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Article 11

Bacterial Isolation and Drug Sensitivity Pattern of Bovine Mastitis in Kashmir

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ABSTRACT

A retrospective analysis of the bovine milk samples screened for causative agents of mastitis at the Institute of Animal Health and Biological Products, Zakura from the year 1997 to 2003 was undertaken. Four hundred and thirty five bovine milk samples received over a period of six years from various places of the Kashmir valley were subjected to bacterial isolation and antibiotic sensitivity. Staphylococci were present in 72% to 76.3%, Streptococci in 12.5% to 14% and mixed infection in 9.7% to 11.2% of the samples tested. The periodic prevalence of Staphylococcal and Streptococcal mastitis and shift in the antibiograms of isolates over the period of these six years was recorded.

INTRODUCTION

Mastitis is the most important disease of high yielding dairy cows. Economic loss to the poor farmers due to loss of milk during current and future lactations is enormous. Wide range of microbes responsible for the disease contribute differently in various climatic zones and managemental practices. The sensitivity pattern of different bacteria to antibiotics also changes over a period of time.

MATERIALS AND METHODS

The samples collected / received from the field , were subjected to cultural examination using standard laboratory procedures of inoculating the samples in nutrient broth overnight and then transferring to solid media i.e. nutrient agar, blood agar, differential agar, strepto-staphylococcal agar (Hi-media). The isolated colonies were studied for the staining character, morphology and hemolytic activity. Selected isolates were then studied for bio-chemical characteristics and identified as Staphylococci or Streptococci as per the Bergey's Manual for Determinative Bacteriology. The antibiotic disks used in the sensitivity studies are given in Table 1.

Table 1
Antibiotic discs used in the study and their concentrations

Antibiotic disc used	Abbreviation	Concentration (µg)
Ampicillin	A	10
Chloramphenicol	C	30
Ciprofloxacin	Cf	5
Cloxacillin	Cx	5
Clorotetracycline	Ct	30
Doxycycline hydrochloride	Do	30
Erythromycin	E	15
Gentamicin	G	10
Kanamycin	K	30
Lincomycin	L	10
Norfloxacin	Nx	10

The Staphylococcus and Streptococcus isolates were grown in separate nutrient broths and aseptically streaked on sterile nutrient agar plates. The antibiotic discs were placed five minutes later selectively with the help of an antibiotic disc dispenser in a clockwise direction. The charged plates were incubated overnight at 37° C by keeping the plates in an inverted position. (Kirby et al, 1966). For determining the sensitivity of each antibiotic for therapeutic purpose, the diameter of zone of inhibition including the disc were measured in millimeters (mm). The organisms were considered sensitive to a particular drug when no growth could be seen in an area of 7mm diameter around the antibiotic discs.

RESULTS AND DISCUSSION

(a) Prevalence

The retrospective analysis was conducted from the years 1997-98 to 2002-03 (six years) wherein the cases of milk samples tested were 56, 100, 80, 71, 56 and 72 respectively. The percentage, distribution of staphylococcal and streptococcal isolates recovered from mastitis milk over the period is depicted in Table 2.

Throughout the study period Staphylococcus was the major bacterial species associated with bovine mastitis followed by streptococci. These two organism thrive in abundance in the vicinity of the animals particularly under poor managerial practices.

The prevalence of Staphylococcal species in causing bovine mastitis was found to be 73%, 72.2%, 72.5%, 73.2%, 73% and 73% in the year 1997-98, 1998-99, 1999-2000, 2000-2001, 2001-2002 and 2002-2003 respectively, which is in agreement with earlier reports of Kalorey et al (1984), Singh et al, (1994) and Tarfarosh, (1996).

Similarly the prevalence of Streptococci was recorded in bovine mastitis as 12%, 13%, 13.7%, 14%, 12.5% and 13.8% in the year 1997-98, 1998-99, 1999-2000, 2000-2001, 2001-2002 and 2002-2003 respectively which is in conformity with the reports of Saini et al, (1994) and Tarfarosh, (1996).

The incidence of mixed infection was recorded as 10.7%, 10%, 11.2%, 11.2%, 10.7% and 9.7% in the year 1997-98, 1998-99, 1999-2000, 2000-2001, 2001-2002 and 2002-2003 respectively which falls in line with the observations of Konte et al, (1988), Kapur and Singh, (1980) and Tarfarosh, (1996).

(b) Drug sensitivity

The results of sensitivity test are presented in Tables 3 and 4.

The area of zone of inhibition with the diameter measured in mm and the quantum of sensitivity was interpreted as follows.

