	CMT, Freiso, MC	112	149 QIR	34.9	2,3,6	
Naipospos-Hutabarat (1986)	CMT	252	152	60.3	1,2	
Pal *et al.* (1979)	WST	189	128	67.7	1	14.84
					2 spp	9.38
					6	46.88
					10	25.78
					15	1.56
					18	9.38
Rasool *et al.* (1985)	MWT	1596	530	33.2±7.84		
Roy *et al.* (1989)	CMT	292 J x H	93	31.9		
		114BS x H	51	44.7		
	242	242 HF x H	152	62.8		
Singh & Baxi (1980)	SLST, MC, CMT, Mastaid	50	27	54		
	Leucocyte count	53 QIR	27.7 QIR			
Siniussi *et al.* (1975)	Negretti Field Test, CM	170	57 QIR	8.8 QIR	1	5.55
					2 spp	52.76
					3	22.22
Tarigan *et al.* (1987)		249	231	93	2 spp	5.5
					2 ag	55
					6	11.1
Verma (1988)	SLST, MC	197	136	43.5		

(Adopted from Allore, 1993)

Abbreviations used in Table 1 are also applicable to this table; Study A: Indonesia; B: citation Hirst *et al.*, Bogor, India (1983)
Breed: J: Jersey; H: Hariana; BS: Brown Swiss; HF: Holstein - Friesian.

Table 3. Incidence of Clinical Mastitis (CM) in Buffaloes

Study	Method of detection	Total Buffalo (No.)	Buffalo with CM		Organisms	
			No.	%	Frequency	%
Dhanda & Sethi (1962)						
A		12	6	50	6	10.6
					Aerobacter spp. (83.4)	
B				15.5	1	25
					2	56.2
					14	18.8
C		171	51	29.9	2	72.6
					6	17.6
					14	9.9
D		90	14	15.6	2	50
					6	50
E				26.9	2	34.5
					6	65.5
F				13	2	66.1
					6	26
					14.6	4.7
Hirpurkar *et al.* (1987)	CMT, MC	26	8	30.8		
Hussain *et al* (1984)	WST, MC	32 to 52	4 to 12	10.0-23.1	2 spp	8.69
					2 ag	40.2
					3	19.56
					6	28.26
Ismail *et al.* (1988)	CMT, MC	56 healthy	0	0	*Campylobacter fetus, jejuni*	
		100 infected	3	3		
Kapur *et al.* (1990)	MC	597	868 QIR	36.3 QIR	1	19.1
					2 spp	21.9
					2 ag	8.1
					3	6.5
					4	18.9
					5	0.7
					6	26.3
					7	2.2
					8	0.3
					9	0.1
					12	1.4
					15	0.5
Kapur & Singh (1978)	MC	60	96 QIR	40.2 QUR	1	10.5
					2 spp	22.05
					2 ag	21
					3	12.63
					4	2.1

					6	25.26
					15	4.21
Raghavan *et al*. (1962)		3322			2	16
Singh *et al.* (1988)	CMT, SLST, Mastaid, SCC, MC	96	94 QIR	42 QIR		
Singh *et al.* (1989)	MC	107	6	5.7	19	5.71
Siniussi *et al.* (1975)	MC, Negretti Field Test	154	17	11.6	2 spp	19.99
			30 QIR	4.9 QIR	2 ag	6.66
					6	56.66
					14	16.66
Wanasingh (1985)	CMT, MC	493	223 QIR	12.1 QIR	1 spp	11.0
					4	0.1
					6	1.0
Yass *et al* (1983)	MC	151	38	25.2	1	1.48
			73 QIR	12.1 QIR	2	33.82
					4	7.35
					6	57.35

(Adopted from Allore, 1993)

Abbreviations used in Table 1 are also applicable to this table.

Study
A: Indian Veterinary Research Institute, Izantnagar (India)
B: Military Farm, Jabalpur (India)
C: Military Dairy Farm, Secunderabad (India)
D: Military Farm, Kirkee (India)
E: Military Farm, Pimpri (India)
F: Military Farm, Banglore (India)

Table 4. Incidence of Subclinical Mastitis (SCM) in Buffaloes

Study	Method of detection	Total Buffaloes (No.)	Buffaloes with CM		Organisms	
			No.	%	Frequency	%
Anwar & Chaudhari (1983)	WST, pH test, Strip Cup, MC	2000	850	47.5	1	40
					2	45.06
					3	25
					5	5
Bhindwale *et al.* (1987)	CMT, MC	295	47 QIR	4.0 QIR	1	46.91
					2	4.93
					4	7.4
					6	39.5
					10	1.23
Hashmi & Muneer (1981)	BTB, WST, CMT, MC	396	178	44.94	1	34.55
					2	34.44
					3	20.0
					4	2.0
Hirpurkar *et al.* (1987)	CMT, MC	26	5	19.23		
Ismail *et al.* (1988)	CMT, MC	56 healthy	1	1.79	*Compylobacter fetus intest inalis*	
		100 infected				
Rasool *et al.* (1988)	MWT	1204	248	20.5±3.14		
Singh & Baxi (1980)	SLST, CMT, Mastaid Leucocyte count MC	88	21	23.8		
			44 QIR	12.5 QIR		
Syamasunder *et al.* (1987)	CMT	179	75	42.3		
Yass *et al.* (1983)	MC	151	46	31.9	2	24.24
			62 QIR	11.6 QIR	3	1.61
					4	4384
					6	69.7

(Adopted from Allore, 1993)

Abbreviations used in Table 1 are also applicable to this table.

Table 5: Effect of type of teat tip on the occurrence of mastitis in cows (crossbred and Sahiwal) and buffaloes.

Type of teat tips	Animals			Quarters		
	Examined	Affected	Per cent	Examined	Affected	Per cent
Crossbred						
Funnel	18	15	88.33	72	35	48.61
Round	36	26	72.22	135	61	45.19
Flat	13	08	61.54	52	17	32.69
Plate	11	05	45.45	42	06	14.23
Sahiwal						
Funnel	07	05	71.43	28	17	60.17
Round	14	08	57.14	54	29	53.70
Flat	10	03	30.00	36	08	22.22
Plate	06	01	16.67	23	04	13.33
Buffalo						
Funnel	08	07	87.59	31	16	51.61
Round	16	12	75.00	60	42	70.00
Flat	08	02	25.00	32	06	18.75
Plate	08	02	25.00	32	03	6.25

Adopted from Shukla *et al.* (1997).

Table 6: Relationship of mastitis Incidence with some factors.

Factors	**Chi square value**
Management (Government/Private)	0.289^{NS}
Species (Cow/Buffalo)	13.070^{**}
Cow breeds (Exotic/Crossbred/ND)	2.580^{NS}
Cow milk yield	34.281^{**}
Buffalo milk yield	4.995^{NS}
Cow lactation order	18.602^{**}
Buffalo lactation order	6.283^{NS}
Cow season	10.030^{**}
Buffalo season	1.320^{NS}

** Significant (P≤0.01)   * Significant (P≤0.05)   NS = Not significant

ND = Non-descript

Adopted from Thirunvukkarasu and Prabaharan (1997).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY SETTING, UNIVERSE AND STUDY POPULATION, SAMPLING PROCEDURES AND PARAMETERS

All 28 Union Councils of Tehsil Sumundri, District Faisalabad of Punjab province comprising of 133 villages constituted the universe of the study population. The study was undertaken over a 3 month period (September – November, 2004).

One village from each Union Council was selected randomly for collection of epidemiologic data. Each selected village was considered a cluster (Thrusfield, 1995) and all dairy producers in a village were included in the survey. A total of 2029 buffaloes and 430 cows were investigated. As per Livestock Census (2006) Tehsil Sumundri included in its livestock strength 193058 buffaloes and 24498 cows. The entire Tehsil is served by 4 Civil Veterinary Hospitals, 7 Civil Veterinary Dispensaries, 26 Veterinary Centers, one Artificial Insemination Center, 10 Artificial Insemination Sub-Centers, one Poultry Diagnostics Laboratory and one Slaughter House. The map of Tehsil Sumundri is given in Appendix 1. The sampling units were adult buffaloes and cows. Two types of determinants viz. host-associated and management associated were studied. Host-associated determinants included: dairy species (cow or buffalo), breed, age, general physical condition, body condition, lactation number, stage of lactation, reproductive disorders, distance between teat tip and ground, teat stenosis, quarters affected, ease of milking, teat injuries, milk leakage, blood in milk, udder oedema, teat oedema, teat shape, teat size, udder shape etc. Similarly, managerial determinants included: condition of floor, type and amount of bedding, frequency of dung removal, quality of drainage, source of drinking water, concentrate fed, stimulus for milk letdown, udder washing, number of animals milked by the same milker, milking technique, and wallowing etc. All information was collected on pre-designed proforma by structured questions and physical examination of udder (Appendix 2).

Diagnosis of mastitis was based on overt manifestations of the disease (clinical mastitis) and results of the Surf Field Mastitis Test (Muhammad *et al.*, 1995) for subclinical mastitis. The following epidemiologic measures were computed to determine the association (if any) between mastitis and the potential determinants associated with host and management.

3.1.1 Linear Regression: Linear regression equation ($y = \alpha + \beta x$) was calculated between the independent (x) and dependent (y) variables. R^2, t value and p value were also measured (Thrusfield, 1999).

3.1.2 Epidemiological measures of association for independent Proportions in 2 x 2 tables.

Strength of Association:

Chi-Square (χ^2): Association between the factor and mastitis was measured by calculating χ^2 values (Thrusfield, 1999). Where the χ^2 value was found significant, the following measures were also calculated (Martin *et al.*, 1987).

Relative risk (RR): Ratio between rate of mastitis in exposed buffaloes/ cows and rate of mastitis in unexposed buffaloes/ cows.

$$RR = (a/a+b)/ (c/c+d)$$

Where

a $\rightarrow$ The exposed animals having mastitis

b $\rightarrow$ The number of non-mastitic animals in the exposed group

c $\rightarrow$ The number of mastitic animals in the unexposed group

d $\rightarrow$ The number of non-mastitic animals in the unexposed group

The variance (Var) of $\log_e RR = [(b/a)/(a + b)] + [(d/c)/(c +d)]$

The 95 % confidence interval of RR = RR exp $(-1.96\sqrt{var})$, RRexp $(+ 1.96 \sqrt{var})$

Population relative risk (RRpop): It indicates the relative impact of the factor in the population:

$$RRpop = \{(a+c)/n\}/\{(c/c+d)\}$$

Where n = a+b+c+d

Effect of Association:

Attributable rate (AR): It is the rate of mastitis in the exposed group minus the rate in the unexposed group:

$$AR = \{(a/a+b)\} – \{c/c+d)\}$$

Attributable fraction (AF): It is otherwise called as etiologic fraction. It is used to know what proportion of mastitis in the exposed group is due to the factor:

$$AF = (RR-1)/RR$$

Total Effect of Association:

Population attributable rate (PAR): It gives the importance of a causal factor in the population and is determined by multiplying its effects (AR) by the prevalence of the factor:

$$PAR = \{(a+b)/n\} \text{ X } AR$$

Population attributable fraction (PAF): It is proportion of mastitis in the population that is attributable to the factor:

$$PAF = (RRpop - 1)/RRpop$$

3.2. ISOLATION OF MASTITIS PATHOGENS FROM MASTITIC BUFFALOES AND COWS

Three hundreds quarter foremilk samples collected from 95 randomly selected buffaloes (clinically mastitic quarters n = 17, sub clinically mastitic quarters n = 183) and 53 cows (clinical n = 11; sub clinical n = 89 quarters) were subjected to microbiological examination. The diagnosis of sub clinical mastitis was based on the results of Surf Field Mastitis Test (Muhammad *et al.*, 1995).

Milk samples were not collected from animals treated with antibiotics by any route till 96 hours of treatment. Quarter-fore milk samples collected at the time of afternoon milking were used. Collected samples were immediately cooled and transported to the Mastitis Research Laboratory, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad in the ice box for microbiological examination. Microbiological examination of milk samples begun within 8 hours of collection.

Procedure described by National Mastitis Council Inc., U.S.A. (1990) was followed for the collection of quarter foremilk samples. Sterile glass vials of 15 mL capacity, labeled as LF (left front), LR (left rear), RF (right front), and RR (right rear) were used. Each teat was scrubbed using pled get of cotton moistened with 70% ethyl alcohol. A separate pledget was used for each teat. While holding the vial as horizontal as possible, the cap was removed without touching the inner surface and held with the inner surface downwards. After discarding the first few streams, about 10 mL of milk was collected aseptically.

Procedures described by National Mastitis Council Inc., USA (1987) were followed for culturing the milk samples and identification of mastitis pathogens. The samples were shaken eight times to get a uniform dispersion of the pathogens. Using a platinum-rhodium loop, 0.01 mL of milk sample was streaked each onto esculin-blood agar and MacConkey's agar plate. Four quarter milk samples were cultured on a 100 mm plate by plating individual quarter samples on one quadrant of plate and incubated at 37°C for 48 hours. Guidelines of National Mastitis Council Inc (1987) on the significance of colony numbers in pure or mixed cultures were used to categorize a sample as infected or contaminated. The representative colonies of the microorganisms were isolated and purified by streaking onto fresh esculin-blood agar plates. Catalase positive, Gram positive coccal isolates were presumptively identified as Staphylococci or micrococci and subjected to the tube coagulase test and speciated using a commercial identification kit viz STAPH-Trac system (BioMerieux-France). Organisms other than staphylococci were identified as per the procedures recommended by National Mastitis Council, Inc. (1990).

CHAPTER 4

RESULTS

An epidemiologic study was conducted on mastitis in buffalo and cattle population of Tehsil Samundri district Faisalabad. Of the total of 133 villages of Tehsil Samundri, 28 villages (one from each Union Council) were randomly selected. Villages were considered as clusters. Every household/farmer of each selected village managing lactating buffaloes and/ or cows was surveyed. Data about various factors relating to host, management and environment were collected on a pre-designed questionnaire. Data were analyzed to determine the frequency of distribution and the risk factors of mastitis. Disease frequency (prevalence) was determined by adding the number of cases of clinical mastitis to the number reacting positive in Surf Field Mastitis Test. The prevalence was stratified on the basis of species, breed, age of the animal, and lactation number. Association between the disease and various factors was determined (where relevant) by calculating chi-square value, relative risk (RR), population relative risk (RR_{POP}), attributable rate (AR), attributable fraction (AF), population attributable rate (PAR) and population attributable fraction (PAF).

Three hundreds randomly selected mastitic quarters of buffaloes and cows were cultured to determine the nature of etiologic agent(s) of mastitis in the study area.

4.1 FREQUENCY AND DISTRIBUTION OF MASTITIS

4.1.1 Overall prevalence of mastitis

The overall prevalence of mastitis in buffaloes and cows was 14.44 and 20.0%, respectively. The prevalence was significantly higher in cows than in buffaloes ($p < 0.1$). The composite (cow plus buffalo) prevalence of mastitis was 15.4% (Table 4.1).

4.1.2 Age-based distribution of mastitis

In case of buffaloes there was variation between the age of the animal and the disease prevalence. It was the lowest in buffaloes of 5 to 6 years of age (3.46 - 4.25%) while maximum in the animals aged 11 years or more (72.72%). Similarly, the prevalence of mastitis increased with the advancing age in cows. The regression analysis showed that there was 12.21 %, 11.285 % highly significant ($p<0.01$) increase in mastitis prevalence with one year increase of age of buffaloes and cows. The coefficient of determination of (R^2) value was .902 and 0.898 in buffaloes and cows, respectively which is indication of good fit of linear regression as shown in Tables 4.2, 4.3 and Figure 4.1, 4.2

4.1.3 Lactation number-based prevalence of mastitis

It was recorded that the occurrence of mastitis increased with the increase in lactation number both in buffaloes and cows. Regression analysis indicated that there was 15.65 % and 15.01 % highly significant ($p<0.01$) increase in mastitis prevalence with one lactation increase in buffaloes and cows, respectively. The coefficient of determination of (R^2) value was 87.8 % and 88.6 % in buffaloes and cows, respectively which is an indication of good fit of linear regression as shown in Table 4.4, 4.5 and Figure 4.3, 4.4

4.1.4 Stage of lactation-based prevalence of mastitis

Prevalence was the highest during first month of lactation (27.0 % and 72.05 %) in buffaloes and cows, respectively. In subsequent months, the prevalence of mastitis varied between buffaloes and cows. Thus in buffaloes, the prevalence fell precipitously till month 4th post calving. After this, a steady increase in prevalence towards the end of lactation was noted. In cows, 4.4 and 8.3 folds decreases in mastitis prevalence were noted in the 2nd and 3rd month of lactation, respectively. At subsequent sampling points, further decrease in prevalence with considerable variation was observed. The relationship between the stage of lactation (months) and mastitis prevalence in buffaloes and cows was significant (p < 0.05) as indicated in Table 4.6, 4.7 and Figure 4.5, 4.6.

4.1.5 Breed-based distribution of mastitis

The prevalence of mastitis was different in different breeds of cows. Highest prevalence (56.00%) was recorded in crossbred/exotic cows followed by Sahiwal (13.54%) and desi (non-descript) cows (5.14%). Only one breed of buffalo (Nili-Ravi) is found in the study area. Therefore, calculations of breed-based prevalence were not relevant in buffalo. The association between mastitis status and breed-based distribution in cow was highly significant (P < 0.01) as chi-square value of 109.175 with 2 df was computed as shown in Table 4.8.

4.1.6 Quarter prevalence rate of mastitis in buffaloes and cows

Of a total of 8116 quarters of 2029 lactating buffaloes, 478 (5.8%) quarters were either clinically mastitic or reacted positive in Surf Field Mastitis Test (sub-clinically mastitic). Similarly, of the 1720 quarters of 430 cows, 146 quarters (8.48%) were found mastitic (clinical and Surf Field Mastitis Test positive) (Table 4.9). As can be seen in Table 4.10, of the 293 mastitic buffaloes, 54.26%, 31.74%, 10.58% and 3.41% respectively had 1, 2, 3 and 4 quarters affected with mastitis.

Eighty six of 430 cows (20%) examined had mastitis either in one (53.48% of affected cows), two (27.90 % of affected cows), three (13.95% of affected cows) and all four (4.65% of affected cows) quarters. The relationship between quarter prevalence in buffaloes and cows with mastitis was significant (P < 0.05). The regression analysis showed that there was 17.4 %, 16.0 % increase in mastitis prevalence. A coefficient of determination (R^2) of 95.8 % and 94.9 % in both the species is the indication of good fit of linear regression as shown in Table 4.11 and Figure 4.7, 4.8.

