

STUDIES ON COMPARATIVE INCIDENCE OF SUB-CLINICAL AND CLINICAL MASTITIS AND *IN VITRO* ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES FROM HOLSTEIN-FRIESIAN AND JERSEY COWS AND BUFFALOES

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ABSTRACT

Data concerning sub-clinical and clinical mastitis in Holstein-Friesian, Jersey cows and buffaloes available for the period from July, 1986 to June, 2002 at Microbiology Division of the Directorate of Research Institute for Physiology of Animal Reproduction Bhunikey (Pattoki) District Kasur were analysed. Year-wise incidence of sub-clinical mastitis in Holstein-Friesian cows was 5.54 to 56.60%. This breed showed 13.19% overall incidence of sub-clinical mastitis, lowest incidence was in the year 1998-99 while it was highest in the year 1989-90. The corresponding values for Jersey cows were 5.93 to 33.73%, 12.89%, years 1995-96 and 1989-90. Lactation-wise incidence of sub-clinical mastitis in Holstein-Friesian cows was 8.33 to 21.90%, with lowest value in 10th lactation, while it was highest in 6th lactation. The corresponding values for Jersey cows were 10.26 to 41.56%, 1st and 7th lactations. Season-wise incidence of sub-clinical mastitis in Holstein-Friesian cows was 6.87 to 16.68%, lowest incidence was in dry hot season, while it was highest in autumn season. The corresponding values for Jersey cows were 8.57 to 16.55 per cent, dry hot and humid hot seasons. Year and season wise frequency of cases of clinical mastitis in buffaloes were 0.17 to 17.03%, lowest numbers of cases were in the year 1992-93, while these were highest in the year 2001-02. Season-wise cases of clinical mastitis in buffaloes were 11.90 to 31.72%, lowest frequency of cases was in dry hot season, while it was highest in humid hot season. Gentamycin showed the highest values of sensitivity for mastitic milk sample cultures of Holstein-Friesian cows and buffaloes (37.22 and 44.93%, respectively). Kanamycin gave the highest values (25.32%) of sensitivity for mastitic milk sample cultures of Jersey cows. It is concluded that years, seasons as well as lactation number showed their effect on incidence of sub-clinical and clinical mastitis in cattle and buffaloes. Gentamycin and kanamycin were found the drugs of first choice on the basis of drug sensitivity tests for the treatment of clinical mastitis in Holstein-Friesian and Jersey cows and buffaloes, respectively.

Key words: Sub-clinical and clinical mastitis, Holstein-Friesian Jersey cows, buffaloes, year, season, lactation.

INTRODUCTION

The dairy industry of Pakistan is comprised both cattle and buffaloes. Both of these species are susceptible to mastitis, which is considered to be the most costly disease of dairy animals. It causes economic losses to the farmers, consumers and milk processors. This is because the milk from the affected animals may harbour the organisms potentially pathogenic for human (zoonosis) and processing of such milk results in sub-optimal output of substandard finished fermented products like yogurt, cheese, etc. Mastitis may be classified into clinical and sub-clinical forms depending upon the presence or absence of overt manifestations of inflammation. Clinical mastitis is

classified according to its severity, rapidity of onset and duration i.e. per acute, acute, sub-acute and chronic forms.

In the clinical form, milk is grossly abnormal and may be bloody, watery or purulent and contains clots, flakes or shreds consisting of fibrin and cellular debris. In the chronic form, there is progressive fibrosis of mammary tissue. Per acute and acute forms of mastitis are usually accompanied by systemic signs. The sub-clinical mastitis is characterized by normal gland and milk appearance. The alterations are only detected by using field screening tests and laboratory methods. The present study was designed to know about the incidence of sub-clinical and clinical mastitis and *in vitro* sensitivity to various antibiotics.