+++	>12mm	Highly sensitive
++	10-12mm	Moderately sensitive
+	7-10mm	Relatively sensitive
0	< 7mm	Resistant

During the year 1997-98, the drugs Cloxacillin and Chloramphenicol were found to be highly effective, but during subsequent years i.e. 1998-99, 1999-2000, 2000-2001, 2001-2002 and 2002-2003 Chloramphenicol, Gentamicin, Kanamycin, Ciprofloxacin were the drugs to which the cultures were highly and moderately sensitive but resistance was encountered for Cloxacillin, Ampicillin, Erythromycin.

Drugs of choice as per the Tables 3 and Table 4 appear to be Chloramphenicol, Gentamicin and Ciprofloxacin which fall in line with the works conducted by Babu et al (1980) Singh and Baxi (1983) Rehman and Boro (1991) Singh et al (1994) and Tarfarosh, (1996).

Table 2
Distribution of causative agents in the bovine milk samples tested

Year	Samples Received	Samples Positive for Mastitis	Causative agents					
			Staphylococci	%	Streptococci	%	Mixed	%
1997-98	56	54	41	73	7	12.5	6	10.7
1998-99	100	95	72	72	13	13	10	10
1999-2000	80	78	58	72.5	11	13.7	9	11.2
2000-01	71	70	52	73.2	10	14	8	11.2
2001-02	56	54	41	73	7	12.5	6	10.7
2002-03	72	70	53	76.3	10	13.8	7	9.7

Table 3
Year-wise use of antibiotics and sensitivity patterns

Year	Isolates tested	Antibiotic discs used	
		Sensitive	Resistant
1997-98	54	C+++ , CX+++ , Cf++ , G++	A, E, Nx
1998-99	95	C+++ , K+++ , G++ , CF++ , DO++	Cx, A, Nx
1999-2000	78	C+++ , K+++ , G++ , CF++	A, Cx, Cl
2000-2001	70	C+++ , G++ , CF++ , NX++	A, E, Cx, L
2001-2002	54	C+++ , G++ , Cf++	A, E, Nx
2002-2003	70	C+++ , G++ , Cf++	A, E, Nx

Table 4
Periodic changes in resistance pattern

Year	Drug
1997-98	Ac, Nx
1998-99	A, Cx
1999-2000	A, Cx, K
2000-2001	A, E, Cx
2001-2002	A, E, Cx
2002-2003	A, E, Nx

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Article 12

Efficacy of Single Dose of Ivermectin Against Psoroptic Mange in Rabbit

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ABSTRACT

The study was conducted on New Zealand white rabbits reared under intensive system of housing suffering from mange where Psorptes cuniculi was detected as the etiological agent. A single dose of Ivermectin was found to be highly effective and safe in treating the psoroptic mange in these rabbits.

INTRODUCTION

In India mange infestation caused by Sarcoptes and Psoroptes species is a common disease of rabbits. The disease is more prevalent during stress. Low ambient temperature and high moisture during the winter months favour development of mange mites (Blood et al, 2002).

Sporadic incidences and outbreaks of mange in rabbits have been reported from India and abroad (Ribbeck, 1976;).

MATERIALS & METHODS

The study was conducted on New Zealand white rabbits reared under intensive system at Animal House, Institute of Animal Health & Biological Products, Zakura. Animals of both sexes aged between 6 months and 3 years revealed symptoms of acute dermatitis, encrustation, loss of hair in face and head region. Skin scrapings were collected and treated with 10% KOH solution. The scrapings were examined for mites following the standard identification technique (Soulsby, 1982).

Thirty five positive cases were randomly allotted to two groups A and B. Animals of group A (n=30) were administered Ivermectin @ 300µg/kg body weight subcutaneously as a single dose treatment and animals of group B (n=5) were not given any treatment.

All animals were kept under identical managemental conditions and observed daily for clinical improvement. Skin scrapings were examined on 7th, 14th, 21st and 28th day post-treatment to determine parasitic load.

RESULTS AND DISCUSSION

The affected rabbits had developed bald patches at the base of ear, face and the head with yellowish grey scab in some cases provoking violent scratching. Scrapings from these lesions showed presence of eggs, larvae and adult stages of *Psoroptes cuniculi* mites. Following treatment with Ivermectin, a significant and progressive decline in parasitic load was observed on 7th, 14th and 21st day and the lesions were found negative for mites and their immature stages on 28th day post treatment.

The animals in untreated control group B continued to suffer with acute infection, lesions extending to involve other body parts, revealing presence of active stages of parasites till the end of the experiment. The clinical findings observed in present study were in accordance with earlier reports of Malcolm and Philip (1979).