4.2 DETERMINANTS/RISK FACTORS OF MATITIS IN BUFFALO AND COW

4.2.1. Distance between teat tip and ground

In general, a positive correlation was observed between the mastitis and distance of teat tip from the ground. As this distance increased, the prevalence of mastitis decreased. Moreover, the regression analysis indicated 5.76 % and 5.24 % (a significant P < 0.05) relation between mastitis status and teat-tip distance from ground as shown in Tables 4.12, 4.13 and Figure 4.9, 4.10.

4.2.2. Udder Shape

Distribution of different shapes of udder in buffaloes and cows respectively is depicted in Table 4.14 and 4.15. The prevalence of mastitis both in buffaloes and cows was the highest in double-leveled udder and lowest in spherical shaped udder. In case of double-leveled udder, prevalences were 96.82 cent and 95.65 per cent in buffaloes and cows, respectively. The relationship between udder shape and mastitis prevalence was highly significant ($P < 0.01$) in both buffaloes and cows as shown in Table 4.16 and 4.17.

4.2.3. Teat Shape

Teats were categorized into 4 types on the basis of shape viz., funnel, round, flat and plate shaped. Flat type teats accounted for 95.67 and 86.62 per cent pattern frequency in buffaloes and cows, respectively (Table 4.18 and 4.19). Round shaped teats had the highest prevalence of mastitis both in buffaloes (61.80%) and cows (56.36%). The study indicated that the relationship between teat shape and mastitis in buffaloes and cows was significant (Table 4.20 and 4.21).

4.2.4 Frequency of dung removal

In buffaloes as well as in cows, the frequency of dung removal seems to affect the prevalence of mastitis appreciably. Regression analysis showed that a significant effect in buffaloes and cows, respectively as shown in Table 4.22 and 4.23 and figure 4.11 & 4.12. In the former species, once, twice, thrice, four times and five times or more than five times daily removal of dung was associated with 27.30, 20.67, 16.12, 7.27 and 7.46% prevalence of mastitis, respectively. The corresponding values of mastitis prevalence (%) associated with these dung removal frequencies in cows were 21.3, 20.73, 20.22, 19.54, and 18.07, respectively.

4.2.5 Floor drainage quality

Effect of the floor drainage quality on the increment of mastitis prevalence appeared to be subtle both in buffaloes and cows. Thus poor, acceptable and proper floor drainage quality, respectively were associated with 15.67, 14.51 and 13.14% prevalence of mastitis in buffaloes. The corresponding percent mastitis prevalence in cow noted with these categories of floor drainage quality was 21.23, 20.0 and 18.65. The association between the mastitis status and drainage quality were found significant ($P < 0.05$) in both the species (Table 4.24 and 4.25).

4.2.6 Nature of milk let down stimulus

Table 4.26 and 4.27 depict the prevalence of mastitis in buffaloes and cows respectively as a function of nature of milk letdown stimuli. Only 2 of 2029 buffaloes were being milked without any milk letdown stimulus. None of the 430 cows was milked with 'No stimulus'. Therefore, buffaloes and cows milked without any milk let down stimulus were disregarded for computation of mastitis prevalence. In buffaloes, the highest mastitis prevalence (38.7%) was recorded in animals milked with exogenous administration of oxytocin, followed by those in which letdown of milk was induced by suckling calves (25.87%) and offering concentrate at the time of milking (2.73%). In the case of cows, the highest prevalence (31.71%) of mastitis was noted in subjects in whom calf suckling was used as a stimulus for letdown of milk followed by those which were enticed to have a letdown of milk by offering concentrate (6.96%). Only two of 430 cows were milked with exogenous parenteral administration of oxytocin and both were bereft of mastitis. The chi square values of milk let down stimulus were 233.102 and 41.326 in buffaloes and cows, respectively. Association between milk let down stimulus and mastitis was found significant ($P < 0.05$) in both the species.

4.2.7 Number of animals milked by a milker

The number of buffaloes and cows milked by the same milker was divided into seven categories i.e., one, two, three, four, five, six and more than six animals milked by the same milker. In general as the number of animals milked by the same milker increased, so did the prevalence of mastitis. The prevalence was found the lowest when a milker milked only one buffalo (12.32%) or cow (9.02 %). In buffaloes, the highest mastitis prevalence (19.23%) and in cows (50%) was recorded when a milker milked seven or more than seven animals. The Relationship between the number of buffaloes and cows milked by a milker and mastitis prevalence rate in both the species was significant (P < 0.05). The regression analysis indicated that there was 0.847 % and 8.15 % mastitis prevalence with the number of animals milked by the same milker in buffaloes and cows, respectively as shown in Table 4.28, 4.29 and Figure 4.13, 4.14.

4.2.8 General physical condition of buffaloes and cows

Association of general physical condition with mastitis in buffaloes and cows has been shown in Tables 4.30and 4.31 the animals were divided into two groups i.e. poor and good. A higher prevalence (90.79% in buffaloes, 88.88% in cows) was observed in animals with poor physical condition. Association between the mastitis prevalence and general physical condition was highly significant ($p < 0.01$) in both the species.

4.2.9 Education of the farmers

In the present study, the prevalence of mastitis was the highest in buffaloes (19.08%) and cows (24.88%) of illiterate owners. In general, as the level of farmer education increased, the prevalence of mastitis decreased both in buffaloes and cows. The regression analysis showed that there was 0.767 %, 1.12 % increase in mastitis prevalence with the farmer's education grading in buffaloes and cows. The association between mastitis status and farmer's education grading was significant in buffaloes as well as in cows Tables 4.32, 4.33 and Figure 4.15, 4.16.

4.2.10 Effect of reproductive disorders

Two hundred and sixteen (82.44%) of 262 buffaloes with a history of 4 common reproductive disorders (metritis, retained placenta, dystokia and prolapse of uterus) were found to be affected with mastitis. Compared to an overall prevalence of 14.44% in 2029 buffaloes sampled in the study area, buffaloes (n = 262) with a history of reproductive disorders had 18.95 times higher prevalence of clinical plus sub clinical mastitis.

In cows, 59 of 69 (85.5%) animals with a history of reproductive disorders were found mastitic. Thus the prevalence of mastitis in cows with reproductive disorders was 11.43 times greater than the overall prevalence of 20% in cows (n = 430) included in the study. The statistical analysis concluded a highly significant association in buffaloes ($P < 0.01$) and significant association in cows ($P < 0.05$) between the mastitis status/prevalence and reproductive disorders as shown in tables 4.34and 4.35 Exotic breed (Holstein-Friesian, Jerseys) of cow and their crosses had a much higher frequencies of reproductive disorders than non-descript (Desi) and Sahiwal cows (Table 4.36).

4.2.11 Teat injury

The value of Relative risk (RR), Population relative risk (RR_{POP}), Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) calculated for the factor of teat injury in cows were 2.138, 1.204, 0.189, 0.532, 0.029 and 0.169. The corresponding values for buffaloes were 1.091, 1.035, 0.038, 0.082, 0.004 and 0.033. The statistical analysis indicated a highly signification association ($p < 0.01$)

between teat injury and mastitis status in both the species as shown in Tables 4.37 - 4.38.

Condition of Floor

The statistical analysis showed a non-significant association (P > 0.05) between mastitis status and unevenness in both the species as chi-square values of 1.623 and 0.220 with 1 df have probability of 0.203 and 0.639 in both the species (Table 4.39-4.40).

Hard milking

The values of Relative risk (RR), Population relative risk, Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) calculated for the factor of hard milking were 1.886, 1.170, 0.109, 0.469, 0.020 and 0.145 in buffaloes and 1.772, 1.197, 0.129, 0.435, 0.032 and 0.164 in cows. The association between mastitis and hard-milking was highly significant (P < 0.01) as chi-square values of 30.077 and 8.359 with 1 df have probability of 0.000 and 0.004 in both the species (Tables 4.41-4.42).

Folded-thumb method of milking

The values of Relative risk (RR), Population relative risk, Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and

Population Attributable fraction (PAF) calculated for the factor of folded-thumb method of hand milking were 1.901, 1.180, 0.110, 0.479, 0.024 and 0.152 in buffaloes and 1.636, 1.136, 0.112, 0.388, 0.037 and 0.119 in cows. The statistical analysis between mastitis status and folded-thumb technique of milking showed a highly significant effect ($p < 0.01$) in buffaloes and a significant effect ($P < 0.05$) in cows (Tables 4.43-4.44).

Udder Oedema

The values of Relative risk (RR), Population relative risk (RR_{POP}), Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) calculated for the factor of udder oedema were 1.800, 1.066, 0.108, 0.444, 0.008 and 0.061 in buffaloes and 1.849, 1.156, 0.147, 0.459, 0.026 and 0.134 in cows, respectively. Statistical association between mastitis prevalence and udder oedema was highly significant ($p < 0.01$) in both the species as shown in Tables 4.45-4.46.

Teat Oedema

The values of relative risk (RR), population relative risk (RR_{POP}), Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) calculated for the factor of teat oedema respectively were 2.701, 1.230, 0.200, 0.629, 0.026 and 0.186 in buffaloes.

The corresponding values in cows were 2.679, 1.307, 0.257, 0.626, 0.046 and 0.234. The statistical analysis showed a highly significant association ($P < 0.01$) between teat oedema and mastitis status in both the species (Tables 4.47-4.48)

Blood in Milk

Prevalence of mastitis was significantly influenced by blood in buffaloes as well as cows. Quantitative measures of association between blood in milk and mastitis in terms of RR, RR_{POP}, AR, AF, PAR and PAF calculated for this factor were: 1.842, 1.085, 0.108, 0.457, 0.010 and 0.078 in buffaloes (Table 4.49). As depicted in Table 4.50, the corresponding values for cows were 1.943, 1.129, 0.167, 0.485, 0.021 and 0.116. The statistical analysis indicated a highly significant association ($P < 0.01$) between blood in milk and mastitis status in both the species

Wallowing

The statistical analysis showed a non significant ($P > 0.05$) association between mastitis status and wallowing practices in buffaloes and cows as shown in Tables 4.51-4.52.

Teat Stenos is

The measures of association between teat stenosis and mastitis status in buffaloes and cows respectively are given in Table 4.53 and 4.54. The values of relative risk (RR), population relative risk (RR_{POP}), Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) calculated for the factor of teat steno sis respectively were 2.737, 1.188, 0.212, 0.634, 0.021 and 0.152 in buffaloes. The corresponding values of cows were 2.797, 1.398, 0.257, 0.642, 0.056 and 0.284. Chi-square analysis indicated a highly significant ($P < 0.01$) relationship between teat steno sis and mastitis status in both buffaloes and cows.

Milk Leakage

Fifteen of 293 (5.12 %) buffaloes suffering from mastitis had milk leakage from one or more teats. Similarly, ten of 86 (8.6 %) were found afflicted with this disorders. Statistically analysis showed a significant ($P < 0.05$) association between milk leakage and mastitis status in both buffaloes and cows (Table 4.55-4.56).

The values of the relative risk (RR), population relative risk (RR_{pop}), attributable rate (AR), population attributable rate (PAR), attributable fraction (AF), and population attributable fraction (PAF) calculated for buffaloes and cows respectively for the teat injuries (1.091 and 2.138;1.035 and 1.204; 0.038 and 0.189; 0.082 and 0.532; 0.004 and 0.029; 0.033 and 0.169), Evenness of floor (1.289 and 1.280; 1.263 and 1.273; 0.033 and 0.044; 0.218 and 0.218; 0.029 and 0.420; 0.208 and 0.214), Hard milking (1.886 and 1.772; 1.170 and 1.197; 0.109

and 0.129; 0.469 and 0.435; 0.020 and 0.032; 0.145 and 0.164), Folded thumb (1.904 and 1.636; 1.180 and 1.136; 0.110 and 0.112; 0.479 and 0.388; 0.024 and 0.037; 0.152 and 0.119), Udder oedema (1.800 and 1.849; 1.066 and 1.156; 0.108 and 0.147; 0.444 and 0.459; 0.008 and 0.026; 0.061 and 0.134), Teat oedema (2.701 and 2.679; 1.230 and 1.307; 0.200 and 0.257; 0.629 and 0.626; 0.026 and 0.046; 0.186 and 0.234), Blood in milk (1.842 and 1.943; 1.085 and 1.129; 0.108 and 0.167 ; 0.457 and 0.485; 0.010 and 0.021; 0.078 and 0.116), Wallowing (1.950 and 1.121; 1.000 and 1.010; 0.008 and 0.108; 0.015 and 0.0015; 0.000 and 0.009), Teat steno sis (2.737 and 2.797; 1.188 and 1.398; 0.212 and 0.257; 0.634 and 0.642; 0.021 and 0.056; 0.152 and 0.284), Milk leakage (1.539 and 1.484; 1.024 and 1.041; 0.076 and 0.093; 0.350 and 0.326; 0.002 and 0.007; 0.023 and 0.039).

4.3 MICROORGANISMS ISOLATED FROM CLINICAL AND SUBCLINICAL CASES OF MASTITIS IN BUFFALOES AND COWS.

Of the 200 quarters foremilk samples collected from 95 buffaloes suffering from clinical (n = 17 quarters) and sub clinical mastitis (n = 183 quarters), 193 (96.5%) yielded growth of different microorganisms when cultured on esculin blood agar and MacConkey's agar plates. A total of 214 isolates of 13 different microbial species were recovered (Table 4.57). *Staphylococcus aureus* was the most frequently recovered bacterial species accounting for 59.53% of all isolates, followed by *Streptococcus agalactiae* (23.83%), *Staphylococcus hyicus hyicus* (8.88%), *Staphylococcus epidermidis* (6.54%), Bacillus species (3.74%), *Staphylococcus hominis* (1.40%), *Escherichia coli* (1.40%), *Staphylococcus xylosus* 1 (0.93%), *Streptococcus dysgalactiae* (0.93%), and Corynebacterial species (0.935 %). Yeast and prototheca (one isolate each) each accounted for 0.47 per cent of 214 isolates.

Of the 193 culture positive milk samples, 170 (88.1%) yielded growth of a single microorganism. Thirteen (6.73% of culture positive) milk samples yielded growth of two microbial species whereas growth of three microbial species in combination was encountered in 6 (3.10%) of the 193 culture positive milk samples. All isolates of *Staphylococcus xylosus*, nontypable coagulase negative Staphylococcus spp, *E.coli* amd Bacillus spp were encountered in association with some other microbial species.

In the case of cows (Table 4.58), of the 100 quarter foremilk samples (clinical n = 11; sub clinical n = 89 quarters), 96 (96%) yielded growth when cultured on esculin blood and MacConkey's agar plates. A total of 105 isolates of 11 different microbial species were recovered. *Staphylococcus aureus* was the most frequently isolated organism (accounting for 44.76% of the total of 105 isolates) followed by *Streptococcus agalactiae* (21.90%), *Staphylococcus epidermidis* (7.62%), nontypable coagulase negative Staphylococci (6.67%), *Staphylococcus hyicus* (3.81%), Corynebacterial spp. (3.81%), environmental streptococci (3.81%), Bacillus spp. (3.81%), *Streptococcus dysgalactiae* (1.90%), *Escherichia coli* (0.95) and Nocardia spp. (0.95%), respectively. All isolates of bacillus species, environmental streptococci and Nocardia spp. were encountered in combination with other microorganism(s).

Table 4.1: Clinical mastitis plus Surf Field Mastitis Test based prevalence of mastitis in buffaloes and cows in Tehsil Samundri, District Faisalabad (Pakistan).

Species	No. of animals examined	No. of affected animals	Mastitis prevalence (%)	z	p
Buffalo	2029	293	14.44	2.67	0.008
Cow	430	86	20.00		
Total	**2459**	**379**	**15.4**		

Table 4.2: Age-based prevalence of clinical plus sub clinical (detected by Surf Field Mastitis Test) mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Age (Years)	No. of buffaloes examined	No. of affected buffaloes	Mastitis prevalence (%)
5	425	18	4.25
6	491	17	3.46
7	392	28	7.14
8	406	65	16.00
9	139	60	43.16
10	121	65	53.71
11	55	40	72.72
Total	**2029**	**293**	**14.44**

<u>Statistical analysis</u>

Linear regression equation is

y = 12.212 x - 69.06

R^2 = 0.9012

t value = 6.75**

p value = 0.01

** = highly significant ($P < 0.01$)

Table 4.3: Age-based prevalence of clinical plus sub clinical (detected by Surf Field Mastitis Test) mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Age (Years)	No. of cows examined	No. of affected cows	Mastitis prevalence (%)
4	4	0	0
5	93	7	7.52
6	121	12	9.91
7	91	16	17.58
8	79	24	30.37
9	17	8	47.06
10	19	15	78.94
11	6	4	66.66
Total	**430**	**86**	**20.00**

