Prevalence of Mastitis in cows and buffaloes from dairy farms in and around Visakhapatnam, Andhra Pradesh, India.

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Abstract
Milk samples from cows and buffaloes were collected from semi urban and urban regions in and around Visakhapatnam and screened for infection with mastitis. Milk samples were tested by employing, California Mastitis Test (CMT), White Side field test (WSFT) and Surf Field Mastitis Test (SFMT). Out of 1050 milk samples collected from cows and buffaloes, 610 samples were found to be mastitis positive. Samples which are detected positive for mastitis with high degree of gel formation were subjected to pathogen isolation on different culture medias such as blood agar, MacConkey agar, Mannitol salt agar, EMB agar, Nutrient Agar and Potato dextrose agar (PDA). The isolated bacteria were identified by performing a panel of preliminary and determinative biochemical tests. The most dominant bacteria isolated and identified from pure cultures were *Staphylococcus spp, E. coli* and *Streptococcus spp* in cows with a prevalence of 30.98%, 24.70% and 2.74% respectively. Where as in buffaloes *E. coli* and *Pseudomonas spp* were dominant with a prevalence of 32.25% and 24.73%, followed by *Staphylococcus spp*. Studies were also conducted to record monthly and seasonal prevalence of mastitis in cows and buffaloes. These studies indicated highest prevalence 74.57% in the month of September in the case of cows, and in buffaloes 76% infection was noted in the month of October 2019. Studies on seasonal prevalence indicated high values in rainy season with a prevalence of 64.65% and 61.25% in cows and buffaloes respectively. The present study is first of its kind from the state of Andhra Pradesh and throws light on the prevalence of mastitis disease in cows and buffaloes from dairy farms situated in and around Visakhapatnam.

Keywords: CMT test, Mastitis, Prevalence, *Staphylococcus spp.*,
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Introduction:

Mastitis is one of the expensive veterinary diseases currently affecting dairy industry next to Foot and Mouth Disease (FMD). It is considered to be a serious problem as it affects the health of cows and buffaloes and quality of milk resulting in huge financial losses (Sharma & Sindhu, 2007). Mastitis is a poly-microbial disease and is mostly caused by bacteria and to some extent fungi. These microorganisms produce toxins which directly damage milk-producing tissue of the mammary glands. Microbes gain entry into the udder from the teats through teat canal and the udder provides favourable conditions for microbial growth, resulting in blockage of teat canal. The presence of bacteria initiates inflammation within the mammary tissue in order to protect the host and eliminate the invading microorganisms. Udder inflammation is found to be common in dairy herds and causes 70% production loss in the farm (Sumathi et al, 2008; Singh et al., 2016).

In India, the economic losses incurred due to mastitis have increased substantially during the last five decades and lately the annual losses due to mastitis have been estimated to the tune of Rs. 7165.51 crore (Bansal et al, 2014). Over last decade ending 2016-17, milk production in Andhra Pradesh has increased to 121.75 lakh tonnes with an annual growth rate of above 7 per cent (NDDB – Andhra Pradesh) (NDDB, 2018). Andhra Pradesh stands 2nd in the milk production according to 2018-2019 report of NDDB (National Dairy Development Board, India) (NDDB,2018-19),however, Visakhapatnam district occupies 9th position in the state.

Mastitis disease brings certain abnormal changes in milk, such as increased somatic cell count (SCC), bloody or flaky milk and is also responsible for certain pathological changes in mammary glands like redness, swelling, hardness and pain (Singh et al., 2016; Rajan et al., 2011). Mastitis is seen as clinical mastitis and sub clinical mastitis in terms of severity. Clinical mastitis shows visible symptoms like swollen udder, inflammation etc. whereas subclinical form does not show any visible symptoms. The cause of mastitis was found to be multifactorial involving several factors like pathological, genetical, physiological or environmental factors. It can spread from animal to animal either by milker's hands or milking machines, besides bedding of animals housed in the farm, milking vessels, water used for cleaning udder, contaminated teat dips, stagnant water, flies, milker's hands which can also be considered as a source for pathogen to gain entry into the host (Constable et al., 2016; Sentitula et al., 2012; Sumathi et al., 2008).