MATERIALS AND METHODS

Milk samples of Holstein-Friesian and Jersey cows maintained at Livestock Experiment Station Bhunikey, District Kasur, have been examined regularly on monthly basis for the detection of sub-clinical mastitis cases using White Side Test at Microbiology Division of the Directorate of Research Institute for Physiology of Animal Reproduction, Bhunikey (Pattoki), District Kasur and in vitro antibiotic sensitivity tests of clinically positive cases, considered as cases of clinical mastitis has also been carried out since 1986. Milk samples of buffaloes and cows received from private sector have also been processed on the same pattern mentioned above at the said Division. Data concerning sub-clinical and clinical mastitis in Holstein-Friesian, Jersey cows and buffaloes available for the period from July 1986 to June 2002 at Microbiology Division of the Directorate of Research Institute for Physiology of Animal Reproduction, Bhunikey (Pattoki), District Kasur were analysed for the present study. These data related to 5626, 6106 and 1756 samples of milk of Holstein-Friesian, Jersey cows and buffaloes, respectively. Drug sensitivity discs used in above mentioned period were: Chloramphenicol (C), Kanamycin (K), Gentamycin (CN), Penicillin (P), Ampicillin (AMP), Amoxycilline (AML), Oxytetracycline (OT), Streptomycin (S), Erythromycin (E), Septran (SXT), Cloxacillin (OB), Neomycin (NE), Polymyxin-B (PB) and Doxycycline (DXT). Zone of inhibition of each antibiotic was measured and compared with the reference values (Anonymous 1990). Zone of inhibition of each antibiotic showing 25, 50, 75 and 100 per cent measurement values of the reference values were graded as +, ++, +++ and +++, respectively.

To determine the effect of season on mastitis, the months of the year were grouped in five seasons, as follows:

Winter	December and January
Spring	February, March and April
Dry hot summer	May and June
Humid hot summer	July, August and September
Autumn	October and November

Following parameters were included in the study:

- Year-wise incidence of mastitis.
- Lactation-wise incidence of mastitis.
- Season-wise incidence of mastitis.
- Antibiotic sensitivity to various drugs.

RESULTS AND DISCUSSION

Year-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows is given in Table 1. Its range in Holstein-Friesian cows was 5.54 to 56.60%. The overall incidence of sub-clinical mastitis in this breed was 13.19%. Data revealed that lowest incidence was in the year 1998-99, while it was highest in the year 1989-90. The corresponding values for Jersey cows were 5.93 to 33.73% in the years 1995-96 and 1989-90, respectively. The overall incidence of sub-clinical mastitis in this breed was 12.89%.

Lactation-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows is given in Table 2. Its range in Holstein-Friesian cows was 8.33 to 21.90%. Data revealed that lowest incidence was in 10th lactation, while it was highest in 6th lactation. Its range in Jersey cows was 10.26 to 41.56 %. Data revealed that lowest incidence was in 1st lactation, while it was highest in 7th lactation.

Season-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows is presented in Table 3. Its range in Holstein-Friesian cows was 6.87 to 16.68%. This breed of cattle showed 13.19% overall incidence of sub-clinical mastitis. Data revealed that lowest incidence was in dry hot season, while it was highest in autumn season. Its range in Jersey cows was 8.57 to 16.55 %. The overall incidence of sub-clinical mastitis in this breed was 12.89%. Data revealed that lowest incidence was in dry hot season, while it was highest in humid hot season.

Year and season-wise frequency of cases of clinical mastitis in buffaloes is presented in Table 4. Year-wise data revealed that the range of frequency of cases was 0.17 to 17.03%. Analysis also revealed that lowest cases were in the year 1992-93, while these were highest in the year 2001-02. The range of season-wise cases of clinical mastitis in buffaloes was 11.90 to 31.72%. Lowest frequency of cases was in dry hot season, while it was highest in humid hot season.

Results of sensitivity to various antibiotics in Holstein-Friesian, Jersey cows and buffaloes are presented in Table 5. Gentamycin showed the highest values of sensitivity for mastitic milk sample cultures of Holstein-Friesian cows and buffaloes (37.22 and 44.93%, respectively). Kanamycin gave the highest values (25.32%) of sensitivity for mastitic milk sample cultures of Jersey cows.