<u>Statistical analysis</u>

Linear regression equation is

y = 11.285 x -52.384

R^2 = 0.8986

t value = 7.299**

p value = 0.000

** = highly significant ($P < 0.01$)

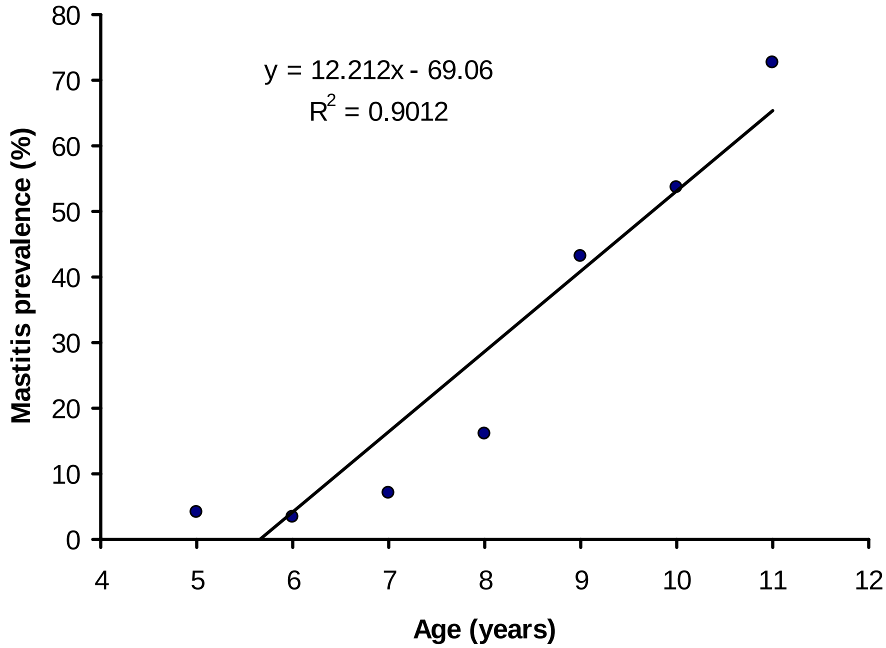

Fig. 4.1 Age based prevalence of mastitis in buffaloes

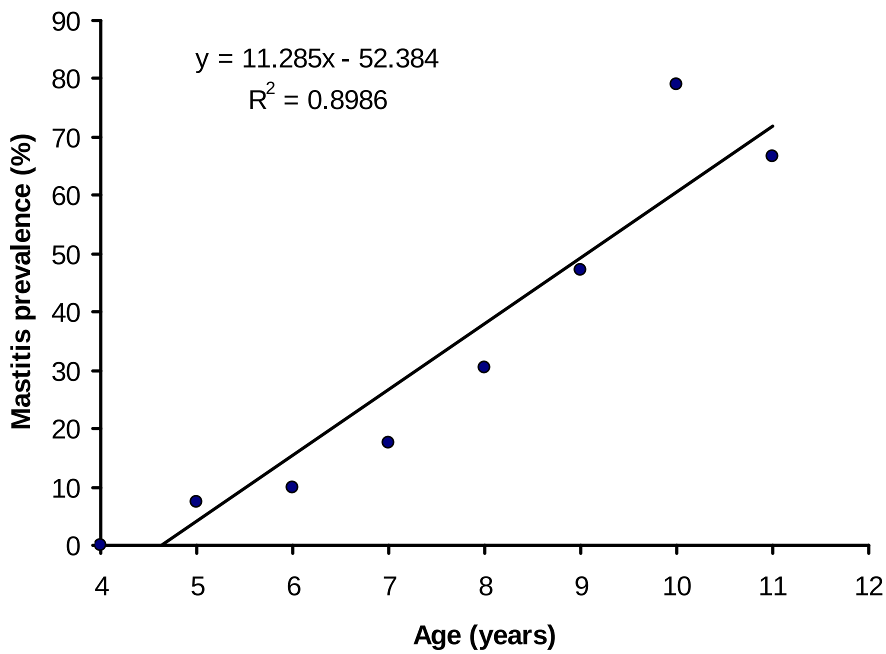

Fig. 4.2 Age based prevalence of mastitis in cows

Table 4.4: Lactation number-based parity prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Lactation No.	No. of buffaloes examined	No. of affected buffaloes	Mastitis prevalence (%)
1	445	19	4.26
2	474	20	4.21
3	487	46	9.44
4	373	75	20.10
5	164	77	46.96
6	77	47	61.03
7	9	9	100.0
Total	**2029**	**293**	**14.44**

Statistical analysis

Linear regression equation is

y = 15.556 x – 27.483

R^2 = 0.8754

t value = 6.01**

p value = 0.002

** = highly significant ($P < 0.01$)

Table **4.5: Lactation number-based prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).**

Lactation No.	**No. of cows examined**	**No. of affected cows**	**Mastitis prevalence (%)**
1	22	1	4.54
2	142	11	7.74
3	119	12	10.08
4	59	18	30.50
5	54	27	45.16
6	28	16	57.14
7	1	1	100
Total	**430**	**86**	**20.00**

<u>Statistical analysis</u>

Linear regression equation is

y = 15.009 x – 23.59

R^2 = 0.8864

t value = 6.25**

p value = 0.002

** = highly significant ($P < 0.01$)

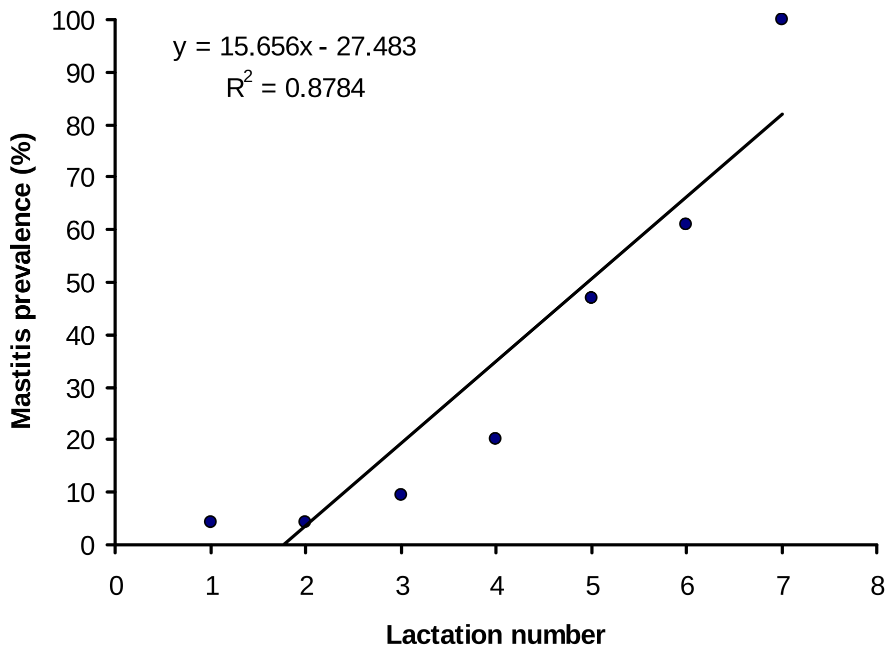

Fig. 4.3 Lactation based prevalence of mastitis in buffaloes

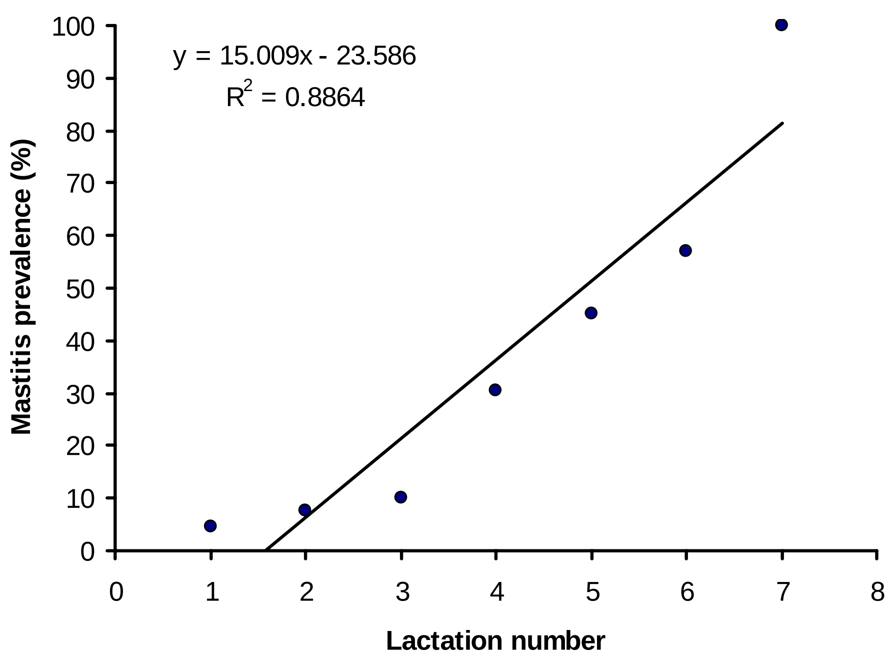

Fig. 4.4 Lactation based prevalence of mastitis in cows

Table 4.6: Stage of lactation-based prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Stage of lactation (Months)	No. of buffaloes examined	No. of affected buffaloes	Mastitis prevalence (%)
1	300	80	27.0
2	295	60	21.0
3	500	80	16.3
4	375	26	6.93
5	325	23	7.0
6	148	16	8.69
7	50	7	14.0
Total	**2029**	**293**	**14.44**

<u>Statistical analysis</u>

Linear regression equation is

y = -2.6043 x + 24.839

R^2 = 0.5444

t value = 02.78

p value = 0.039

* =-significant (P < 0.05)

Table 4.7: Stage of lactation-based prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Stage of lactation (Months)	No. of cows examined	No. of affected cows	Mastitis prevalence (%)
1	68	49	72.05
2	117	19	16.23
3	174	15	8.62
4	47	1	2.12
5	19	1	5.26
6	3	1	7.69
7	3	0	0
Total	**430**	**86**	**20.00**

Statistical analysis

Linear regression equation is

y = -8.4496 x + 49.794

R^2 = 0.5221

t value = 2.34 *

p value = 0.047

***** = significant (P < 0.05)

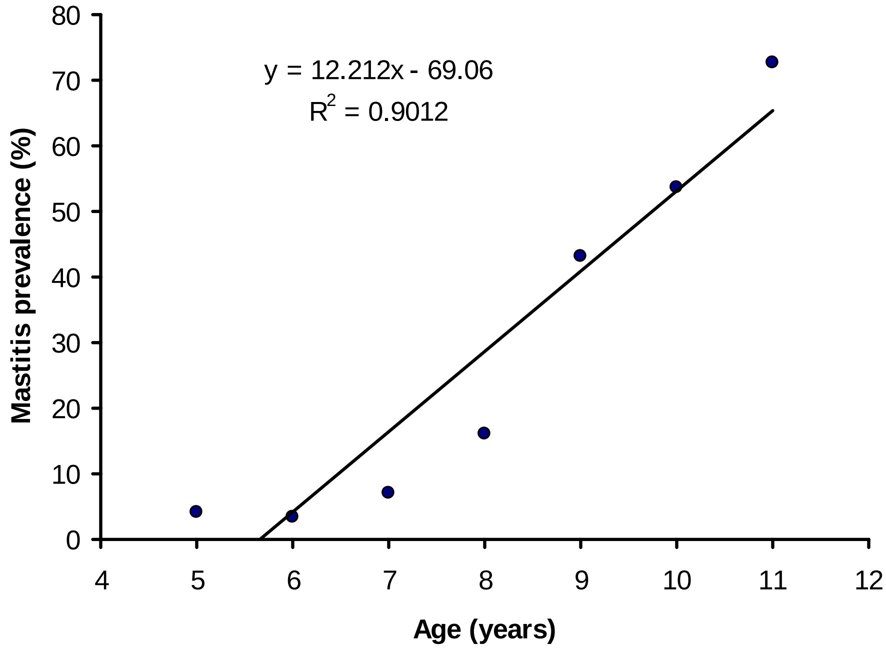

Fig. 4.1 Age based prevalence of mastitis in buffaloes

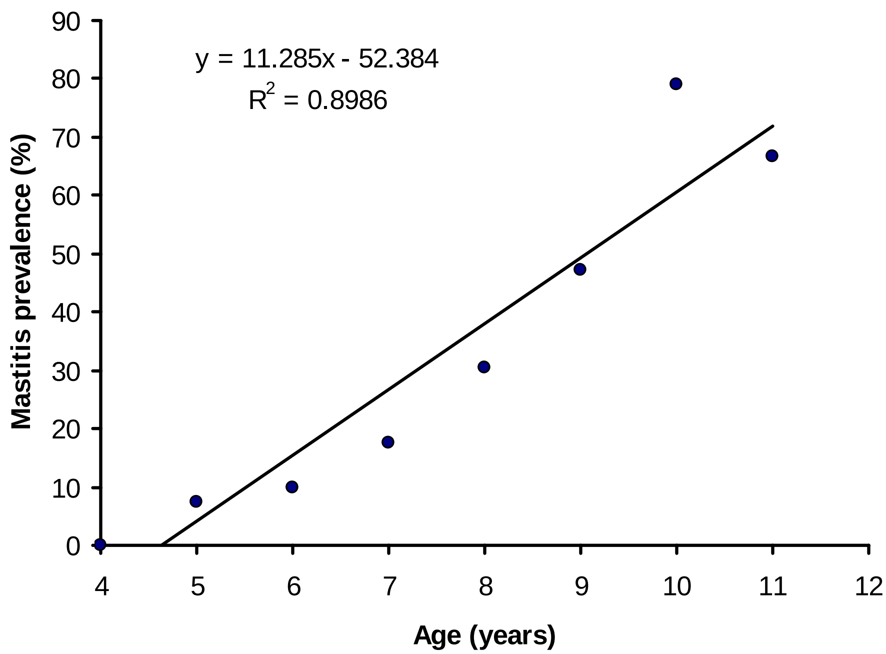

Fig. 4.2 Age based prevalence of mastitis in cows

Table 4.8: Breed-based distribution of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Breed of cow	No. of cows examined	No. of affected cows	Mastitis prevalence (%)
Desi (Non-descript)	175	9	5.14
Sahiwal	155	21	13.54
Crossbred/Exotic	100	56	56.00
Total	**430**	**86**	**20.00**

$\chi^2(2) = 109.175$ ($P < 0.01$)

Table 4.9: Quarter prevalence rates of mastitis in buffaloes and cows in Tehsil Samundri, District Faisalabad (Pakistan).

S. No.	Species	Total number of quarters examined	No. of mastitic animals	No. of mastitic quarters	Quarter prevalence rate (%)
1	Buffalo (n = 2029)	8116	293	478	5.88
2	Cow (n = 430)	1720	86	146	8.48

Table 4.10: Number of buffaloes with one, two, three and four mastitic quarters in Tehsil Samundri, District Faisalabad (Pakistan).

No. of mastitic quarter(s)	**No. of affected buffaloes**	**percent buffaloes**
1	159	54.26[a]
2	93	31.74[b]
3	31	10.58[c]
4	10	3.41[d]
Total = 478	**293**	

[*]Total number of buffaloes examined = 2029
Number of clinical quarters = 67
Number of sub clinically mastitis quarters = 411
(i.e. Surf Field Mastitis Test +ve quarters)

<u>Statistical analysis</u>
Linear regression equation is
y = -17.371 x + 68.425
R^2 = 0.9576
t-value = 6.72*
p-value = 0.02
* = significant ($P < 0.05$)

Values having different superscripts vary significantly

Table 4.11: Number of cows with one, two, three, and four mastitic quarters in Tehsil Samundri, District Faisalabad (Pakistan).

No. of mastitic quarters	No. of affected cows	Percent cows affected
1	46	53.48[a]
2	24	27.90[b]
3	12	13.95[c]
4	4	4.65[d]
Total = 146	**86**	

[*]Total number of cows = 430
Number of clinical quarters = 20
Number of sub clinically mastitic quarters = 126
(i.e. Surf Field Mastitis Test +ve quarters)

<u>Statistical analysis</u>
Linear regression equation is
y = – 16.044x +65.105
R^2 = 0.9493
t-value = 6.12*
p-value = 0.02
* = significant ($P < 0.05$)

Values having different superscripts vary significantly

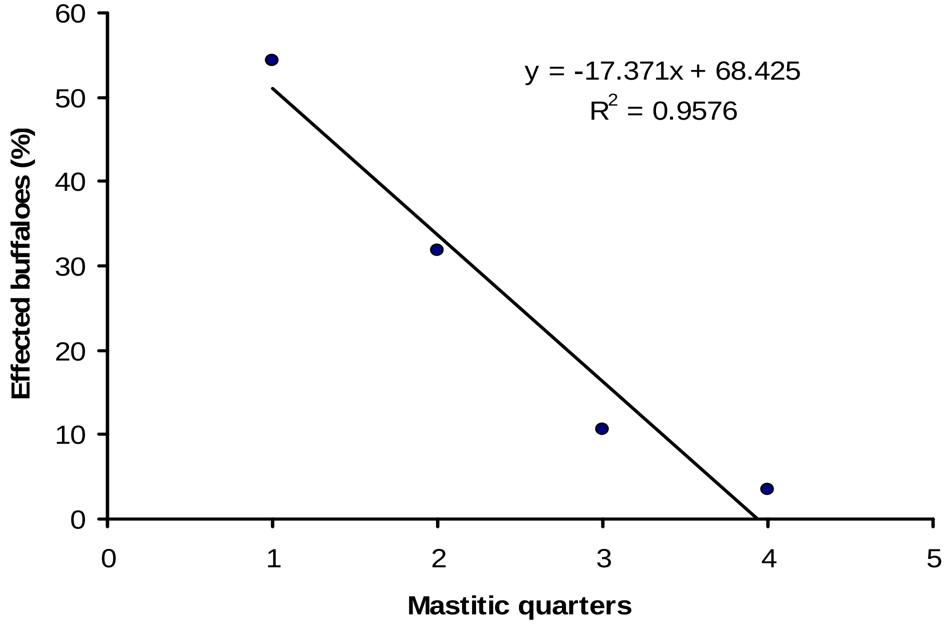

Fig. 4.7 Prevalence of mastitis vis-à-vis position of quarter in buffaloes

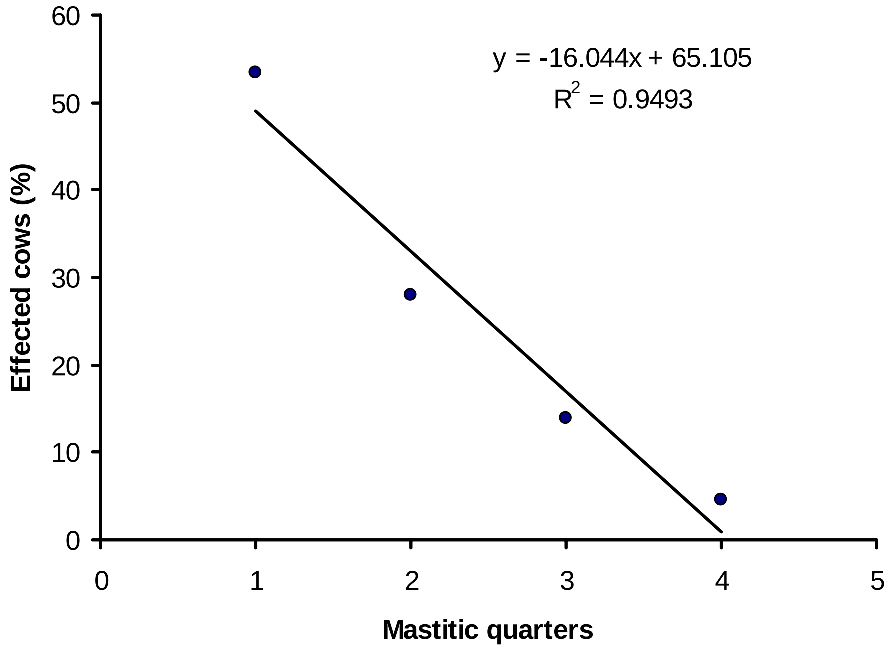

Fig. 4.8 Prevalence of mastitis vis-à-vis position of quarter in cows

Table 4.12: Prevalence of mastitis in buffaloes *vis-à-vis* distance between teat tip and ground in Tehsil Samundri, District Faisalabad (Pakistan).