A broad range of microbial fauna such as *Staphylococcus spp, Streptococcus spp, Proteus spp, E. coli, Klebsiella spp, Enterobacter spp, Corynebacterium spp, Leptospira borgpetersenii, Micrococcus spp, Candida spp, Aspergillus spp, Penicillium spp, Geotrichum spp, Mycoplasma spp,* Yeast, Viruses and Algae have been reported to be the causative agents of mastitis disease in cattle (Sentitula et al., 2012; Sumathi et al., 2008; Ranjan et al., 2011; de Casia & Marin, 2005; Pachauri et al., 2013; Das & Joseph, 2005; Gangwal et al., 2017, Waseem et al., 2020).

Bacterial culture is the gold standard method for identification of mastitis. However, there are some reliable cow side tests used for screening clinical and sub clinical mastitis. CMT (California Mastitis Test), WSFT (White Side Field Test), SFMT (Surf Field Mastitis Test) and SCC (Somatic Cell Count) are the simple, rapid and inexpensive tests for detecting mastitis. However, the financial and logistic considerations involved working with all fresh milk samples adopt these methods for screening of intra-mammary infection (IMI) in cattle (Badiuzzaman et al., 2016; Sargeant et al., 2001; Bhaskara et al., 2018).

The present study has been undertaken to investigate the prevalence of mastitis in lactating cows and buffaloes from dairy forms situated in and around Visakhapatnam and also to isolate and identify the various causative agents involved, especially microbial pathogens.

Materials and Methods:

Study Area:

Visakhapatnam of Coastal Andhra Pradesh, India was selected for the present study. A total of 1050 milk samples were collected from cows and buffaloes from different areas of urban and semiurban farms situated in and around Visakhapatnam. The sampling areas were selected based on the availability of milk samples from dairy farms with lactating cows and buffaloes. The major sampling areas selected for the present study are dairy farms from Vepagunta, Hanumanthawaka, Gajuwaka, Anandapuram and Chinawaltair.

Experimental design:

Sampling was carried out throughout the year from March 2019 to February 2020, collecting on an average 70 – 80 milk samples per month. During sampling, details such as age, parity, stage of lactation and incidence of mastitis in cows and buffaloes and hygienic practices followed by the farmer was recorded and the data sheet is provided in the form of a table (Table 2). Approximately 10-15ml of milk sample from each animal was collected in a sterile screw capped container and placed in an ice box and transported to the laboratory for further analysis.

Table 2: Sample data sheet used for collection of details on host and dairy farm at the time of sample collection

Data Sheet						
S. No	Data collection parameters		Sample 1	Sample 2	Sample 3	Sample 4
1	Type	Cow	√		√	
		Buffalo		√		√
2	Breed		Jersey	Murrah	Shaiwal	Murrah
3	Region	VSP	Vepagunta		Gajuwaka	Hanuamanthawaka
4	Age		3yrs	4yrs	2yrs	4yrs
5	Stage of Lactation		Early	Middle	Late	Early
6	Parity		1st calving	3rd calving	1st calving	2nd calving
7	Feeding type	Dry	√	√		
		Wet	√	√	√	√
8	Frequency of milking per day		Twice	Twice	Twice	Twice
9	Milk yielding per day		6-8 litres	8-12 litres	8 liters	9-12 litres
10	Relation between yield of milk and age		Age ↑ Milk production ↓		Age ↑ Milk production ↓	Age ↑ Milk production ↓
11	Farming type	Organised	√	√		
		Unorganised			√	√
		Dairy Farm				
12	Total no. of animals in the farm		10	10	9	7
13	Hygiene practices	Animals	Washed at the time of milking		Washed at the time of milking	Washed at the time of milking
		Farm	Floor and walls washed regularly		-	-
14	Duration of animal housed in the farm		2yrs	2yrs	1yr	2yrs

All milk samples collected were tested by employing standard tests such as California Mastitis Test (CMT), White Side Field Test (WSFT) and Surf field Mastitis Test (SFMT) to check the occurrence of mastitis and the state of severity of the infection. The following are the details of tests performed.