The findings of the present study regarding incidence of sub-clinical mastitis in cattle are not in agreement with the findings of Singh and Baxi (1980), Rasool *et al.* (1985), Hirpurkar *et al.* (1987), Mahmound (1988), Verma (1988) and Roy *et al.* (1989), who reported 54.0, 32.2, 32.7, 34.9, 43.5, and

Table 1: Year-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows

Years	Holstein-Friesian			Jersey		
	Total samples tested	Samples found positive	Percentage	Total samples tested	Samples found positive	Percentage
1986-87	84	27	32.14	130	35	26.92
1987-88	299	80	26.76	462	61	13.20
1988-89	149	33	22.15	273	30	10.99
1989-90	53	30	56.60	83	28	33.73
1990-91	156	24	15.38	316	47	14.87
1991-92	242	26	10.74	362	47	12.98
1992-93	304	28	9.21	600	50	8.33
1993-94	194	11	5.67	164	18	10.98
1994-95	392	44	11.22	382	49	12.83
1995-96	592	44	7.43	540	32	5.93
1996-97	637	53	8.32	644	89	13.82
1997-98	437	92	21.05	529	103	19.47
1998-99	289	16	5.54	260	39	15.00
1999-00	506	66	13.04	402	57	14.18
2000-01	621	79	12.72	462	48	10.39
2001-02	671	89	13.26	497	54	10.87
Overall	5626	742	13.19	6106	787	12.89

Table 2: Lactation-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows

Lactation No.	Holstein-Friesian			Jersey		
	Total samples tested	Samples found positive	Percent positive	Total samples tested	Samples found positive	Percent positive
1	2241	196	8.75	1959	201	10.26
2	1301	201	15.45	1421	194	13.65
3	1075	194	18.05	1209	159	13.15
4	561	71	12.66	647	83	12.83
5	255	48	18.82	406	60	14.78
6	105	23	21.90	283	44	15.55
7	59	5	8.47	77	32	41.56
8	15	3	20.00	71	9	12.68
9	---	---	---	18	2	11.11
10	12	1	8.33	14	3	21.43
11	2	---	---	---	---	---
12	---	---	---	1	---	---
Total	5626	742	---	6106	787	---

44.7% incidence of sub-clinical mastitis in cattle, respectively. The findings of the present study regarding incidence of sub-clinical mastitis in cattle are close to the findings of Chanda *et al.* (1989), who reported 16.9 per cent incidence of sub-clinical mastitis in cattle. The findings of the present study regarding the frequency of clinical mastitis cases in buffaloes are in agreement with the findings of Yass *et al.* (1983),

Wanasingh (1985), Hussain *et al.* (1984), and Singh *et al.* (1989), who reported the incidence of clinical mastitis in buffaloes as 12.1, 12.1, 10.0, and 5.7, per cent, respectively. Information on lactation and season-wise incidence of sub-clinical and clinical mastitis in dairy animals is scanty.

The findings of the present study regarding results of *in vitro* sensitivity tests to various antibiotics in cows

Table 3: Season-wise incidence of sub-clinical mastitis in Holstein-Friesian and Jersey cows

Seasons	Holstein-Friesian			Jersey		
	Total samples tested	Samples found positive	Percent positive	Total samples tested	Samples found positive	Percent positive
Winter	873	95	10.88	900	93	10.33
Spring	1498	197	13.15	1623	177	10.91
Dry hot	932	64	6.87	945	81	8.57
Humid hot	1298	215	16.56	1523	252	16.55
Autumn	1025	171	16.68	1115	184	16.50
Total	5626	742	13.19	6106	787	12.89