Distance (inches) between teat tip and ground	No. of buffaloes examined	No. of affected buffaloes	Mastitis prevalence (%)
10	20	15	75
11	36	15	41.66
12	57	14	38.88
13	84	26	30.95
14	105	32	30.47
15	180	40	22.10
16	441	58	13.15
17	415	45	10.84
18	413	29	7.02
19	263	18	6.84
20	15	1	6.67
Total	**2029**	**293**	**14.44**

Statistical analysis

Linear regression equation is

y = -5.7641x + 112.24

R^2 = 0.837 %

t value = 6.794**

p value = 0.000

** = Highly Significant (P < 0.01)

Table 4.13: Prevalence of mastitis in cows *vis-à-vis* distance between teat tip and ground in Tehsil Samundri, District Faisalabad (Pakistan).

Distance (inches) between teat tip and ground	No. of cows examined	No. of affected cows	Mastitis prevalence (%)
11	10	5	50
12	20	10	50
13	30	11	36.66
14	32	10	31.25
15	42	9	21.42
16	95	17	17.89
17	96	15	15.62
18	63	6	9.54
19	30	2	6.66
20	12	1	8.33
Total	**430**	**86**	**20.00**

Statistical analysis

Linear regression equation is

y = -5.239 x +105.94

R^2 = 0.932

t value = 10.49**

p value = 0.000

** = Highly Significant ($P < 0.01$)

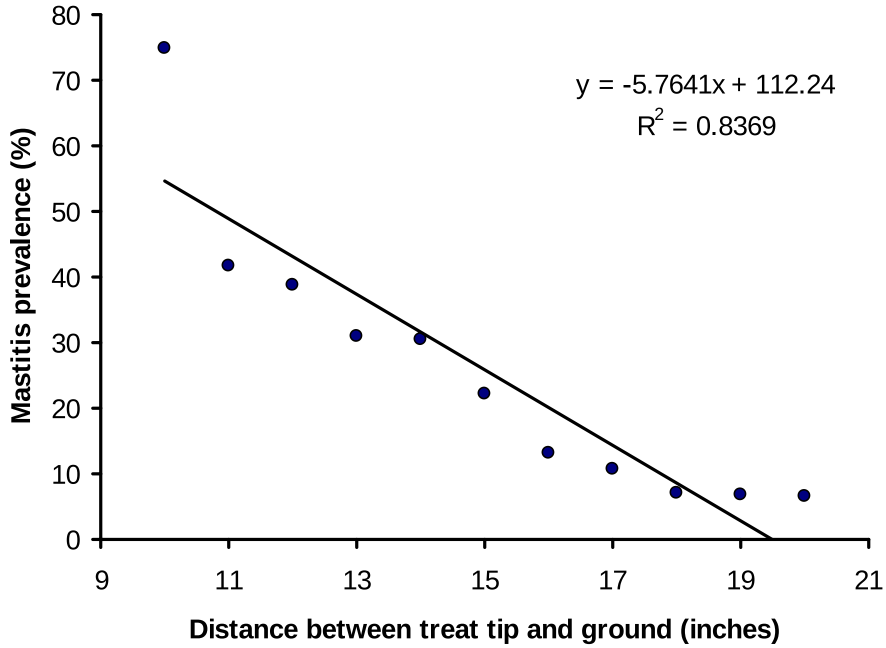

Fig. 4.9 Prevalence of mastitis in buffaloes vis-à-vis distance between teat tip and ground

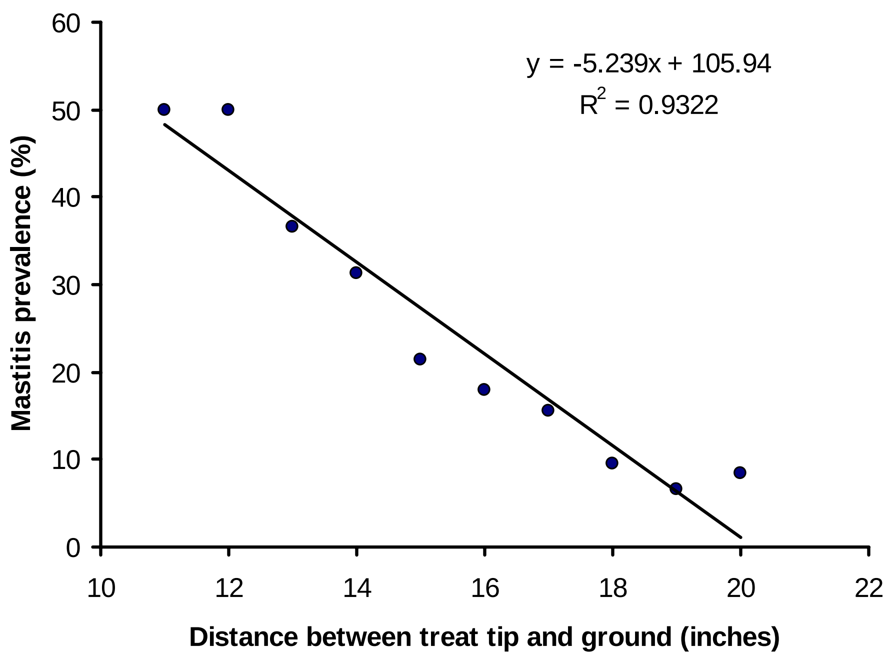

Fig. 4.10 Prevalence of mastitis in cows vis-à-vis distance between teat tip and ground

Table 4.14: Distribution of udder shape in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Udder shape (Saleh & Khamis, 1969)	No. of buffaloes having a particular udder shape	Frequency (%)
Spherical	1850	91.18
Double- Leveled	126	6.21
Hanging	53	2.61
Total	**2029**	

Table 4.15: Distribution of udder shape in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Udder shape (Saleh & Khamis, 1969)	No. of cows having a particular udder shape	Frequency (%)
Spherical	407	94.65
Double- Leveled	23	5.34
Hanging	0	0
Total	**430**	

Table 4.16: Udder shape in relation to prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Udder shape (Saleh & Khamis, 1969)	No. of buffaloes with a particular udder shape	No. of affected buffaloes	Mastitis prevalence (%)
Spherical	1850 (91.17%)	129	6.97
Hanging	53 (2.61%)	42	79.24
Double- Leveled	126 (6.20%)	122	96.82
Total	**2029**	**293**	**14.44**

χ^2 (2) = 955.818 ($P < 0.01$)

Table 4.17: Udder shape in relation to prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Udder shape (Saleh & Khamis, 1969)	No. of cows having a particular udder shape	No. of affected cows	Mastitis prevalence (%)
Spherical	407 (94.65%)	64	15.72
Hanging	0	0	0
Double- Leveled	23 (5.34%)	22	95.65
Total	**430**	**86**	**20.00**

χ^2 (2) = 86.921 ($P < 0.01$)

Table 4.18: Shape based distribution of teats in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Teat Shape (Shukla *et al.*, 1997)	**No. of quarters**	**Frequency (%)**
Funnel	8	0.09
Round	343	4.22
Flat	7765	95.67
Plate	0	0
Total	**8116**	

Table 4.19: Pattern of teat shape in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Teat Shape (Shukla *et al.*, 1997)	**No. of teats with a particular teat shape**	**Frequency (%)**
Funnel	6	0.34
Round	220	12.79
Flat	1490	86.62
Plate	4	0.23
Total	**1720**	**100**

Table 4.20: Teat shape in relation to prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Teat shape (Shukla *et al.*, 1997)	**No. of particular teat shape**	**No. of affected quarters**	**Mastitis prevalence (%)**
Funnel	8 (0.098%)	4	50.00
Round	343 (4.23%)	212	61.80
Flat	7765 (95.67%)	262	3.37
Plate	0	0	0
Total	**8116**	**478**	

<u>Statistical analysis</u>

χ^2 = 2051.697

Table 4.21: Teat shape *vis-à-vis* prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Teat shape (Shukla *et al.*, 1997)	No. of teats with a particular shape	No. of affected quarters	Mastitis prevalence (%)
Funnel	6	3	50
Round	220	124	56.36
Flat	1490	17	1.14
Plate	4	2	50
Total	**1720**	**146**	

<u>Statistical analysis</u>

χ^2 = 774.884

Table 4.22: Frequency of dung removal in relation to prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Frequency of dung removal (times/day)	No. of buffaloes examined	No. of affected buffaloes	Mastitis prevalence (%)
1	304	83	27.30
2	295	61	20.67
3	496	80	16.12
4	371	27	7.27
5 or more	563	42	7.46
Total	**2029**	**293**	**14.44**

Statistical analysis

Linear regression equation is

y = -5.308 x + 31.688

R^2 = 0.944

t value = 7.13 *

p value = 0.006

* =significant (P < 0.05)

Table 4.23: Frequency of dung removal in relation to prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Frequency of dung removal (times/day)	No. of cows	No. of affected cows	Mastitis prevalence (%)
1	89	19	21.3
2	82	17	20.73
3	89	18	20.22
4	87	17	19.54
5 or more	83	15	18.07
Total	**430**	**86**	**20.00**

Statistical analysis

Linear regression equation is

y = -0.765 x+22.267

R^2 = 0.943

t value = 7.07 *

p value = 0.006

* = Significant ($P < 0.05$)

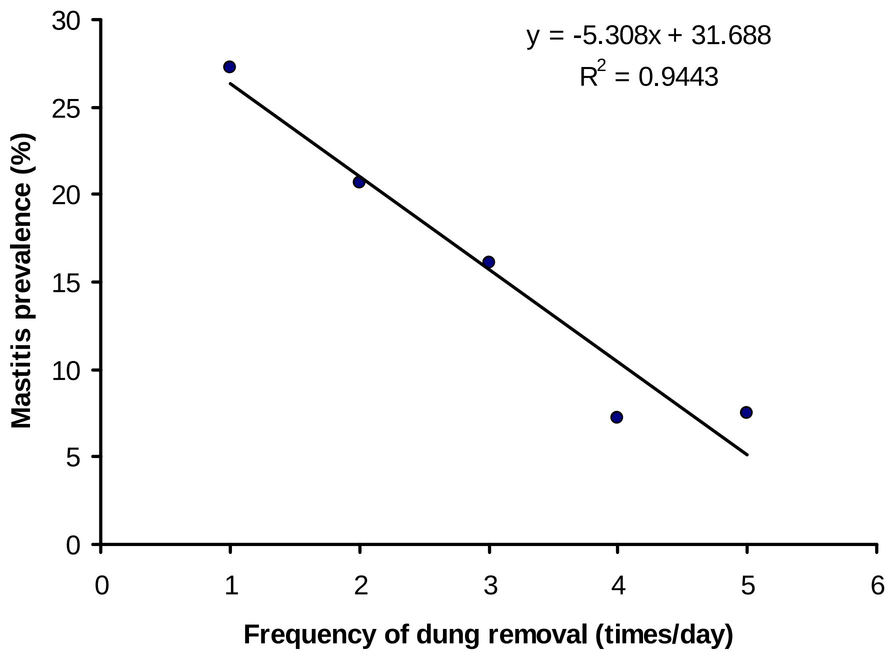

Fig. 4.11 Frequency of dung removal in relation to prevalence of mastitis in buffaloes

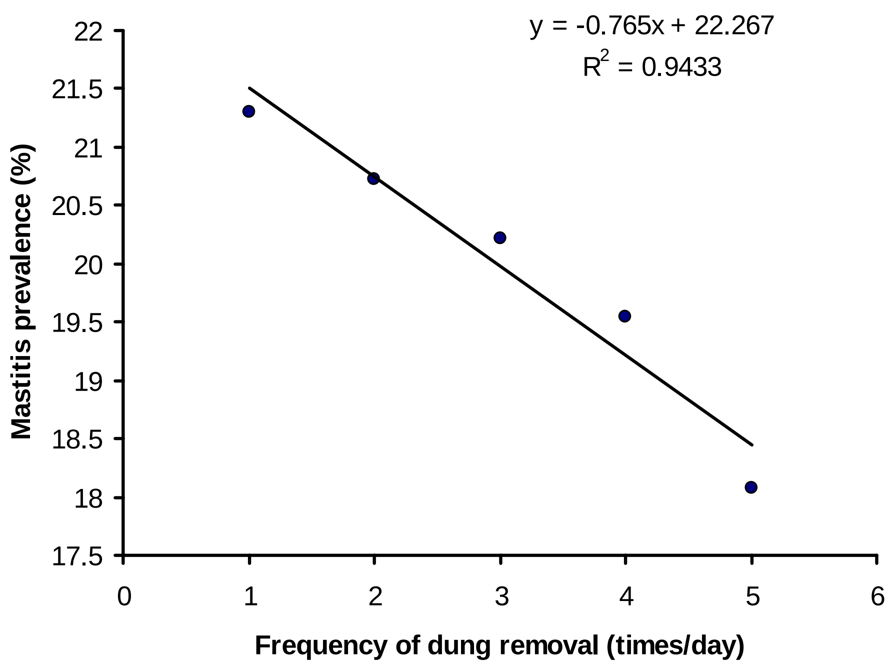

Fig. 4.12 Frequency of dung removal in relation to prevalence of mastitis in cows

Table 4.24: Floor drainage quality in relation to prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Floor drainage quality	No. of buffaloes	No. of affected buffaloes	Mastitis prevalence (%)
Poor*	670	105	15.67
Acceptable**	682	99	14.51
Proper***	677	89	13.14
Total	**2029**	**293**	**14.44**

χ^2 (2) = 1.743 (P < 0.05)

Poor* = Water/urine keeps standing on the floor for more than 2 hours after washing floor or after urination.

Acceptable** = Water/urine keeps standing on the floor for less than 2 hours after washing floor or after urination.

Proper*** = Floor dries up quickly within 1 hour after washing or after urination.

Table 4.25: Floor drainage quality in relation to prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Floor drainage quality	No. of cows	No. of affected cows	Mastitis prevalence (%)
Poor*	146	31	21.23
Acceptable**	150	30	20.00
Proper***	134	25	18.65
Total	**430**	**86**	**20.00**

χ^2 (2) = 0.290 (P < 0.05)

Poor* = Water/urine keeps standing on the floor for more than 2 hours after washing floor or after urination.

Acceptable** = Water/urine keeps standing on the floor for less than 2 hours after washing floor or after urination.

Proper*** = Floor dries up quickly within 1 hour after washing or after urination.

Table 4.26: Prevalence of mastitis in relation to nature of milk let down stimulus in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Nature of milk let down stimulus	**No. of buffaloes**	**No. of affected buffaloes**	**Mastitis prevalence (%)**
Calf suckling	974	252	25.87
Concentrate	1022	28	2.73
Oxytocin injection	31	12	38.70
No. Stimulus	2	1	50.0
Total	**2029**	**293**	**14.44**

χ^2 (3) = 233.102 ($P < 0.05$)

Table 4.27: Prevalence of mastitis in relation to nature of milk let down stimulus in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Nature of milk let down stimulus	No. of cows	No. of affected cows	Mastitis prevalence (%)
Calf suckling	227	72	31.71
Concentrate	201	14	6.96
Oxytocin injection	2	0	0
No. Stimulus	0	0	0
Total	**430**	**86**	**20.00**

χ^2 (2) = 41.326 (P < 0.05)

Table 4.28: Prevalence of mastitis in buffalos *vis-à-vis* the number of animals milked by a milker in Tehsil Samundri, District Faisalabad (Pakistan).

No. of buffaloes milked by the same milker	No. of buffaloes	No. of affected buffaloes	Mastitis prevalence (%)
1	1014	125	12.32
2	400	67	16.75
3	289	45	15.57
4	178	30	16.85
5	82	14	17.07
6	40	7	17.50
7 or more than 7	26	5	19.23
Total	**2029**	**293**	**14.44**

<u>Statistical analysis</u>

Linear regression equation is

y = 0.847 x + 13.08

R^2 = 0.736

t value = 3.74 *

p value = 0.013

* = Significant (P < 0.05)

Table 4.29: Prevalence of mastitis in cows' *vis-à-vis* the number of animals milked by a milker in Tehsil Samundri, District Faisalabad (Pakistan).

No. of cows milked by the same milker	No. of cows	No. of affected cows	Mastitis prevalence (%)
1	20	2	10.00
2	144	13	9.02
3	116	14	12.06
4	60	16	26.66
5	59	26	44.06
6	17	8	47.05
7 or more than 7	14	7	50.
Total	**430**	**86**	**20.00**

Statistical analysis

Linear regression equation is

y = 8.145 x – 4.204

R^2 = 0.908

T-value = 7.05 *

P-value = 0.001

* = significant (P < 0.05)

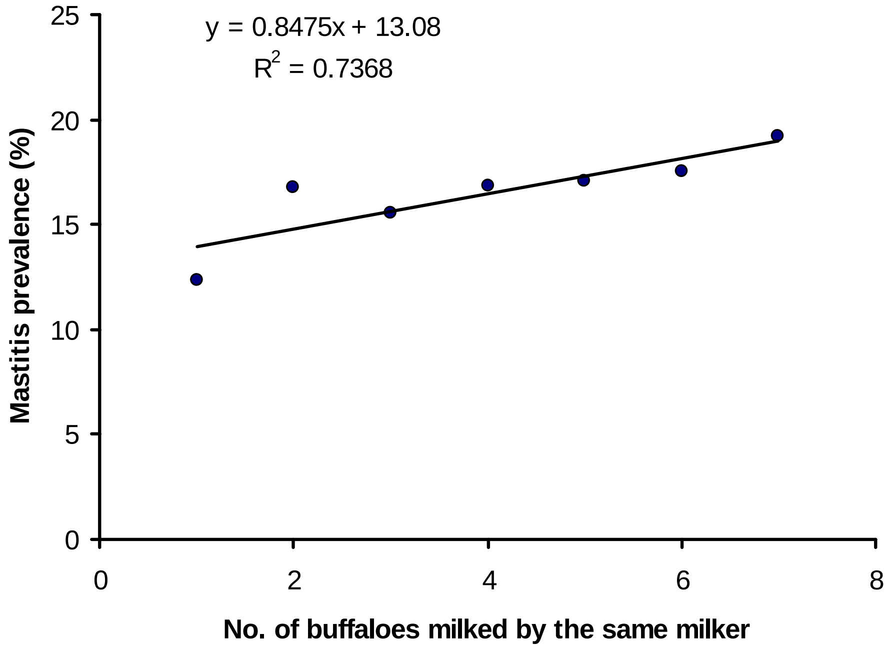

Fig. 4.13 Prevalence of mastitis in buffaloes in relation to number of animals milked by milker

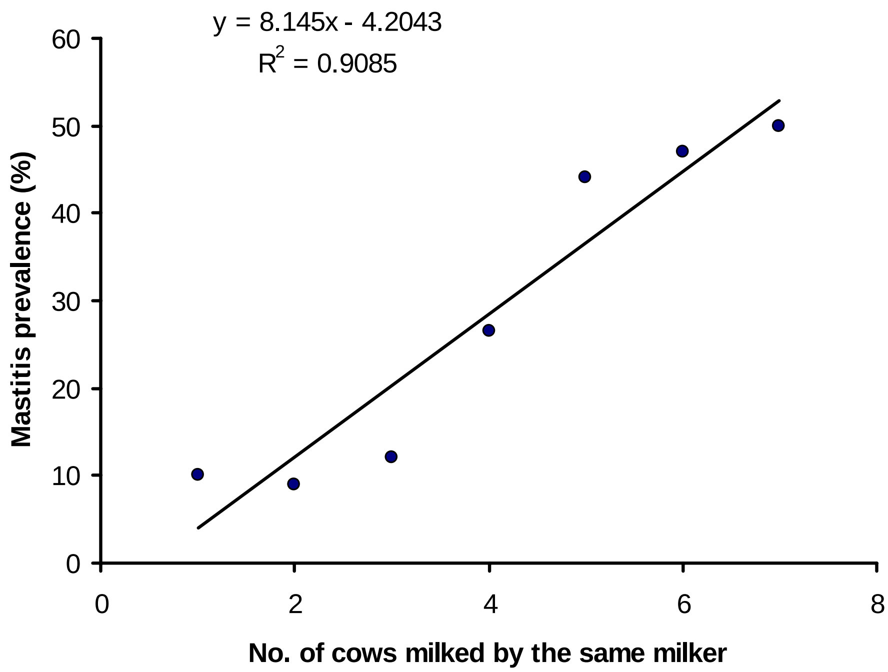

Fig. 4.14 Prevalence of mastitis in cows in relation to number of animals milked by milker

Table 4.30: General physical condition of buffaloes in relation to prevalence of mastitis in Tehsil Samundri, District Faisalabad (Pakistan).