California Mastitis Test (CMT) – This test was performed by taking equal quantity of milk sample and the CMT reagent in a CMT paddle, mixed thoroughly and the result is noted. Based on the percentage of gel formation, samples were marked as negative and as trace 1, 2 & 3 (Positive samples). The CMT score is proportional to the severity of the infection, and a score of 3, indicates higher infection rate. (Jasper & Kroger 1967, Iqbal et al., 2006; Rakesh et al., 2018)

Wite Side Field Test (WSFT) – In this test, 4% NaOH solution was prepared and equal quantity of milk and NaOH solution is mixed and stirred rapidly with a toothpick for about 20 – 25 secs. Appearance of milk into flakes, shreds and viscid mass indicates a positive reaction. The results were noted as negative, 1+ (weak positive), 2+ (Distinct positive), 3+ (Strong Positive) (Islam et al., 2010; Muhammad et al, 2009)

Surf Field Mastitis Test (SFMT) – This is a very simple and economically viable test. in which 3% of test solution was prepared by using household detergent with clean tap water. Then equal quantity of test solution and milk was taken in a cup and swirled up to one minute. Appearance of clumps or gel formation was interpreted as SFMT positive (Islam et al., 2010; Muhammad et al., 1995; Iqbal et al., 2006) and no other gradation was given for positive samples. Details of grading of milk samples for preliminary tests, mentioned in the table 1

Table 1: Testing and Grading of milk samples employing three standard tests

Test	Grading				
	-ve Negative	+ve Positive			
		Trace	1	2	3
CMT	No formation of gel	Slight gel formation and disappears quickly	Distinct gel formation and disappears with paddle movement	Distinct gel formation	Strong gel formation
WSFT	No Visible changes	Milky appearance, fine particles, gel formation	Coagulated particles, with gel formation	Watery, Large coagulated particles, with gel formation	Formation of thick coagulum
SFMT	No formation of gel	Formation of gel	Formation of gel	Formation of gel	Formation of gel

Culture Studies: Milk samples identified as positive and showing high infection scores for mastitis were used for culture studies. For initial isolation positive milk samples were inoculated into nutrient broth and incubated for about 18hrs – 24hrs for the growth of pathogens. A loopful of sample was streaked simultaneously onto different media plates viz, nutrient agar, blood agar plate (containing 5% sheep blood) and MacConkey agar plate, Mannitol salt agar plate, EMB agar and PDA agar and incubated at 37 °C for 24 to 48 hrs. After the stipulated time period all plates were examined for the growth of bacterial colonies and any other pathogens like fungi. Though fungal growth was noticed in some culture plates along with bacterial colonies, they were not cultured further. Isolation of the bacterial colonies in fresh culture was carried out until pure cultures with single colonies were obtained. The morphological characteristics of bacterial colonies were recorded and separate cultures were maintained for each variety of colony. Preliminary tests such as gram staining, motility test, oxidase, catalase and oxidative-fermentation test were performed to know the bacterial characteristics Later these tests were subjected to determinative biochemical tests with 24 hrs pure cultures by following the standard procedures given in (Cowan, 2004, Sneath et al., 1986).

Results& Discussion:

Studies on Overall Prevalence of Mastitis in Cows and Buffaloes:

A total of 1050 milk samples (Cows – 710 and Buffaloes – 340) collected from dairy farms situated in and around Visakhapatnam were tested for mastitis employing standard tests like CMT, WSFT, and SFMT. Out of 710 cow milk samples, 426 tested positive and out of 340 buffaloes milk samples 184 samples tested positive. The prevalence of mastitis in cows was found to be high showing 60.00% whereas in buffaloes 54.11% prevalence was recorded. Over all prevalence of mastitis in both cows and buffaloes was found to be 58.09% (Table 3).

Table 3: Overall prevalence of infection in cow and buffalo from Visakhapatnam district

Year	Host	Total no. of milk samples	No. of positive samples	Prevalence of infection
March 2019 to Feb-2020	Cow	710	426	60.00%
	Buffalo	340	184	54.11%
	Total	1050	610	58.09%

Studies on Monthly Prevalence of Mastitis in Cows and Buffaloes:

Month wise prevalence of mastitis in cow and buffalo milk samples was calculated individually and also collectively and is presented in tables 4, 5 & 6 and figure 1 & 2. In the case of cows' highest prevalence of 74.57% was recorded in the month of September 2019 and a lowest prevalence of 50.70% was seen in the month of May 2019. Whereas in buffaloes, 76 % prevalence was recorded in the month of October 2019, and lowest is seen in the month of May at 25. 92 %. Overall prevalence of the data also indicated highest prevalence in the month of September recording 68. 60 % and lowest in the month of May with 43.87%. Statistical analysis was carried out by employing Two-way ANOVA with level of significance p = 0.05 between months and hosts to see the significance of differences in prevalence between cows and buffaloes. (Table 7). Prevalence between cows and buffaloes and also between different months was found to be significantly different at p > 0.05.