Table 4: Year-wise and season-wise cases of clinical mastitis in buffaloes

	Positive samples	Frequency of cases (%)
Years		
1987-88	5	0.28
1988-89	8	0.46
1989-90	12	0.68
1990-91	6	0.34
1991-92	0	0.00
1992-93	3	0.17
1993-94	12	0.68
1994-95	18	1.03
1995-96	150	8.54
1996-97	194	11.05
1997-98	287	16.34
1998-99	206	11.73
1999-00	285	16.23
2000-01	271	15.43
2001-02	299	17.03
Total	1756	100.00
Seasons		
Winter	295	16.80
Spring	363	20.67
Dry hot	209	11.90
Humid hot	557	31.72
Autumn	332	18.91
Total	1756	100.00

and buffaloes are partially in agreement with the findings of Ahmad and Ahmad (2001), who reported that Norfloxacin and Gentamycin were most effective antibiotics for the treatment of mastitis, followed by Amoxicillin, Tetracycline and Chloramphenicol. They also added that most of the bacterial isolates of mastitic

milk samples were resistant to Penicillin and Streptomycin. Ahmad (2001) reported that *in vitro* sensitivity revealed Kanamycin to be highly effective against various isolates, followed by Gentamycin which is in agreement with the findings of the present study.

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Table 5: Results of sensitivity tests of various antibiotics in cows and buffaloes

Results	C	K	CN	P	AMP	AML	OT	S	E	SXT	OB	NE	PB	DXT
Holstein-Friesian cows														
+	32	37	52	15	13	0	15	26	8	---	1	15	---	6
++	84	121	145	16	44	8	81	54	31	---	2	10	---	---
+++	66	84	77	12	22	6	69	35	22	2	2	6	---	---
++++	13	13	4	2	1	5	17	1	6	---	---	---	---	---
Total positive	195	255	278	45	80	19	182	116	67	2	5	31	---	6
Percent positive	26.1	34.18	37.22	6.03	10.74	5.35	24.46	15.61	9.42	33.3	2.26	9.63	---	4.6
Resistant	553	491	469	701	665	336	562	627	644	4	216	291	217	126
Total	748	746	747	746	745	355	744	743	711	6	221	322	217	132
Jersey cows														
+	20	29	54	12	10	2	27	33	7	1	---	5	1	7
++	82	135	144	21	30	14	85	81	33	2	2	15	---	---
+++	91	99	62	15	27	13	96	61	30	1	2	---	---	---
++++	11	12	6	6	7	1	7	2	9	---	---	---	1	1
Total positive	204	275	266	54	74	30	215	177	79	4	4	20	2	8
Percent positive	20.1	25.32	24.7	6.24	8.36	8.09	20.96	17.91	9.22	0.98	1.37	4.73	0.69	3.9
Resistant	607	536	545	757	737	311	596	634	699	400	285	383	284	188
Total	1015	1086	1077	865	885	371	1026	988	857	408	293	423	288	204
Buffaloes														
+	74	123	174	15	33	13	38	77	22	2	---	2	---	---
++	260	326	396	26	64	28	147	131	87	5	---	8	---	---
+++	263	208	203	38	72	32	188	83	64	9	---	1	---	---
++++	25	11	15	11	13	5	30	2	12	1	---	1	---	---
Total positive	622	668	788	90	182	78	403	293	185	17	---	12	---	---
Percent positive	35.4	38.06	44.93	5.13	10.36	4.44	22.95	16.69	10.5	0.97	---	0.68	---	---
Resistant	1134	1087	966	1667	1574	1678	1353	1463	1571	1739	---	1744	1756	---
Total	1756	1755	1754	1756	1756	1756	1756	1756	1756	1756	---	1756	1756	---

C= Chloramphenicol K= Kanamycin CN= Gentamycin P= Penicillin AMP= Ampicillin AML= Amoxycillin OT= Oxytetracycline S= Streptomycin
 E= Erythromycin SXT= Septran OB= Cloxacillin NE= Neomycin PB= Polymyxin-B DXT= Doxycillin

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