Physical condition	No. of buffaloes	No. of affected buffaloes	Mastitis prevalence (%)
Poor*	163	148	90.79
Good**	1866	145	12.86
Total	**2029**	**293**	**14.44**

χ^2 (1) = 836.376 ($P < 0.01$)

Poor* = Cache tic condition

Good ** = slightly emaciated condition

Table 4.31: General physical condition of cows' *vis-à-vis* prevalence of mastitis in Tehsil Samundri, District Faisalabad (Pakistan).

Physical condition	No. of cows	No. of affected cows	Mastitis prevalence (%)
Poor*	36	32	88.88
Good**	394	54	13.70
Total	**430**	**86**	**20.00**

χ^2 (1) = 116.534 ($P < 0.01$)

Poor* = Cache tic condition

Good ** = slightly emaciated condition

Table 4.32: Education of farmers in relation to prevalence of mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Education level of farmer	No. of buffaloes	No. of affected buffaloes	Mastitis prevalence (%)
Illiterate	922	176	19.08
Primary (5^{th} Grader)	14	2	14.28
Middle (8^{th} Grader)	196	21	10.71
Matric (10^{th} Grader)	470	49	10.42
Intermediate (12^{th} Grader) & Above	427	45	10.53
Total	**2029**	**293**	**14.44**

<u>Statistical analysis</u>

Linear regression equation is

y = 0.767 x +18.373

R^2 = 0.914

t-value = 5.67 *

p-value = 0.010

* = significant ($P < 0.05$)

Table 4.33: Education of farmers in relation to prevalence of mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Education level of farmer	**No. of cows**	**No. of affected cows**	**Mastitis prevalence (%)**
Illiterate	209	52	24.88
Primary (5^{th} Grader)	8	2	25
Middle (8^{th} Grader)	27	6	22.22
Matric (10^{th} Grader)	102	17	16.66
Intermediate (12^{th} Grader) & Above	84	9	10.71
Total	**430**	**86**	**20.00**

Statistical analysis

Linear regression equation is

y = -1.118 x +27.722

R^2 = 0.7281

t-value = 2.83 *

p-value = 0.046

* =significant (P <0.05)

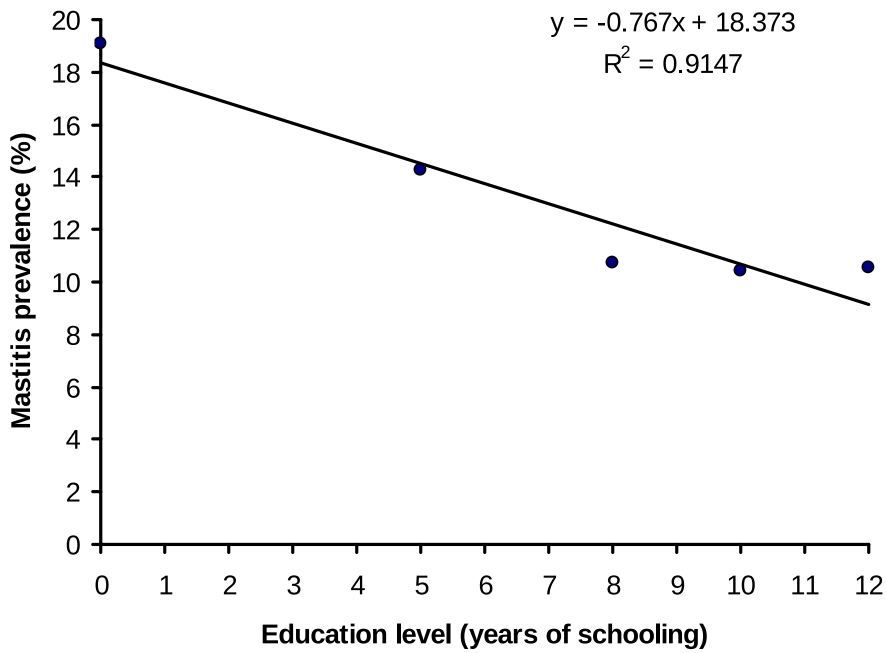

Fig. 4.15 Education of farmers in relation to prevalence of mastitis in buffaloes

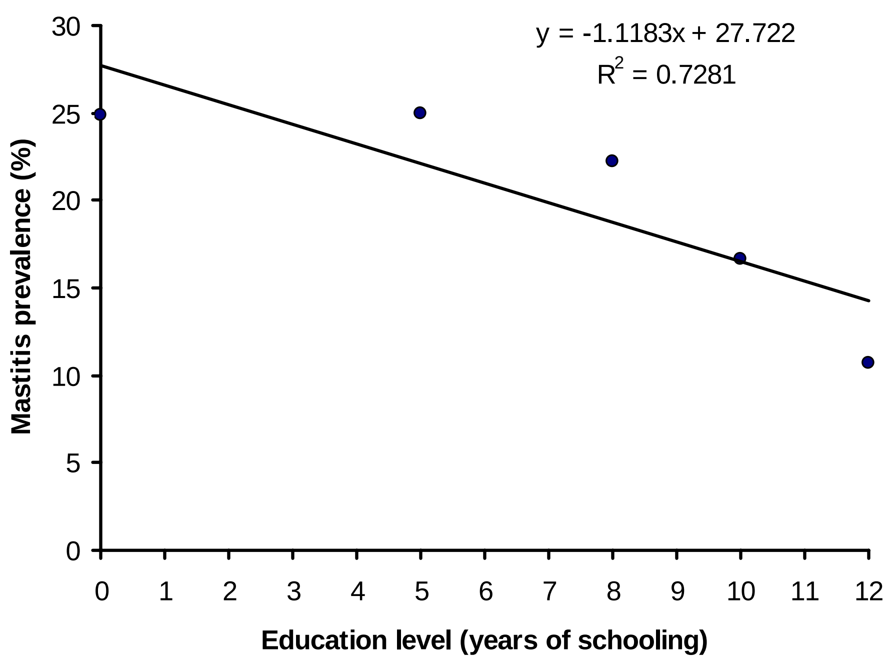

Fig. 4.16 Education of farmers in relation to prevalence of mastitis in cows

Table 4.34: Prevalence (%) of mastitis among buffaloes as a function of reproductive disorders in Tehsil Samundri, District Faisalabad (Pakistan).

Reproductive disorder	No. of buffaloes with reproductive disorder	No. of buffaloes with mastitis	Mastitis prevalence (%)	Mastitis prevalence (times (x) that in unaffected animals)
No	1767	77	4.35	NA
Metritis	121	112	92.56	21.28
Retained placenta	102	79	77.45	17.8
Dystokia	16	8	50	11.49
Prolapse of uterus	23	17	73.91	16.99
Total	**2029**	**293**	**14.44**	**18.95**

χ^2 (4) = 1153.07 ($P < 0.01$)

Two hundred and sixteen of 262 buffaloes with reproductive disorders had mastitis i.e. 82.44% of animals with reproductive disorders were found affected with mastitis

Table 4.35: Prevalence (%) of mastitis among cows as a function of reproductive disorders in Tehsil Samundri, District Faisalabad (Pakistan).

Reproductive disorder	No. of cows with reproductive disorder	No. of cows with mastitis	Mastitis prevalence (%)	Mastitis prevalence (times (x) that of unaffected animals)
No	361	27	7.48	NA
Metritis	40	34	85.00	11.36
Retained placenta	25	21	84.00	11.22
Dystokia	1	1	100	13.4
Prolapse of uterus	3	3	100	13.37
Total	**430**	**86**	**20.00**	**11.43**

χ^2 (4) = 220.99 (P < 0.05)

Fifty nine of 69 cows (85.5 %) with reproductive disorders were found affected with mastitis

Table 4.36: Distribution of reproductive disorders in various breeds of cattle in Tehsil Samundri, District Faisalabad (Pakistan).

Disorder	Desi (local non-descript)	Sahiwal	Crossbred/pure exotic
Metritis	2	11	22
Retained placenta	0	0	25
Dystokia	1	1	1
Prolapse of uterus	0	1	0
Total	**175**	**155**	**100**

Table 4.37: Association between teat injury and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Teat injury	+	46	213	259
	-	247	1523	1770
		293	1736	**2029**

χ^2 (1) = 2.649 (P < 0.01)

A: Strength

i. Relative risk (RR) = 1.09

Limits of 95 % confidence interval of RR = 0.826, 1.808

ii. Population relative risk (RRpop) = 1.035

B: Effect

i. Attributable rate (AR) = 0.038

ii. Attributable fraction (AF) = 0.082

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.004

ii. Population attributable fraction (PAF) = 0.033

Table. 4.38: Association between teat injury and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Teat injury	+	27	49	76
	-	59	295	354
		86	344	**430**

χ^2 (1) = 13.09 (P < 0.01)

A: Strength

i.	Relative risk (RR)	= 2.138
	Limits of 95 % confidence interval of RR	=1.47, 3.129
ii.	Population relative risk (RR_{POP})	= 1.204

B: Effect

i.	Attributable rate (AR)	= 0.189
ii.	Attributable fraction (AF)	= 0.532

C: Total Effect (importance)

i.	Population attributable rate (PAR)	= 0.029
ii.	Population attributable fraction (PAF)	= 0.169

Table 4.39: Association between condition of floor and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Uneven Floor	+	23	178	201
	-	270	1558	1828
		293	1736	**2029**

χ^2 (1) = 1.623 (P > 0.05)

Table 4.40: Association between condition of floor and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Uneven Floor	+	3	16	19
	-	83	328	411
		86	344	**430**

χ^2 (1) = 0.220 (P > 0.05)

Table 4.41: Association between hard milking and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

		Mastitis Status +	Mastitis Status -	
Hard Milking	+	90	297	387
	-	203	1439	1642
		293	1736	**2029**

χ^2 (1) = 30.077 ($P < 0.01$)

<u>A: Strength</u>

i. Relative risk (RR) = 1.886

Limits of 95 % confidence interval (RR) = 1.519, 2.332

ii Population relative risk (RR_{POP}) = 1.170

<u>B: Effect</u>

i. Attributable rate (AR) = 0.109

ii. Attributable fraction (AF) = 0.469

<u>C: Total Effect (importance)</u>

i. Population attributable rate (PAR) = 0.020

ii. Population attributable fraction (PAF) = 0.145

Table 4.42: Association between hard milking and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

		Mastitis Status		
		+	-	
Hard Milking	+	32	76	108
	-	54	268	322
		86	344	**430**

χ^2 (1) = 8.359 (P < 0.01)

<u>A: Strength</u>

i. Relative risk (RR) = 1.772

Limits of 95 % confidence interval (RR) = 1.213, 2.585

ii. Population relative risk (RR_{POP}) = 1.197

<u>B: Effect</u>

i. Attributable rate (AR) = 0.129

ii. Attributable fraction (AF) =0.435

<u>C: Total Effect (importance)</u>

i. Population attributable rate (PAR) = 0.032

ii. Population attributable fraction (PAF) = 0.164

Table 4.43: Association between folded-thumb milking technique and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Folded-Thumb method of hand milking	Mastitis Status +	Mastitis Status -	Total
+	93	307	400
-	200	1429	1629
	293	1736	**2029**

χ^2 (1) = 31.294 (P < 0.01)

A: Strength

i. Relative risk (RR) = 1.901

Limits of 95 % confidence interval (RR) = 1.544, 2.337

ii. Population relative risk (RR_{POP}) = 1.180

B: Effect

i. Attributable rate (AR) = 0.110

ii. Attributable fraction (AF) = 0.479

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.024

ii. Population attributable fraction (PAF) = 0.152

Table 4.44: Association between folded-thumb milking technique and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

		Mastitis Status +	Mastitis Status -	
Folded-Thumb method of hand milking	+	26	64	90
	-	60	280	340
		86	344	**430**

χ^2 (1) = 5.621 (P < 0.05)

A: Strength

i. Relative risk (RR) = 1.636

Limits of 95 % confidence interval (RR) = 1.104, 2.421

ii. Population relative risk (RR_{POP}) = 1.136

B: Effect

i. Attributable rate (AR) = 0.112

ii. Attributable fraction (AF) = 0.388

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.037

ii. Population attributable fraction (PAF) = 0.119

Table 4.45: Association between udder oedema and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

		Mastitis Status		
		+	-	
Udder Oedema	+	40	124	164
	-	253	1612	1865
		293	1736	**2029**

χ^2 (1) = 14.294 ($P < 0.01$)

A: Strength

i. Relative risk (RR) = 1.800

Limits of 95 % confidence interval (RR) = 1.357, 2.386

ii Population relative risk (RR_{POP}) = 1.066

B: Effect

i. Attributable rate (AR) = 0.108

ii. Attributable fraction (AF) = 0.444

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.008

ii. Population attributable fraction (PAF) = 0.061

Table 4.46: Association between udder oedema and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Udder Oedema	+	25	53	78
	-	61	291	
		86	344	**430**

χ^2 (2) = 352.49 (P < 0.01)

<u>A: Strength</u>

i. Relative risk (RR)	= 1.849
Limits of 95 % confidence interval (RR)	= 1.248, 2.736
ii. Population relative risk (RR_{POP})	= 1.156
<u>B: Effect</u>	
i. Attributable rate (AR)	= 0.147
ii. Attributable fraction (AF)	= 0.459
<u>C: Total Effect (importance)</u>	
i. Population attributable rate (PAR)	= 0.026
ii. Population attributable fraction (PAF)	= 0.134

Table 4.47: Association between teat oedema and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

Teat Oedema		+	-	
	+	85	183	268
	-	208	1553	1761
		293	1736	**2029**

χ^2 (1) = 74.590 (P < 0.01)

A: Strength

i. Relative risk (RR) = 2.701

Limits of 95 % confidence interval (RR) = 2.195, 3.321

ii. Population relative risk (RR_{POP}) = 1.230

B: Effect

i. Attributable rate (AR) = 0.200

ii. Attributable fraction (AF) = 0.629

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.026

ii. Population attributable fraction (PAF) = 0.186

Table 4.48: Association between teat oedema and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

		Mastitis Status +	Mastitis Status -	
Teat Oedema	+	32	46	78
	-	54	298	352
		86	344	**430**

χ^2 (1) = 26.327 (P < 0.01)

A: Strength

i. Relative risk (RR) = 2.679

Limits of 95 % confidence interval (RR) = 1.866, 3.818

ii. Population relative risk (RR_{POP}) = 1.307

B: Effect

i. Attributable rate (AR) = 0.257

ii. Attributable fraction (AF) = 0.626

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.046

ii. Population attributable fraction (PAF) = 0.234

Table 4.49: Association between blood in milk and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Blood in Milk	+	50	154	204
	-	243	1582	1825
		293	1786	**2029**

χ^2 (1) = 18.612 (P < 0.01)

A: Strength

i. Relative risk (RR) = 1.842

Limits of 95 % confidence interval (RR) = 1.383, 2.401

ii. Population relative risk (RR_{POP}) = 1.085

B: Effect

i. Attributable rate (AR) = 0.108

ii. Attributable fraction (AF) = 0.457

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.010

ii. Population attributable fraction (PAF) = 0.078

Table 4.50: Association between blood in milk and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Blood in Milk	+	20	38	58
	-	66	306	372
		86	344	**430**

χ^2 (1) = 8.789 (P < 0.01)

A: Strength

i. Relative risk (RR) = 1.943

Limits of 95 % confidence interval (RR) = 1.294, 2.924

ii. Population relative risk (RR_{POP}) = 1.129

B: Effect

i. Attributable rate (AR) = 0.167

ii. Attributable fraction (AF) = 0.485

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.021

ii. Population attributable fraction (PAF) = 0.116

Table 4.51: Association between wallowing and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Wallowing	+	12	191	203
	-	281	1545	1826
		293	1736	**2029**

χ^2 (1) = 1.09 (P > 0.05)

Table 4.52: Association between wallowing and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

Wallowing		+	-	
	+	6	21	27
	-	80	323	403
		86	344	**430**

χ^2 (1) = 0.089 (P > 0.05)

Table 4.53: Association between teat Stenosis and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Teat Stenosis	+	70	139	209
	-	223	1597	1820
		292	1736	**2029**

χ^2 (1) = 68.453 (P < 0.01)

A: Strength

i. Relative risk (RR) = 2.737

Limits of 95 % confidence interval (RR) = 2.21, 3.35

ii. Population relative risk (RR_{POP}) = 1.188

B: Effect

i. Attributable rate (AR) = 0.212

ii. Attributable fraction (AF) = 0.634

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.021

ii. Population attributable fraction (PAF) = 0.152

Table 4.54: Association between teat Stenosis and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

Teat Stenosis		+	-	
	+	38	57	95
	-	48	287	335
		86	344	**430**

χ^2 (1) = 30.485 (P < 0.01)

A: Strength	
i. Relative risk (RR)	= 2.797
Limits of 95 % confidence interval (RR)	= 1.96, 3.93
ii. Population relative risk (RR_{POP})	= 1.398
B: Effect	
i. Attributable rate (AR)	= 0.257
ii. Attributable fraction (AF)	= 0.642
C: Total Effect (importance)	
i. Population attributable rate (PAR)	= 0.056
ii. Population attributable fraction (PAF)	= 0.284

Table 4.55: Association between milk leakage and mastitis in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Milk Leakage	+	15	54	69
	-	278	1682	1960
		293	1736	**2029**

χ^2 (1) = 3.080 (P < 0.05)

A: Strength

i. Relative risk (RR) = 1.539

Limits of 95 % confidence interval (RR) = 0.966, 2.417

ii. Population relative risk (RR_{POP}) = 1.024

B: Effect

i. Attributable rate (AR) = 0.076

ii. Attributable fraction (AF) = 0.350

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.002

ii. Population attributable fraction (PAF) = 0.023

Table 4.56: Association between milk leakage and mastitis in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Mastitis Status

		+	-	
Milk Leakage	+	10	25	35
	-	76	173	395
		86	344	**430**

χ^2 (1) = 0.055 (P < 0.05)

A: Strength

i. Relative risk (RR) = 1.484

Limits of 95 % confidence interval (RR) = 0.864, 2.530

ii. Population relative risk (RR_{POP}) = 1.041

B: Effect

i. Attributable rate (AR) = 0.093

ii. Attributable fraction (AF) = 0.326

C: Total Effect (importance)

i. Population attributable rate (PAR) = 0.007

ii. Population attributable fraction (PAF) = 0.039

Table 4.57: Frequency distribution of isolates (n = 214) recovered from 200 mastitic (clinical n = 17) and sub clinically (n = 183) quarters of 95 buffaloes in Tehsil Samundri, District Faisalabad.