Table 4: Month wise prevalence of mastitis in Cow milk samples collected from dairy farms situated in and around Visakhapatnam.

Month & Year	Total no. of milk samples	No. of positive samples	No. of negative samples	Prevalence of infection
Mar-19	80	41	39	51.25%
Apr-19	50	33	17	66.00%
May-19	71	36	35	50.70%
Jun-19	54	30	24	55.55%
Jul-19	53	33	20	62.26%
Aug-19	53	33	20	62.85%
Sep-19	59	44	15	74.57%
Oct-19	50	29	21	58.00%
Nov-19	62	33	29	53.22%
Dec-19	57	35	23	60.52%
Jan-20	62	40	17	64.51%
Feb-20	59	39	20	66.10%

Table 5: Month wise prevalence of mastitis in Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam.

Month & Year	Total no. of milk samples	No. of positive samples	No. of negative samples	Prevalence of infection
Mar-19	52	18	34	34.61%
Apr-19	23	10	13	43.47%
May-19	27	7	20	25.92%
Jun-19	23	14	9	60.86%
Jul-19	28	21	7	75.00%
Aug-19	27	15	12	55.55%
Sep-19	27	15	12	55.55%
Oct-19	25	19	6	76.00%
Nov-19	42	21	21	50.00%
Dec-19	30	12	18	40.00%
Jan-20	24	16	8	66.66%
Feb-20	33	16	17	48.48%

Table 6: Overall monthly prevalence of mastitis in Cow and Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam

Month & Year	Total no. of milk samples	No. of positive samples	No. of negative samples	Prevalence of infection
Mar-19	132	59	80	44.69%
Apr-19	73	43	30	58.90%
May-19	98	43	55	43.87%
Jun-19	77	44	33	57.14%
Jul-19	81	54	27	66.66%
Aug-19	80	48	32	60.00%
Sep-19	86	59	27	68.60%
Oct-19	75	48	27	64.00%
Nov-19	104	54	50	51.92%
Dec-19	87	47	40	54.02%
Jan-20	86	56	30	65.11%
Feb-20	92	55	37	59.78%

Table 7: Two-way ANOVA with bovine hosts (cows and buffaloes) and months

Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	0.20099	11	0.018272	1.712601	0.192938	2.81793
Columns	0.036372	1	0.036372	3.409072	0.091897	4.844336
Error	0.117359	11	0.010669			
Total	0.354721	23				

Fig1: Month wise prevalence of mastitis in Cow and Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam

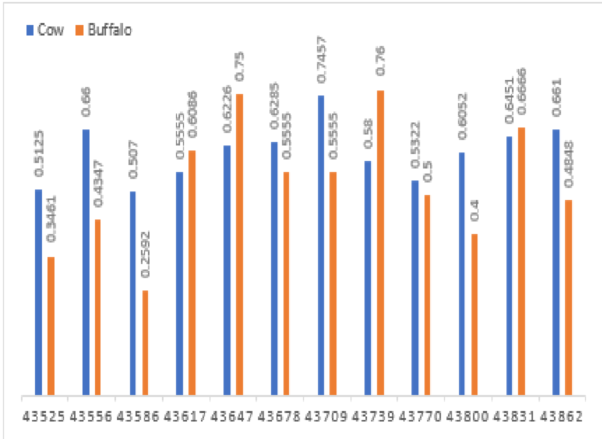
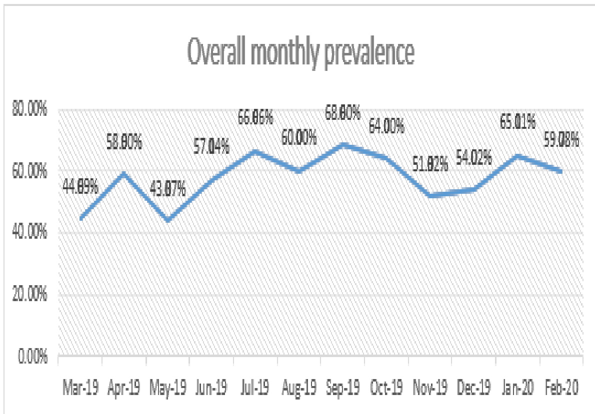


Fig 2: Overall monthly prevalence of mastitis in Cow and Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam.



Studies on Seasonal Prevalence of Mastitis in Cows and Buffaloes:

Studies on seasonal prevalence of mastitis in cows and buffaloes revealed high prevalence during rainy season showing 64.65 % and 65.42%, followed by winter with 61.25% and 50.38% and summer with 54.90% and 32.23% respectively (Table 8 & Fig 3). Two – Way ANOVA was carried out between seasons and host to see whether the difference in prevalence between cows and buffaloes during different seasons is significant or not (Table 9).

Table 8: Seasonal prevalence of mastitis in in Cow and Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam

Period	Host	Season		
March 2019 - February 2020		Summer	Winter	Rainy
	Cows	54.90%	61.25%	64.65%
	Buffaloes	32.23%	50.38%	65.42%

Fig 3: Seasonal prevalence of mastitis in in Cow and Buffalo milk samples collected from dairy farms situated in and around Visakhapatnam

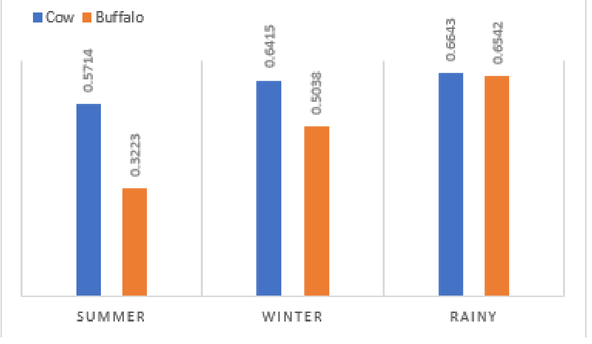


Table 9: Two-way Anova between the hosts (cows and buffaloes) and seasons

Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	0.046402	2	0.023201	3.378126	0.228408	19
Columns	0.017898	1	0.017898	2.605971	0.247816	18.51282
Error	0.013736	2	0.006868			
Total	0.078036	5				

Culture Studies:

Milk samples were screened using CMT, WSFT and SFMT and positive percentages were calculated (Table 10). Positive samples with more than 50% severity were subjected to microbial culture studies on various culture media employing standard procedures. Rich growth of bacterial and fungal pathogens was noticed in most of the samples, and culture plates showing good microbial growth were subjected to further isolation on different media, specific for bacteria commonly reported in mastitis infections. Samples with more than 50% severity for mastitis (255 + ve samples from cows and 93 +ve from buffaloes) were cultured for pathogen isolation and identification. Both bacterial colonies and fungal growth was noticed in most of the culture plates. Though fungal growth was noticed in culture plates of mastitis positive milk samples, present study was focused only on bacterial pathogens and their isolation and identification. Altogether 6 varieties of bacterial isolates viz. *Staphylococcus spp, Streptococci spp, E. coli, Pseudomonas spp, Corynebacterium spp and Klebsiella spp* were isolated and identified up to genus level. Studies on prevalence of bacterial infections showed *Staphylococcus spp* with a prevalence of 30.98% to be the most dominant bacteria in milk samples of cows followed by *E. coli* (24.70%) and *Streptococcus spp.* (22.74%), whereas *Klebsiella spp* was least represented with only 1.96%. On the other hand, in buffalo milk samples *E. coli* was found to be the most abundantly occurring bacterial pathogen with 32.25% followed by *Pseudomonas spp* (24.73%), whereas *Streptococcus spp* (7.52%) was the least represented one. The prevalence of various bacterial pathogens in cows and buffaloes was provided in the table (Table 11).

Pure cultures with single colonies were tested for purity by preparing smears and observing under the microscope. After confirmation of the purity, each culture was subjected to a series of confirmatory biochemical tests for identification. A panel of 14 biochemical tests were performed and 6 varieties of bacterial pathogens were identified up to genus level. The results of these tests were presented in the following table (Table 12).

Table 10: Occurrence of mastitis by various diagnostic procedures in cows and buffaloes from Visakhapatnam district.