S. No	Species	No of isolates	Frequency (%)
1.	*Staphylococcus aureus*	106	59.53
2.	*Streptococcus agalactiae*	51	23.83
3.	*Staphylococcus hyicus hyicus*	19	8.88
4.	*Staphylococcus epidermidis*	14	6.54
5.	*Staphylococcus hominis*	3	1.40
6.	*Staphylococcus xylosus* 1	2	0.93
7.	*Undifferentiable (nontypable) Coagulase negative Staphylococcus species*	2	0.93
8.	*Streptococcus dysgalactiae*	2	0.93
9.	*Corynebacterial species*	2	0.93
10.	*Escherichia coli*	3	1.40
11.	*Bacillus spp.*	8	3.77
12.	Yeast	1	0.47
13.	Prototheca	1	0.47
	Total	**214**	

Table 4.58: Frequency distribution of isolates (n = 105) recovered from 100 mastitic (clinical n = 11; sub clinical n = 89) quarters of 53 cows in Tehsil Samundari, District Faisalabad.

S. No	Species	No of isolates	Frequency (%)
1	*Staphylococcus aureus*	47	44.76
2	*Streptococcus agalactiae*	23	21.90
3	*Staphylococcus epidermidis*	8	7.62
4	Nontypable coagulase negative staphylococci	7	6.67
5	*Staphylococcus hyicus*	4	3.81
6	*Corynebacterial spp.*	4	3.81
7	Environmental streptococci (Esculin positive, CAMP-ve streptococci)	4	3.81
8	*Bacillus spp.*	4	3.81
9	*Streptococcus dysgalactiae*	2	1.90
10	*Escherichia coli*	1	0.95
11	Nocardia spp.	1	0.95
	Total	**105**	

Table 4.59: Epidemiologic measures of association between factors and mastitis status in buffaloes in Tehsil Samundri, District Faisalabad (Pakistan).

Measurements of Association	Factors									
	Teat injury	Evenness of floor	Hard milking	Folded thumb	Udder oedema	Teat oedema	Blood in milk	Wallow -ing	Teat stenosis	Milk leakage
A: Strength										
i. Relative risk (R.R)	1.091	1.289	1.886	1.904	1.800	2.701	1.842	1.950	2.737	1.539
ii. Population relative risk (RR_{POP})	1.035	1.263	1.170	1.180	1.066	1.230	1.085	1.000	1.188	1.024
B: Effect										
i. Attributable rate (AR)	0.038	0.033	0.109	0.110	0.108	0.200	0.108	0.008	0.212	0.076
ii. Attributable fraction (AF)	0.082	0.218	0.469	0.479	0.444	0.629	0.457	0.009	0.634	0.350
C: Total Effect (importance)										
i. Population attributable rate (PAR)	0.004	0.029	0.020	0.024	0.008	0.026	0.010	0.015	0.021	0.002
ii. Population attributable fraction (PAF)	0.033	0.208	0.145	0.152	0.061	0.186	0.078	0.000	0.152	0.023

Table 4.60: Epidemiologic measures of association between factors and mastitis status in cows in Tehsil Samundri, District Faisalabad (Pakistan).

Measurements of Association	Factors									
	Teat injury	Evenness of floor	Hard milking	Folded thumb	Udder oedema	Teat oedema	Blood in milk	Wallow -ing	Teat stenosis	Milk leakage
A: Strength										
i. Relative risk (R.R)	2.138	1.280	1.772	1.636	1.849	2.679	1.943	1.121	2.797	1.484
ii. Population relative risk (RR_{POP})	1.204	1.273	1.197	1.136	1.156	1.307	1.129	1.010	1.398	1.041
B: Effect										
i. Attributable rate (AR)	0.189	0.044	0.129	0.112	0.147	0.257	0.167	0.024	0.257	0.093
ii. Attributable fraction (AF)	0.532	0.218	0.435	0.388	0.459	0.626	0.485	0.108	0.642	0.326
C: Total Effect (importance)										
i. Population attributable rate (PAR)	0.029	0.420	0.032	0.037	0.026	0.046	0.021	0.0015	0.056	0.007
ii. Population attributable fraction (PAF)	0.169	0.214	0.164	0.119	0.134	0.234	0.116	0.009	0.284	0.039

CHAPTER 5

DISCUSSION

Mastitis is one of the major diseases causing huge economic losses to the dairy industry. This disease is the outcome of the interaction of determinants associated with host, pathogen(s) and environment. The local information on the epidemiological dimensions of mastitis in Pakistan is extremely inadequate. This information is imperative for planning an intervention strategy for this costly disease. Without knowing the epidemiology, it is very difficult, rather impossible to control the disease.

The present study was, therefore, designed to determine the prevalence of bubaline (= dairy buffaloes) and bovine(cow) mastitis in one of the major rural dairying areas of Pakistan (Tehsil Samundri, District Faisalabad) and to determine the association of important potential risk factors with mastitis. For this purpose, epidemiological information of randomly selected 2029 buffaloes and 430 cows was collected and analyzed. It is anticipated that inferences deduced from this study would help the farmers, veterinarians and other concerned authorities in the control of this disease.

The overall prevalence of mastitis was 14.44% in buffaloes and 20% in cows. Almost all microorganisms, which cause mastitis enter the udder through the teat opening (= teat meatus). Owing to stronger smooth muscles around the teat opening (Uppal, 1994), dairy buffaloes are considered to be less susceptible to mastitis than dairy cows. Several workers have documented a lower susceptibility of dairy buffaloes to mastitis than diary cows (Allore, 1993; Kalara and Dhanda, 1964; Saini *et al.*, 1994; Bansal *et al.*, 1995; Thirunvukkarasu and Prabaharan, 1998). The present findings indicated a significant association between age of animals and prevalence of clinical and sub clinical mastitis in buffaloes and cows. The prevalence was maximum (72.72%) in buffaloes aged ≥ 11 while in cows the prevalence (78.94%) was highest in 10-yr age group. The increase in prevalence rate with the advancing age may be due to gradual suppression of immune system of the body and structural changes in udder and teats . The present findings are supported by those of Rasool *et al.* (1985) and Khalaf (1983) who reported that rate of occurrence of mastitis increased with age. Al-Shawabkech and Abdul-Aziz (1987) also reported that mastitis was more likely to occur in older animals.

In the present study, the regression analysis and coefficient of determination values indicated a highly significant increase in mastitis prevalence with the increase in lactation number. Thus it was maximum in lactation number 6 and above in buffaloes as well as cows. This may be due to higher milk production paralleling in general with advancing lactation number which often tends to predispose the udder to mastitis (Stableforth and Galloway, 1959). Similar results were also reported by Rasool *et al.* (1985), Al-Shawabkeh and Abdul-Aziz (1987), Saini *et al.* (1994) and Premachand *et al.* (1995), who reported that prevalence of mastitis tended to increase with the increasing lactation number. Similar disease pattern in bovines was observed by Gonzalez *et al.* (1980), Didonet *et al.* (1986), and Pluvinage *et al.* (1988), and Ramachandraiach *et al.* (1981).

Stage of lactation based (= month in lactation) stratification prevalence of mastitis indicated the highest prevalence rates during the first month of lactation both in buffaloes (27.00 %) and cows (72.05%). The highest prevalence rate during the first month of lactation is an indication of infection probably prior to freshening. It may also be reflection of important changes in endocrine, nutritional and metabolic status which occur in the peri parturient period. These changes predispose the cows and buffaloes to parturition disease complex (ketosis, milk fever, retained placenta, fatty liver syndrome, dystokia, metritis, and displaced abomasums). Mastitis is also an important component of the peri parturient disease complex (Spain and Scheer, 2004). Goff and Kimura (2002) reviewed the relationship between transition cow nutrition, metabolic disorders and metabolism. These authors described the strong associations in describing the relationships between milk fever, ketosis and increased risk of mastitis. According to Pluvinage *et al.* (1990), 35 percent occurrence was in the first month of lactation. Similar picture of disease was reported by Rasool *et al.* (1985), Khalaf (1985), Gonzalez *et al.* (1980).

In the present study, chi-square analysis revealed a significant association between the reproductive disorders and mastitis prevalence in buffaloes and cows. Two hundred and sixteen of 262 buffaloes had mastitis (82.44%) with reproductive disorders (metritis, retention of placenta, dystokia and prolepses of uterus). Similarly, fifty nine of 69 cows had mastitis (85.5%) with these reproductive disorders .Out of all reproductive disorders, metritis showed the most strong association with mastitis in dairy buffaloes. The association of mastitis with reproductive disorders is in line with the findings reported previously. Schukken *et al.* (1989) reported that cows with retained placenta (n = 62) were three times more likely to develop mastitis during hospitalization than animals without retained placenta (n = 134). Esmat and Badr (1996) investigated lactation failure and purulent uterine discharge (assumed to be due to metritis) in 127 diary cows (from 3 dairy farms) in relation to mastitis. Eighty seven cows (68.5%) had acute mastitis and 23 (18.1%) were affected with sub clinical mastitis. Bacteriological examination of the udder and uterine secretion of cows showed mastitis-matritis agalactia syndrome. The possibility of this syndrome may explain in part at least the much higher frequency of mastitis in animals affected with metritis and retained placenta as compared with those animals not affected with these reproductive disorders. It can also be conjunctured that animals affected with mastitis and metritis/retained placenta were in a state of immunosuppression. Therefore, they were vulnerable not only to mastitis but also to metritis and retention of placenta. According to Marcos *et al.*

(1988), 83% of cows with endometritis following parturition developed acute mastitis. Prabkakar *et al.* (1988) examined milk samples and uterine contents of 5 buffaloes (that developed mastitis concurrently with metritis) for the presence of *S. aureus, S. epidermidis, Str. agalactiae* and *E. coli*. The same organisms were found in both samples for 87.7% of the animals.

The present study revealed a highly significant association (P< 0.01) between the mastitis status and breed based distribution in cows. However, a cross bred / exotic cows had a higher (56.0%) prevalence of mastitis than Sahiwal (13.54%) and nondescript breeds (5.14%). Different breeds of cattle are known to differ in their susceptibility to mastitis. The indigenous desi-breeds are comparatively more resistant to the disease. Breeds difference in prevalence of mastitis were also reported by Dutta *et al.* (1990) who concluded that the risk ratio for the development of mastitis was 1.21 to 1.89 times greater in Jersey cows than the crossbred cows. The difference may be due to inheritance as well as due to more development of udder and teats in exotic breeds of cows. According to Nooruddin *et al.* (1997), the highest prevalence of the disease was recorded in crossbred (28.1%) followed by exotic pure bred (26.1%) and indigenous cows/desi cow (5.96%). Similarly, Roy *et al.* (1993) reported that the prevalence/

incidence of mastitis in crossbred cows were 76% as compared with 11% in the indigenous cows.

The present findings concluded a highly-significant association between mastitis prevalence and teat-tip distance from the ground in buffaloes and cows. Rosenberger (1979) documented that the distance between teat tip and ground should be at least 16-18 inches in cows of the German Black pied breed.

In the present study a highly significant association ($P<0.01$) was found between udder shape and the mastitis status in buffaloes and cows. The maximum prevalence rate was observed in double-leveled shape udder in buffaloes and cows followed by hanging and spherical shaped udder. Spherical udder type was the predominant udder form in buffaloes and cows followed by double-leveled and hanging shape udder. Similar findings were also documented by Saleh *et al.* (1969). These workers examined 500 lactating buffaloes in order to classify the different morphological patterns of the udder form. Only 82 animals (16.4%) showed an ideal udder form and the predominant udder form among the buffaloes was the hanging form (54.8%).

In the present study a significant relationship was found between the factors and mastitis prevalence. The teats with round shape had a higher prevalence of mastitis as compared with funnel and flat type teats. The reasons for rounded shaped teat tips more prone to infection could be more chances of exposure to environmental contamination and injuries. Similarly, a fairly high rate of prevalence in funnel shape teat tips could be due to the retention of some amount of milk which facilitates the microbial growth to establish mastitis. Indian workers (Shukla *et al.*, 1997) attempted to determine the relationship of teat type, teat length and quarters affected with the occurrence of mastitis in a prevalence study. The study was conducted on 154 animals (597 quarters) representing 78 crossbred (302 quarters), 36 Sahiwal cows (145 quarters) and 40 Murrah buffaloes (155 quarters). The teat tips were categorized into four types viz., funnel, round, flat and plate shaped. Teat length was categorized into small (<5.5 cm), medium (5.6-7.5 cm) and large (> 7.5 cm). Quarter milk samples were examined by performing California mastitis test, somatic cell counts and microbiological examination of milk. Maximum prevalence of mastitis was recorded in case of animals with funnel shaped teat tip and was attributed to the retention of some milk which facilitates the microbial growth to establish mastitis. However, the results of the total somatic cell counts showed the round shaped teat tips to be equally susceptible to infection as they are more frequently exposed to environmental contamination and are also more likely to be injured. The investigators opinioned that plate type teat tips should be preferred over funnel and round shaped teat tips in breeding programme to decrease the incidence of mastitis. According to Rosenberger *et al.* (1979) teat shape should be rounded or hemispherical. The disadvantage of other shapes of teat tip, such as funnel and plate shapes is that a drop of milk tends to hang at the teat tip after milking, favouring colonization of the teat canal by mastitis pathogens. Cows with pointed teat tips tend to be hard milkers. The opening of the teat canal must be in the center of the teat tip, and not to one side. The formation of a small wall around the teat opening, due to prolapse of the mucous membrane of the teat canal, also predisposes to udder infection. Rathore *et al.* (1976) documented that cows with cylindrical shaped teats had a significantly higher prevalence of mastitis, possibly due to a higher incidence of teat cup crawl.

In the present study a regression analysis revealed a significant association in buffaloes and cows between the frequency of dung removal and the mastitis prevalence. The prevalence rate was maximum (27.30% and 21.3% in buffaloes and cows respectively) when the dung removal was done once/ day in buffaloes and cows. Similar results have been documented by Carrol (1977). Ahmed (1981) also founded that with once a day cleaning of sheds, the incidence of mastitis in buffaloes was 21.06% where as when cleaning of sheds was done twice a day, 15.76 % of animals developed mastitis. When cleaning of sheds was practiced more than twice a day, 9.38% incidence of mastitis was observed. Only a marginal decrease in mastitis with increasing frequency of dung removal may be explained by the fact that mastitis in Pakistan and other developing countries lacking the application of standard mastitis control is predominantly contagious in nature (Allore, 1993). Environmental mastitis pathogens with reservoirs in dung, floor, bedding etc. are only occasionally associated with mastitis. Therefore, mastitis control practices directed against environmental mastitis pathogens are not likely to be as potentially rewarding as practices aimed at controlling contagious pathogens.

The present findings documented a significant association between the drainage quality and mastitis status in buffaloes and cows. Only a marginally higher prevalence of mastitis in buffaloes and cows (15.67%

and 21.23%) were managed under poor floor drainage quality than those managed with proper drainage (13.14% and 18.65%) may also be explained along the same lines of reasoning. Oltenacu *et al.* (1990) reported that the herds with liquid system were at highest prevalence rate risk of mastitis than herds with solid systems. Similarly, Dodd and Phipps (1985) observed that attention towards drainage and cleanliness of bedding was means of minimizing mastitis.

The present study concluded a chi-square value as 233.102, 41.326 in buffaloes and cows respectively. The association of 4 different milk let down stimuli (calf suckling, concentrate, oxytocin injection, No stimulus) with prevalence of mastitis was investigated. In the present study only two buffaloes and one cow fell under 'No stimulus' category, the relationship of this "stimulus" with mastitis was not calculated. For the remaining 3 milk let down stimuli, the highest mastitis prevalence (38.70%) was recorded in buffaloes milked by exogenous parenteral administration of oxytocin, followed by those in which letdown of milk was induced by suckling calves (25.87%) and offering concentrate at the time of milking (2.73%). The highest prevalence (31.71%) of mastitis was recorded in cows in which sucking calf was used as a milk letdown stimulus, followed by those which were enticed to have a letdown of milk by offering concentration (6.96%) injection.