Preliminary tests	Cows			Buffaloes		
	No. of samples tested	No. of samples tested positive	Percentage of +ve samples	No. of samples tested	No. of samples tested positive	Percentage of +ve samples
CMT	710	426	60.00%	340	184	54.11%
WSFT	710	419	59.01%	340	171	50.29%
SFMT	710	405	57.04%	340	157	46.17%

CMT: California Mastitis Test; **WSFT**: White Side Field Test; **SFMT**: Surf Field Mastitis Test

Table 11: Prevalence of various bacterial pathogens isolated from milk samples of cows and buffaloes +ve for mastitis.

Bacteria isolated from Mastitis milk samples	Host					
	Cows			Buffaloes		
	Total no. of isolates	Individual isolates	Prevalence	Total no. of isolates	Individual isolates	Prevalence
Staphylococcus spp	255	79	30.98%	93	15	16.12%
Streptococcus spp		58	22.74%		5	7.52%
E. coli		63	24.70%		30	32.25%
Pseudomonas spp		33	12.94%		23	24.73%
Corynebacterium spp		12	4.70%		10	10.75%
Klebsiella spp		5	1.96%		10	10.75%
Fungal spp.		5	1.96%		0	0

Table 12: List of Biochemical tests performed for confirmation of bacteria isolated from milk samples of cows and buffaloes.

Biochemical Tests / Organism	*Staphylococcus spp*	*Streptococcus spp*	*E. coli*	*Pseudomonas spp*	*Corynebacterium spp*	*Klebsiella spp*
Motility	-	-	+	+	-	-
Staining	+	+	-	-	+	-
Catalase	+	-	+	+	+	+
Oxidase	-	-	-	+	-	-
Coagulase	+	-	-	-	-	
Indole	-	-	+	-	-	-
Methyl Red	+	+	+	-	+	-
Voges Proskauer	+	-	-	-	-	+
Citrate	+	-	-	+	-	+
Nitrate	+	-	+	+	+	+
Oxidative fermentative	Fermentative	Fermentative	Fermentative	Oxidative	Fermentative	Fermentative
H_2S	-	-	-	-	+	-
Urease	+	-	-	-	-	+
Glucose	+	+	-	-	+	+

Results and Discussion:

The present investigation has been carried out to study the prevalence of mastitis in cows and buffaloes from dairy farms situated in and around Visakhapatnam, the causative agents involved and their prevalence. The study revealed high prevalence 74.57% in case of cows and 76.00% in buffaloes was reported. High prevalence was noticed during September – October months in both bovines and low prevalence was noticed in April – May months. Similarly, seasonal data also indicated high infection during rainy season and low in summer season.

A study on prevalence of clinical and sub clinical mastitis in buffaloes and crossbred cows showed an overall prevalence of sub clinical mastitis (SCM) and clinical mastitis, as 27% and 4% in buffaloes, and as 36% and 5.5% in crossbred cows respectively (Khan and Muhammad, 2005). Bhanot et al 2012 in their study for a period of 6 years i.e., from 2004 – 2009 reported a very high prevalence of mastitis in both cows and buffaloes (85.3% and 78.1% respectively). During the present investigation also, high prevalence was noted in cows when compared to buffaloes. Redite et al. 2013 in one of his surveys reported that prevalence of mastitis in cows was 39.5% and at herd level it was 73.4%. Salveen et al. (2020) reported 90.80% prevalence of mastitis in heifers' cows from Jammu region, reporting 227 out of 250 milk samples as positive.

Sachin Joshi & Suresh Gokhale reported high incidence of mastitis in both cows and buffaloes during rainy season (15.05 % & 8.89%) when compared to summer season (13.97% & 7.09%) respectively. Tufani et.al., (2012) reported that cross bred cows showed highest incidence of mastitis in spring (42.86%) followed by winter (34.92%), summer (15.875) and least during autumn (6.35%). Malik, 2009 noted high incidence of mastitis in winter (18.8%) followed by summer (16.6%) in bovine from Kashmir valley. Sinha et al. (2014) also made similar observations in the course of his studies on mastitis. The present study showed highest prevalence of infection during rainy season in both cows (64.25%) and buffaloes (65.42%) followed by winter 61.25% in cows and 50.38% in buffaloes and 54.90% in cows and 32.23% during summer.