Oxytocin injection is used quite commonly by the farmers in Pakistan for let down of milk. This particularly is the case with buffaloes that are reportedly deficient in oxytocin. As far as could be ascertained there is no report on the epidemiologic association between the use of oxytocin and mastitis. Newbould (1970) hypothesized that during active suckling, mechanical and oxytocin stimulation could act together to dilate ducts and contract alveoli. Newbould (1970) cited Espe and Cannon (1942) who had earlier observed that at let down of milk there was an increased tonicity of smooth muscle in the wall of the teat and the teat cistern tended to balloon. Newbould (1970) suggested that the stimulus for these phenomenons also affects the smooth muscle around the proximal part of the teat duct near Furstenberg Rosette resulting in its dilation. Thus instead of keeping the duct closed, the smooth muscle around the duct under stimulation, open the proximal end, and allow direct access of microorganisms to the cistern. In theory at least, animals milked by exogenous administration of oxytocin are at a greater risk to develop mastitis than animals not receiving this milk let down hormone. This may explain an apparently higher prevalence of mastitis in buffaloes milked with oxytocin injection than those milked with other milk letdown stimuli. A strong association of suckling calves is supported by the observations of Socci and Redaelli (1973) who reported suckling calves as possible agency of microorganims transmission. Prabhakar *et al.* (1990) also isolated mastitis causing organisms from calf pharynx. It is a common practice in Punjab to use the buffalo calf for stimulating the udder. After the calf has suckled for a short while, it is dragged away (Egenolf, 1990).

In the present study a significant associations were observed between the number of animals milked by a milker and mastitis prevalence in buffaloes and cows. In the developing countries like Pakistan, dairy animals are predominantly hand milked. Milk is often used as a lubricant during milking and milker's hands are often heavily soiled with milk during this process. As stated above, mastitis in Pakistan and other developing countries is predominantly caused by contagious mastitis pathogens which are transmitted usually at the time of milking. Infectious agents of mastitis may be transmitted from infected to uninfected animals through milkers' hands (Philpot, 1975; Oliver, 1975) especially owning to the proclivity of using milk foam in the milking pail as a lubricant. Studies conducted at National Institute for Research in Dairying (UK) revealed that 50% milkers' hands were infected before milking compared to 100% during milking (Dodd *et al.*, 1966 cited by Philpot, 1975). Motie *et al.* (1985) reported that mastitis in hand-milked cows was nearly twice as frequent as in machine-milked ones (25.1 versus 14.6%). Keeping this in perspective, one can expect an increase in mastitis prevalence with the increase in the number of animals milked by a milker.

The present findings revealed a highly significant association between the general physical condition and mastitis prevalence in buffaloes and cows. Anonymous (1987) reported that increased milk production by an animal of good health might be one of the risk factors. It was suggested that high milk yield might predispose animals to udder infections. Rehman *et al.* (1997) also concluded that poor health management may be responsible for the higher prevalence of mastitis in small herds.

The present study concluded a significant association between number of mastitis quarters in buffaloes and cows. In the present study, 293 mastitic buffaloes and 86 mastitic cows were encountered. Single quarter involvement was noted in 159 (54.26%) buffaloes whereas 2, 3 and 4 quarters were found mastitic in 93 (31.74%), 31 (10.58%) and 10 (3.41%)

buffaloes respectively. Similarly 1, 2, 3 and 4 quarters were involved in 46 (53.48), 24 (27.9%), 12 (13.95%) and 4 (4.65%) mastitic cows. The quarter's prevalence rate in buffaloes and cows was 5.88 and 8.48% respectively. Wilson and Richards (1980) reported that as per the criteria of International Dairy Federation, 9.6% of quarters were infected in a national survey of mastitis conducted in UK. Based on 17 months national survey of clinical and sub clinical mastitis, Jamaican workers (Zingerser *et al.*, 1991) found that 56% of all quarters were positive in CMT, 0.8% showed clinical mastitis and 3.2% were blind. A research study conducted in 1994 by Saini *et al.* involving 123 crossbred cows and 241 buffaloes indicated the presence of sub clinical mastitis in 4.87 and 2.59% quarters and 17.33 and 9.57% animals of the respective dairy species. Single quarter involvement was seen in maximum number of animals and this is congruent with the findings of the present study.

Bilal and Muhammad (2004) recorded a higher incidence in hind quarters (73.3% and 63.1%) vs fore-quarters (26.6 and 36.8%) in buffaloes in peri urban and rural areas, respectively while Langoni and Domingues (1998) observed higher incidence in left hind (34.0%) followed by right front (28.0%), right hind (21.8%) and left front (15.3%). Thirunavukkarasu and Prabaharan (1997) documented a higher involvement of hind quarters (255, 58.76%) than fore quarters (179, 41.24%) further, among hind quarters, left hind quarters was found to be more susceptible to udder infection with 144(33.18%) out of 434 clinical quarters, followed by right hind quarters (25.58%). Right fore quarter involvement was found to be the least (19.35%) among the four quarters. It needs to be stressed that buffaloes also exhibited a very similar pattern. Out of 67 clinical quarters observed in buffaloes, left hind quarters were involved on 23(34.33%) occasions, right hind on 17(25.37%), left fore on 15(22.39%) and right fore on 12(17.91%) occasions. Rasool *et al.* (1985) reported higher incidence in hind quarters. Didonet *et al.* (1986) and Adkinson *et al.* (1993) also reported higher incidence in hind quarters than fore quarter. Egan and Meancy (1987) also reported the similar results.

The present findings concluded a significant association between education grading and mastitic prevalence in buffaloes and cow. In the present study, the prevalence of mastitis was higher in buffaloes (19.08%) and cows (24.88%) kept by the illiterate farmers than those kept by the educated farmers. Similar finding were reported by Venkatasubramanian *et* *al.* (1997). The higher prevalence of mastitis may be related to the poorer management of animals by the illiterate farmers as compare educated ones. A statistical analysis indicated a highly significant association in buffaloes and cows between teat injury and mastitis status. Oltenacu *et al.* (1990) reported that trampled teats, udder injuries were the most serious risk factors for clinical mastitis. In the present study, of the 259 buffaloes with a history of teat injuries, 46(17.76%) developed mastitis. Similarly, 27 of 76(35.5%) cows with a history of teat injuries were found mastitic. The epidemiological measures of association between teat injury and mastitis were computed in the study. The greater values of the relative risk (RR) indicated a stronger association between the factor and the mastitis. Computation of epidemiologic measures of association indicated that mastitis in buffaloes and cows with injured teat was1.09, 2.138 times more frequent than in the subjects of the corresponding species without teat injuries. Population relative risk (RR_{POP}) 1.035, 1.204 in buffaloes and cows for teat injury would indicate that the rate of mastitis in both the species would increase by1.035, 1.204 times as compared with animals without this factor. Attributable rate (AR) of mastitis attributed to teat injury was 0.038, 0.189 in buffaloes and cows. The higher values of attributable fraction in cows reflect a stronger effect of the factor as compared with buffaloes. The calculated population attributable rates (PAR) indicate the rate of mastitis in the milch animal population that is ascribable to the teat injury 0.4%, 2.9% in buffaloes and cows. Population attributable fraction (PAF) calculated for teat injury implied that 3.03%, 16.9% of all mastitis in buffaloes and cows population is attributable to teat injury. Similar epidemiologic measures of association between factors and incidence of mastitis were studied by Thirunavukkarasu *et al.* (1998). The factors e.g. milk yield, stage of lactation, udder abnormalities, teat abnormalities, season, stall hygiene, and milking hygiene in cows and buffaloes were found to be significantly associated with mastitis. The measures of association Relative risk (RR), Population relative risk (RR_{POP}), Attributable rate (AR), Attributable fraction (AF), Population attributable rate (PAR) and Population attributable fraction (PAF) were observed as 1.757, 1.602, 0.056, 0.43, 0.044 and 0.376 for exotic blood in cows. The epidemiologic values were as 00, 00, 0.133, 1.00, 0.130 and 1.00 for udder teat abnormalities in cows and measures were found as 1.407, 1.075, 0.257, 0.289, 0.047 and 0.070. Similar epidemiological measures reported in less hygienic stalls and in less hygienic milking of cows factors were as 1.297, 1.149, 0.035, 0.238, 0.017 and 0.130, and 1.287, 1.127, 0.033, 0.223, 0.015 and 0.115 respectively. Study indicate greater the departure of epidemiological measures stronger the association between the factors and mastitis status.

In the present study a non-significant association between un evenness of floor and mastitis were observed in buffaloes and cows. The data showed that chi-square value for mastitis was higher in buffaloes than in cows.

In the present findings a highly significant association was observed between the hard milking and mastitis status with the higher chi-square values in buffalo's vs cows. The limits of 95 % confidence interval RR were recorded as 1.519, 2.332 and 1.213, 2.585 in both the species. In hard milking association with mastitis attributable fraction were found higher in buffaloes than in cows. Similarly population relative risk (RR_{POP}), population attribute rate (PAR) and population attributable fraction (PAF) were found to be higher in cows as compared in buffaloes. Hard milking could be due to constriction of the teat sphincter as a result of any teat lesion/ injury or genetics of the animal. Thirunavukkarasu and Prabaharan (1998) documented a similar statement that greater the epidemiological measures computed stronger the association between the factor and the disease.

The present study concluded a significant association between folded thumb and mastitis status in buffaloes and cows. The data showed that the chi-square values 31.294, 5.621 and the relative risk for mastitis were 1.901, 1.636 and the limits of confidence interval at 95 % level were found as 1.544, 2.322 and 1.104, 2.421 in both the species. The values of relative risk greater than 1.00 showed an association between the factor and the mastitis. RR_{POP} in the factor was increased 1.180, 1.136 times greater as compared with or without factors. In the present study greater the effect of factors observed due to increase the AR values in cows and attributable fraction (AF) values in buffaloes and lesser the attributable fraction (AF) values in cows and higher the value in buffaloes showed a higher effect of the factor in buffaloes vs cows. Population attributable rate attributed to the folded thumb was 2.4 %, 3.7 % in both the species. PAR, PAF values were observed slightly higher in buffaloes as compared in cows. Ahmed (1980) reported that the use of folded thumb technique predisposed to higher incidence of mastitis in animals. More over the use of “folded thumb” the teat cistern is injured and has often been considered to be conducive to the development of adverse effects on teat canal.

The present study concluded a highly significant association between mastitis status and predisposing factors like udder oedema, teat oedema and blood in milk. The limits of 95 % confidence interval (RR) (1.357, 2.386),(1.248,2.736),(2.195,3.321),(1.866,3.818)and(1.383,2.401),(1.294,2.924) were observed in both the species respectively. The relative risks for mastitis to teat oedema were found to be higher in buffaloes as in cows. The RR values for buffaloes and cows were 1.800, vs 1.849, 2.701, vs 2.679 and 1.842, vs 1.943 respectively. Similarly, values of other epidemiological measures e.g. RR_{POP} (1.066, 1.156), (1.230, 1.307) and (1.085, 1.129), AR (0.108, 0.147), (0.200, 0.257) and (0.108, 0.167), AF (0.444, 0.459), (0.629, 0.626) and (0.457, 0.485), PAR (0.008, 0.026), (0.026, 0.046) and (0.010, 0.021) and PAF (0.061, 0.134), (0.186, 0.234) and (0.078, 0.116) were found in buffaloes and cows. The findings of the present study were also inline with those of Thirunvukkarasu and Prabaharan (1998s) reported that the measures of association for the factor implied that the rate of mastitis in cows having udder/ teat abnormalities would increase by 1.075 (RR_{POP}) times of the cow in question was having abnormal udder/ teat. The rate of disease in cows that might be attributed to udder/ teat abnormality was 25.70% (AR) and 28.9% (AF) of mastitis in cows with udder abnormalities could be attributed to such abnormalities. According to the (Grohn *et al.*, 1999, Slettbakk *et al.*, 1995) found observation the udder oedema to be associated with the increased risk of clinical mastitis in cows. More-over the risk of clinical mastitis caused by *Staph. aureus* was not associated with the udder oedema, however, teat oedema was a significant risk factor.

The association between increased risk of clinical mastitis in both teat oedema and udder oedema are biologically plausible. An oedematous, rigid teat is less likely to be reacting normally to machine; hand milking e-g. teats in which tissue elasticity is reduced may be more vulnerable to forces generated by changes in pressure during pulsation. Waage *et al.*, (2001) reported that the presence of blood in the milk, udder oedema and teat oedema at the time of parturition were highly significant risk factors for clinical mastitis in heifers during the first 2 weeks post-partum. Blood in milk and teat oedema were also associated with the increased risk of clinical mastitis caused by *Staphylococcus aureus*.

In the present findings a non-significant association in buffaloes and cows between wallowing and mastitis prevalence were observed with the chi-square values of 1.09, 0.089.

The present study revealed a highly significant association between teat stenosis and mastitis prevalence with a chi-square values of 68.453, 30.485 and at the 95 % confidence interval of RR 2.21, 3.35 and 1.96, 3.93 in buffaloes and cows. The higher values of RR, RR_{POP}, AR, AF, PAR and PAF were observed in cows as compared with buffaloes. The present findings are in agreement with that of Thirnvukkarasu and Prabaharan (1998). Pyorala *et al.* (1992) reported that the most of the mastitis problems were often preceded by teat stenosis.

The present study, indicated a significant ($p < 0.05$) association between milk leakage and mastitis status in buffaloes and cows. The chi-square values of 3.080, 0.055 were observed in both the species. The present findings were closely correlated with the findings of (Thirunvukkarasu *et al.*, 1998) Waage *et al.* (2001) More over the present findings were fully agreed with the study of Myllys and Rautala (1995), Waage *et al.* (1998) (Schukken *et al.*, 1990, Van de Geer *et al.*, 1988).

Although, mastitis is an inflammation of mammary glands regardless of the cause, different types of pathogens in particular bacteria are by far the most frequently associated etiologic agents of the disease. Mastitis is the outcome of various factors associated with the host, environment and the pathogens. In the present study, *Staphylococcus aureus* was the frequently encountered pathogen (accounting for 59.53% of 214 isolates from 200 mastitis quarters of dairy buffaloes and 44.76 % of 105 isolates recovered from 100 mastitic quarters of cows). In buffaloes as well as in cows, *Streptococcus agalactiae* was the second most frequently isolated mastitis pathogen accounting for 23.83 % of all isolates recovered from dairy buffaloes and 21.9 % of all isolates recovered from cow. Together, these two contagious mastitis pathogens accounted for 83.36 and 66.66 % of isolates recovered from buffalo and cow quarters, respectively The present finding in agreement with the findings of , USA worker (Allore, 1993) while reviewing the important studies conducted in countries (India, Pakistan, Indonesia, Sri Lanka and Egypt) endowed with both dairy buffalo and cow also concluded that clinical as well as sub clinical mastitis in dairy animals in these countries is predominantly contagious in nature. The present study are also in broad agreement with the findings of several previous workers (Zingerser *et al.* , 1991; Prabhakar *et al.,* 1995; Madsen *et al* , 1974; Mitra *et al.,* 1995; Esmat and Badr, 1996; Fazal-ur-Rehman, 1995, Oliver, 1975, Ghuman, 1967; Chander and Baxi, 1975; Hashmi *et al.,* 1980; Anwar and Chaudary, 1983; Lafi, *et al.,* 1994; Iqbal, 1992; Gonzalez *et al.,* 1980; Pyorata, and Syvajarvi, 1987; Al-Shawabkeh and Abdul Aziz, 1987; Hogan *et al.,* 1989; Ahmad *et al.,* 1991, Khan *et al.,* 2004, and Ali et al., 2008). The preponderance of contagious mastitis in buffaloes as well as in cows may be ascribed to the total lack of such contagious mastitis pathogens control practices as post-milking antiseptic teat dipping, dry period antibiotic therapy, culling of chronically infected animals in the herd as well to the rife proclivity of using milk foam in the milking pail to lubricate the teat during milking. Keeping in view the finding of the present study in perspective, it may be recommended that the mastitis control in the study area (Tehsil Samundri, District Faisalabad) should focus on control practices aimed at controlling contagious pathogens (e.g., post-milking antiseptic teat dipping, dry period antibiotic therapy, segregation of infected from non-infected animals, fly control etc.). In view of the preponderance of contagious pathogens as the etiologic agents of mastitis in dairy buffaloes and cows, workers at the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan (Shakoor, 2005; Athar, 2006 and Butt, 2005) evaluated mono (*S. aureus* only) and polyvalent (containing *S. aureus*, *Str. agalactiae* and *E. coli*) mastitis vaccines for the control of mastitis in buffaloes and cows. These vaccines were found to be effective as prophylactive as well as curative against these pathogens.

Staphylococcal species other than *S. aureus* (*S. hyicus hyicus*, *S. epidermidis*, *S. hominis*, *S. xylosus* 1, and nontypable coagulase negative staphylococcus species) accounted for 18.68 and 18.10 % of isolates recovered from mastitis quarters of buffaloes and cows, respectively. Coagulase negative staphylococci are the most frequently isolated pathogens in dairy herds practicing the mastitis control recommended by National Mastitis council, Inc (USA) and other mastitis monitoring bodies e.g., Milk Marketing Board, UK (Bartlett *et al*, 1992 and Timms and Schultz, 1987).

Jamaican workers (Zingerser *et al.*, 1991) reported the results of a 17-month national survey of clinical and sub clinical mastitis between April 1985 and August 1986.

Bansal *et al.* (1995) investigated milk samples obtained from 154 cows in different herds, and 117 buffaloes in 5 herds in the Indian Punjab province. Sub clinical mastitis was found in 48% of cows and 27.05% cow udder quarters, and 23.93% of buffaloes and 11.32% of buffalo's udder quarters.

Prabhakar *et al.* (1995) studied the incidence of clinical mastitis and its etiologic agents at five farms. The overall monthly incidence was found to be 4.06 percent. Of the total of 421 buffaloes, 40 were affected with clinical mastitis in 76 quarters. Staphylococci were the major causative organisms (34.21% *S. aureus* and 13.16% coagulase-negative Staphylococci), followed by *Str. agalactiae* (14.74%), *E. coli* (10.53%), *Pseudomonas* spp (7.89%), *Str. pyogenes* (3.95%), *Klebsiella* spp. (3.95%), *Str. dysgalactiae* (2.63%), *Proteus* spp. (2.63%), *Str. uberis*, Diptheroids and mixed infections (1.31% each). No organism could be isolated from 2.63% quarters.

To investigate the prevalence of clinical and sub clinical mastitis in buffalo in Hyderabad (Pakistan), Soomro *et al.* (1997) conducted a study on 785 milk samples collected from 200 buffaloes during 1994-95. The physical examination of the udder of these animals revealed 6% clinical (chronic) and 1.0% congenital abnormalities of udder. Three chemical tests viz., Bromothymol blue test, chloride test and Whiteside test were applied on milk, which respectively revealed 19.7%, 15.9% and 13.85% quarters affected with sub clinical mastitis. In general, 33.3 percent animals had sub clinical mastitis. More recently, Khan *et al.* (2004) using Surf field mastitis test and bacteriological examination of quarter milk samples of 50 buffaloes documented 27, 4, 10 per cent prevalence for sub clinical mastitis, clinical mastitis and blind quarters, respectively.