Badiuzzaman et.al., 2015 conducted screening studies on 111 crossbred dairy cows from Bangladesh employing CMT, SCC, WST and SFMT tests to check their efficacy in detecting sub-clinical mastitis during 2010-2011. The sensitivity of the field tests CMT, SCC, WST & SFMT was found to be 80.08%, 86.60%, 60.54% and 57.47%, specificity 69.40%, 97.81%, 63.38% and 60.66%; percentage accuracy 75.68%,91.22%, 61.71% and 58.78%; positive predictive value 78.87%, 98.26%, 70.22% and 67.57%; and negative predictive value 70.95%, 83.64%, 52.97% and 50%respectively. CMT was concluded to be the most reliable field diagnostic test after cultural isolation and SCC as they require laboratory facilities and technical skill. Bhaskara Reddy et.al., 2018 in their study comparison of different diagnostic tests in subclinical mastitis in dairy cattle of Andhra Pradesh aims to find out the specificity, sensitivity and predictive values of CMT, EC (electrical conductivity) and SCC taking cultural test as standard. Out of quarter 135 milk samples. Percent accuracies were found to be 73.33, 70.37 and 71.00 for CMT, EC and SCC. Present study also finds that CMT test is more reliable field test. Altogether, out of 710 cow milk samples and 340 buffalo milk samples, 426 and 184 samples tested +ve with CMT followed by 419 and 171 +ve samples with WSFT and SFMT showing 405 and 157 +ve samples respectively.

De cassia & Joseph (2005) during their study on microbial isolation, especially *Candida* spp from bovine mastitic milk in Brazil, reported the presence of various bacterial pathogens, *E. coli* (26.5%), *Coagulase Positive Staphylococcus* (25%), *Streptococcus* (8.1%). The fungal isolates consist of 17.3% from 45 positive mastitis milk samples out of 260 milk samples. Getahun et. al., 2008 reported *Staphylococcus aureus* (42.6%) to be the most predominantly isolated bacteria in subclinical mastitis milk samples. Sumathi et al., 2008 reported high prevalence of *Staphylococcus aureus* (24%) followed by *E. coli* (20%), *Staphylococcus epidermidis* (16%), *Streptococcus spp* (16%) and *Klebsiella spp* (10%) from a total of 75 bacterial isolates from 60 clinical mastitic milk sample from dairy cattle in and around Bangalore. Mekbib et.al., (2009) during his studies also showed similar results and reported *Klebsiella spp.* (3.20%) to be the least represented. Grima et.al., (2012) in their study found that *Staphylococcus spp* (47.11%) is the most dominantly isolated bacteria next to *Streptococcus spp* (31.40%), *E. coli* (9.92%), whereas *Klebsiella* to be the least isolated bacteria from clinical and sub clinical mastitis. Sentitula et al (2012) during his studies on the occurrence of *Staphylococci* & *Streptococci* pathogens in mastitic milk samples from Sahiwal Cow and Murrah Buffaloes, screened 117 milk samples with California Mastitis Test, and recovered 109 and 90 cultures showing the growth of *Staphylococci* and *Streptococci* respectively. Redite et al. 2013 reported *Staphylococcus aureus* (26.8%) was the major pathogen followed by *Coagulase Negative Staphylococcus* (CNS) (18.7%). The present study is also in agreement that *Staphylococcus spp* (30.98%) is the most dominantly isolated bacteria followed by *E. coli* (24.70%) and *Streptococcus spp* (22.74%) in cows. Whereas in buffaloes *E. coli* (32.25%) is the most dominantly isolated bacteria followed by *Pseudomonas spp* (24.73%) and *Staphylococcus spp* (16.12%)

Conclusion:

The present study shows that over a period of time, mastitis may become the major problem in cows and buffaloes in Visakhapatnam district, Andhra Pradesh, if serious studies are not taken up immediately. *Staphylococcus spp, Streptococcus spp, E. coli* and *Pseudomonas spp* to be the most dominant bacteria isolated from mastitis positive samples from both cows and buffaloes. Seasonal wise variations were observed during the study and found that in rainy season prevalence of mastitis was noted to be high in both cows and buffaloes. In order to understand & implement effective prophylactic measures, the need of the hour is to undertake more in depth epidemiological and microbiological studies.

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