Qazi *et al.* (1999) surveyed 45 different small livestock units/herds in Lahore (Pakistan) for epidemiologic data on mastitis. Analysis of data showed a prevalence of 8.8% in herds. The prevalence in lactating animals was 8.3%. Of 1000 quarters milk samples, 14.3% were positive for sub clinical mastitis. Highest prevalence of mastitis was recorded in 6-8 year old cows and buffaloes. Out of positive cases the prevalence of cases was highest (53.63%) during early lactation followed by middle (21.97%) and late lactation (24.4%). Prevalence was higher in high yielding animals. Surgically manipulated animals were more prone to disease (4%).

CHAPTER 6

SUMMARY

Mastitis is considered to be the most costly disease of dairy animals worldwide. This disease complex is the outcome of interaction of various factors associated with the host, pathogens and the environment. Infectious agents, in particular various species of bacteria are the most important etiologic agents of mastitis. Local information on the epidemiology of mastitis in Pakistan is extremely scanty. This information is imperative for devising an appropriate intervention on the control of this economically significant disease. The present study was, therefore designed to determine the frequency distribution of mastitis in dairy buffaloes and cows and to determine the association of some host, environment and pathogen(s) related determinants with the disease.

A cross-sectional study was undertaken in 28 randomly selected villages (one from each Union Council) of Tehsil Samundri, District Faisalabad over a 3 month-period (September - November, 2004). Each selected village was considered a cluster and all dairy farmers managing cow/buffaloes in the selected village were included in the survey. A total of 2029 hand milked buffaloes and 430 cows were investigated. No mastitis control measures (e.g., teat dipping, dry period antibiotic therapy, etc) were in place in the study area. Two types of determinants viz. host-associated and management associated were studied. Host-associated determinants included: dairy species (cow/buffalo), breed, age, general physical condition, lactation number, stage of lactation, reproductive disorders, distance between teat tip and ground, teat stenos is, quarters affected, ease of milking, teal injuries, milk leakage, blood in milk, udder oedema, teat oedema, teat shape, teat size, udder shape, etc. Similarly, managerial determinants included: condition of the floor, type and amount of bedding, frequency of dung removal, quality of drainage, stimulus for milk letdown, udder washing, and number of animals milked by the same milker, milking technique, and wallowing practices, etc. All information was collected on pre-designed pro forma by structured questions and physical examination of udder. Diagnosis of mastitis was based on overt manifestations of the disease (clinical mastitis) and results of the Surf Field Mastitis Test for sub clinical mastitis.

The epidemiologic measures computed to determine the association between mastitis status and potential determinants included relative risk (RR), population relative risk (RR_{pop}), attributable rate (AR), attributable fraction (AF), population attributable rate (PAR), and population attributable fraction (PAF). To determine the nature of pathogens associated with mastitis in dairy buffaloes and cows, 300 quarter foremilk samples collected from 95 randomly selected mastitic buffaloes (clinically mastitic quarters n = 17; sub clinically mastitic quarters n = 183) and 53 mastitis affected cows (clinical n = 11; subclinical n = 89 quarters) were subjected to microbiological examination. The diagnosis of subclinical mastitis was based on the results of Surf Field Mastitis Test (a California Mastitis Test like animal-side test).

The overall prevalence of mastitis (clinical and subclinical) was 14.14% in buffaloes and 20% in cows. The composite (cow and buffalo) prevalence was 15.4%. The prevalence was the lowest (3.46-4.25%) in buffaloes of 4-6 years of age and the highest in animals aged 11 years and more (72.72%). Similarly, the prevalence registered an increment with increasing the age in cows. The prevalence of mastitis was increased with the increase in lactation number both in buffaloes and cows respectively;

being maximum in animals of lactation number 6 and or above. When the occurrence of mastitis was stratified according to the stage of lactation, it was found that the prevalence was the highest during the first month of lactation both in buffaloes (27.0%) and cows (72.05%). In cattle, highest the prevalence (56.0%) was recorded in crossbred/exotic cows following by Sahiwal (13.54%) and desi (non-descript) cows (5.14%).

Of a total of 8116 quarters of 2029 lactating buffaloes, 478 (5.8%) quarters were either clinically mastitic or reacted positive in Surf Field Mastitis Test. Similarly, of the 1720 quarters of 430 cows, 146 quarters (8.48%) were found mastitic (clinical and Surf Field Mastitis Test positive). Of the 293 mastitic buffaloes, 54.27%, 31.74%, 10.58%, and 3.41%, respectively had 1, 2, 3, and 4 quarters affected with mastitis. Eighty six of 430 cows (20%) examined had mastitis either in one (53.4% of affected cows), two (27.90% of affected cows), three (13.95% of affected cows), and all four (4.65% of affected cows) quarters. As the distance between teat tip and ground increased, the prevalence of mastitis was decreased. Dairy buffaloes as well as cows with double leveled udder had the highest prevalence of mastitis (96.82% in buffaloes, 95.65% in cows) whereas the lowest prevalence rate (6.97% in buffaloes, 15.72% in cows) was recorded in animals with spherical shaped udder. Of the 4 categories of teat shape (funnel, round, flat, and plate), round shaped teats had the highest prevalence both in buffaloes (61.80%) and cows (56.3%). Frequency of dung removal and quality of floor drainage also seem to affect the prevalence of mastitis appreciably. In buffaloes, the highest mastitis prevalence (38.7%) was recorded in animals milked with exogenous parenteral administration of oxytocin, followed by those in which letdown of milk was induced by suckling calves (25.87%) and offering concentrate at the time of milking (2.73%). In cows, the highest prevalence (31.71%) of mastitis was noted in subjects to which calf suckling was used as a stimulus for letdown of milk followed by those which were enticed to have a letdown of milk by offering concentrate (6.96%). Only two of 430 cows were milked with exogenous parenteral administration of oxytocin and both were bereft of mastitis. In general as the number of animals hand milked by the same milker increased, so did the prevalence of mastitis.

Two hundred and sixteen (82.44%) of 262 buffaloes with a history of 4 common reproductive disorders (metritis, retained placenta, dystokia, and prolapse of uterus) were found to be affected with mastitis. Compared to an overall prevalence of 14.44% in 2029 buffaloes sampled in the study area, buffaloes (n = 262) with a history of reproductive disorders had 18.95 times higher prevalence of clinical plus subclinical mastitis. In cows, 59 of 69 (85.5%) animals with a history of reproductive disorders were found mastitic. Thus the prevalence of mastitis in cows with reproductive disorders was 11.43 times greater than the overall prevalence of 20% in cows (n = 430) included in the study.

The values of the relative risk (RR), population relative risk (RR_{pop}), attributable rate (AR), population attributable rate (PAR), attributable fraction (AF), and population attributable fraction (PAF) were calculated for buffaloes and cows respectively for the teat injuries (1.091 and 2.138;1.035 and 1.204; 0.038 and 0.189; 0.082 and 0.532; 0.004 and 0.029; 0.033 and 0.169), Hard milking (1.886 and 1.772; 1.170 and 1.197; 0.109 and 0.129; 0.469 and 0.435; 0.020 and 0.032; 0.145 and 0.164), Folded thumb (1.901 and 1.636; 1.180 and 1.136; 0.110 and 0.112; 0.479 and 0.388; 0.024 and 0.037; 0.152 and 0.119), Udder oedema (1.800 and 1.849; 1.066 and 1.156; 0.108 and 0.147; 0.444 and 0.459; 0.008 and 0.026; 0.061 and 0.134), Teat oedema (2.701 and 2.679; 1.230 and 1.307; 0.200 and 0.257; 0.629 and 0.626; 0.026 and 0.046; 0.186 and 0.234), Blood in milk (1.842 and 1.943; 1.085 and 1.129; 0.108 and 0.167 ; 0.457 and 0.485; 0.010 and 0.021; 0.078

and 0.116), Teat steno sis (2.737 and 2.797; 1.188 and 1.398; 0.212 and 0.257; 0.634 and 0.642; 0.021 and 0.056; 0.152 and 0.284), Milk leakage (1.539 and 1.484; 1.024 and 1.041; 0.076 and 0.093; 0.350 and 0.326; 0.002 and 0.007; 0.023 and 0.039).

The Present findings concluded a highly significant association ($P<0.01$) in age, lactation number, general physical condition, reproductive disorders, hard milking, udder oedema, teat oedema, blood in milk, teat tip distance from ground, udder shape, breed based, teat injury, folded thumb, and teat steno sis between mastitis, prevalence in buffaloes and cows. While a regression and chi-square analysis depicts a significant ($P< 0.05$) association between mastitis status and factors e.g., stage of lactation, dung removal time, animal milked by a milker, education of the farmers, mastitics quarters with mastitics prevalence and floor drainage in both the species.

Moreover a statistical analysis indicated a non significant ($p > 0.05$) association between conditions of floor and wallowing practices factors and mastitis prevalence in both the species.

In cows, it has been suggested that teat traits with a high hereditary status should be taken into account in selection studies, in order to reduce mastitis prevalence (Hamann and Burvenich, 1994). Moreover, there is strong correlation between teat status and udder health (Loppnow, H, 1959; Stocker *et al.,* 1989).

It was suggested that understanding the association between TC length and udder health in dairy cows may provide valuable advantages in terms of udder health (Stocker *et al.,* 1989).

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Appendix II

Epidemiology of mastitis in buffaloes and cows with in Tehsil Summandri of District Faisalabad

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Part A. A field survey of risk factors of mastitis in buffaloes and cows in Tehsil Summandri, District Faisalabad

(I) Serial No. Village [ ] Farmer [ ] Animal [ ] **(II) Date of data collection** [ ] [ ] [ ]

(III) Information regarding owner/farm

(1).Name --------------------------------- (2) Size of agriculture land owned--------------

(3) Education---------------------- (4) Experience of farming (Years) ----------------------

(IV)Host-Associated determinants

1-Since when the particular animal is being kept Y [ ] M [ ] period. 2- Species [ ]

3- Breed [ ] 4- Age (years) [ ]

5-General physical conditions

Very poor	Poor	Good	Very good

6- Body condition score

1	2	3	4	5

7- Lactation Number [ ] 8- Stage of lactation [ ] 9- Stage of pregnancy [ ]

10-Dry period length during previous gestation (months) [ ]

11-Reproductive disorders

None	Metritis	Retained placenta	Dystokia	Vaginal prolapse	

12- Any other disease problem present concomitantly [ ]

13- Mastitis occurred in the wake of [ ]
(Only one week period is relevant)

14- Distance between teat tip and ground (inches) [ ]

15- Height of the animal

ft	in

from the point of withers.

16-Teat stenosis

Present	Absent

17- Milk letdown time (minutes) [ ]

18- History of mastitis in the genetic lineage

Yes	No

19-Quarter(s) reported by the farmer to be affected

LF	LR	RF	RR	No

20- Ease of milking

Easy	Hard

21- Teat injuries

Present	Absent

22-Milk leakage

Present	Absent

23-Blood in Milk

Present	Absent

24- Udder oedema

Absent	Present Prepartum --------	Present Postpartum --------

25-Teat oedema

Present	Absent

26-Supernumerary teat

Present	Absent

27-Skin lesions between udder and thigh region

Present	Absent

28-Teat shape (As per Shukla *et al*, 1997)

LF	LR	RF	RR

(Fu→ Funnel, R→ Round, Fl→ Flat, P→ Plate)

29-Teat size (As per Shukla *et al*, 1997) (inches)

LF	LR	RF	RR

30-Udder shape (As per Saleh and Khamis,1969)

Spherical	Double-leveled	Hanging

(V) Management and Housing-Associated Determinants

1-Type of housing with hours of stay on each type

Backyard housing	Street	Open area

2-Type of floor with hours of stay on each type

Katcha	Brick	Cemented

3-Condition of floor

Even	Uneven

4- Type and amount of bedding

	Cut straw	Sand	Arori	

5-Frequency of dung removal per day ☐ 6-Drainage

Poor	Acceptable	Proper

7-Source of drinking water

Pond	Under ground	Canal	River	

8-Salt used (Amount) ☐ Frequency of use ☐ Salt lump ☐

9-Concentrate used (Amount per day) ☐ Ingredients

Cotton seed cake	Wheat bran	Bread crums

10-Stimulus for milk letdown

Calf suckling	Concentrate	Oxytocin

If calf suckling, is it at the beginnin ☐ or at the end ☐ or both ☐

11-Udder washing

Practiced	Not practiced

12-Gender of milker

Male	Female

13-Number of animals milked by the same milker ☐

14-Wet hand milking | Practiced | Not practiced |

15-Type of 'lubricant' used to soften the teats at the time of milking | Saliva | Milk 'surf' | Milk | No |

16-Milking technique | Whole hand | Folded thumb |

17-Milking | Complete | Incomplete |

18-Milking order | Mastitic animal first | Nonmastitic animal first | No consideration |

19- Post milking teat dipping | Yes | No |

20-Wallowing practiced | Yes | No |

21-Grazing | Yes | No |

(VI) Disease Status

Result of Surf field Mastitis test | Positive | Negative |

Mastitis animals' disease data

1- Mastitis episode No. during the current lactation [ ]

2-No. of episodes of Mastitis during the previous lactation [ ]

3-Systemic reaction, Temp [ ] Pulse [ ] Respiration [ ]

4-Reduction in feed intake | No | 25% | 50% | 75% |

5-Duration of disease

LF			RF
LR			RR

6-Position of affected quarters and disease severity based on Surf field mastitis test score

LF			RF
LR			RR

7- Mastitis status at drying off

LF			RF
LR			RR

8-Atrophy of quarter(s)

LF	LR	RF	RR	No

9-Atrophy of teat(s)

LF	LR	RF	RR	No

10-No. and Position of blind quarter(s)

LF	LR	RF	RR	No

11-Quarter(s) voiding Pus

LF	LR	RF	RR	No

12-Thelitis

Present	Absent

If Present

LF	LR	RF	RR

13-Effect of Mastitis on milk production

Milk yield/day/animal (L) Before Mastitis [ ] After Mastitis [ ] After 'recovery' [ ]

14-Effect of Mastitis on milk characteristics.

(a)Taste

Normal	Salty

(b) Colour

Normal	

(c) Odour

Normal	

(d) Appearance of milk

Normal	

15- Microbiological examination of milk:

LF--

LR---

RF---

RR---

16-Indigenous medication practiced regularly/before seeking professional advice

Yes	No

If yes

(a)Type of indigenous medicine used ---

(b) Response to indigenous medicine ---

(c)In the opinion of the owner, what factor(s) predispose to mastitis ---------------------

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(d) In the opinion of the owner where does mastitis rank Visa –a– viz other important health problems? ---

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(VII) Allopathic Treatment

Any allopathic treatment undertaken

Yes	No

(a)If yes nature of the allopathic R_x ---

(b) Duration of allopathic R_x --

(c) What was the basis of selection of the drugs? ---

(d) Who advised the R_x?--

(e)Who administered the therapy?--

(VIII) Any other remarks ---

Appendix III

Part B. ***Clinico-Microbiological examination of bubaline and bovine clinical mastitis in Tehsil Summandri of District Faisalabad.***

S. No. --------------   Date ----------------

Name of the owner and address ---

No of lactating cows --------------, buffaloes ------------ ID of the Animal(if any) ---------

Age of the Animal --------------- Lactation No ---------------- Stage of lactation ------------

Stage of pregnancy -------------- Dry period length during previous gestation(months) ----

Reproductive disorders ------------------------------------ Any other disease problem present

concomitantly ------------------ Mastitis occurred in the wake of ---------------------------

Milk yield before mastitis ___L/day. Milk yield after development of mastitis ____ L /day
Antibiotic therapy given / not given so far. Temperature ______F^0
Feed in take ________
Inspection (Rosenberger, 1979)

Udder shape

All 4 quarters of the same size Yes/No

All 4 quarters not the same size "(Kanna Havana)" Yes/No

Pendulous ("Dhalka Huwah"or "Dheelah") Non pendulous

Teat shape (Shukla *et al*, 1997)
Funnel Round Flat Plate

Teat & Udder Palpation Findings (Rosenberger, 1979)
(after milking)
Teats

Streak Canal	LF	LR	RF	RR
Warm				
Normal				
Bead				

Teat Cistern

	LF	LR	RF	RR
(i) Cord (present or Absent)				
(ii) Milk stone, blood clots, clumps of fibrin or pus, fibropapilloma, polyps (present or absent)				

Teat Fistula (present or absent)

LF	LR	RF	RR

Udder
(Milked-out udder)

	LF	LR	RF	RR
Warm				
Cold				
Tender (Painful)				
Oedema				

Key to Udder Glandular Tissue Consistency

n = normal fine-grained and soft feel (when milked out)
I = udder tissue contains coarse-grained parts and is firm
II = udder tissue is coarse-grained and firm all over, with occasional lumps
III = udder tissue is lumpy all over.
IV = udder tissue is lumpy with patches of diffuse hardening
V = udder tissue diffusely hardened throughout
VI = udder tissue acutely swollen (unusually warm and tender)
VII = udder tissue cannot be felt because of oedema of the udder skin

Key to the coding of examination of mammary gland secretion
(Rosenberger, 1979)

n = normal milk. Colostrums is normally yellow and viscous. (The secretion from pregnant heifers and dry cows is normally serous or honey-like.)

A = bluish discoloration and watery consistency (temporary change seen during the feeding of a ration containing a lot of water, and during digestive disorders and chronic mastitis)
B = looks like milk, but is bluish and watery, with fine flakes present
C = looks like milk, but has a few large flakes
D = looks like milk, but contain many large flakes
E = no longer resembles milk; consists mainly of flakes or floccules
F = no resemblance to milk; consists of pus (Corynebacterium pyogenes mastitis or mixed infections), blood (may be regarded as physiological if it occurs during the first ten days of lactation; this form passes off spontaneously; other forms are pathological), flakes of serum or fibrin (E. coli mastitis). During severe febrile diseases), the udder secretion may become viscous and slimy resembling colostrums or dry cow secretion.

Key to Clinical severity Grading
(Fall and Hughes 1985)
Grade 1 = a quarter with visible charges in the milk (usually a few clots in the fore-milk) plus many neutrophils and usually pathogens. The quarter, however, feels normally and the cow is not ill.
Grade 2 A = a quarter with visible and palpable charges but the cow is not ill. If the quarter is swollen, hot, painful and sometimes discolored, the mastitis is acute.
Grade 2 B = If the quarter is hard and lumpy and not painful (it may be charged or contracted = ‘high’), the mastitis is chronic.
Grade 3 = a quarter that is 2A and the cow is ill.

Microbiological examination of milk (NMC, Inc. 1990)