Ivermectin has been found highly effective at very low concentrations against ectoparasites in domestic animals. Our observations are in concurrence with earlier reports of Pathak and Chaurasia, (2002) and Ramprabhu et al, (2004). During present study *Psorptes cuniculi* was detected, a common mange mite affecting both sexes of all age groups of rabbits raised under intensive system. A single dose of Ivermectin was found highly effective and safe in treating Psoroptic mange in rabbits.

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Efficacy of Different Anthelmintics Against Gastro-Intestinal Nematodes of Sheep in Kashmir Valley

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ABSTRACT

Forty weaner sheep divided randomly into four groups of ten animals each were administered various anthelmintics. The efficacy was accessed by calculating the reduction in eggs per gram (EPG) percentage. The result showed ivermectin to be the most effective followed by fenbendazole and albendazole respectively.

INTRODUCTION

The sheep of the Kashmir valley are reared on an extensive pattern, in which they are dependent mostly on the green lush pasture of the meadows. This results in high parasitic infestation especially due to gastro-intestinal (GI) nematodes. The infested animals show anorexia, reduced feed intake, alteration in protein metabolism along with loss of blood and plasma protein in the gastro-intestinal tract. Great economic losses due to decreased growth rate and wool production, sub-optimal reproduction and sometimes death of the animals are encountered.

Keeping in view the adverse effects of these helminth parasites in sheep, the present study was undertaken to investigate the therapeutic efficacy of some anthelmintics against the natural infection of gastro-intestinal nematodes in weaners at Sheep Breeding Farm, Goabal, Kashmir.

MATERIALS AND METHODS

Forty sheep (weaners) of either sex having naturally acquired infestation of GI nematodes were selected for the study and divided into four groups named A, B, C, & D. (Table 1)

Animals of group B, C and D were orally administered ivermectin (0.2 mg/kg), fenbendazole (5 mg/kg) and albendazole (7.5 mg/kg) immediately after collecting the fecal samples on day 0 (zero). The animals of group A were not administered any anthelmintic and served as control.

Fecal samples from the different groups were collected at day 0 (pre-treatment) and thereafter on day 3, 7, 14, 21 and 28 (post-treatment). All samples were examined to determine the number of eggs per gram (EPG) of samples as per the Stoll's egg counting technique (Soulsby 1986).

The efficacy of anthelmintics was calculated by comparing the means of EPG values pre and post-treatment over the different groups. The experiment was continued up to 28th day post-treatment and the treated animals were also observed for tolerance to the drugs and any other untoward symptoms. At the end of the study period percent reduction in EPG was calculated as per the standard formula:

$$= \frac{\text{Mean EPG pre treatment} - \text{Mean EPG post treatment}}{\text{Mean EPG pre treatment}} \text{ (Soulsby, 1982)}$$

RESULTS AND DISCUSSION

The result of the drug trial in terms of percent decrease in EPG (mean) of treated animals is given in Table 1.

Table 1
Comparative efficacy of various anthelmintics in sheep

Groups	Drug	Dose Rate/ Route	No. of Animals	Mean EPG						Percent reduction in EPG
				Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	
A	Untreated (control)	-	10	2700	2750	2800	2800	2900	2950	-
B	Ivomec (Ivermectin 0.085 w/v solution, Ethicare)	0.2 mg/kg body weight, orally	10	2475	200	160	160	160	160	93.21
C	Fendazole (Fenbendazole 10% suspension w/v, Panacur)	5 mg/kg body weight, orally	10	2612	400	300	300	300	200	88.91
D	Aldazole (Albendazole 2.5% suspension w/v, Ethicare)	7.5 mg/kg body weight, orally	10	2683	800	600	600	600	400	77.63

Ivermectin administered to animals of group C was found the most effective as the EPG value decreased from 2475 to 160 resulting in a 93.21% reduction. These results were in agreement with the findings of Wescott and Leamaster (1982), Swan et al (1984) and Singh et al (2001).

Fenbendazole in animals of Group D also proved effective against gastro-intestinal nematodes with decrease in EPG count from a pre-treatment mean value of 2612 to a post-treatment mean of 300. A decrease of 88.91% in EPG values was thus noticed in this group of animals. Similar results have been reported by Srivastav et al (1983) and Yaz Winski et al (1983) for sheep infected with nematodiasis.

Albendazole in weaners of group E was least effective against gastro-intestinal nematodes with a decrease in EPG from a pre-treatment mean value of 2683 to 600 at the end of the trial period. The reduction in EPG was only 77.63% in the animals of this group.

On the basis of these results it was concluded that ivermectin proved the most effective drug against ovine GI nematodiasis. Fenbendazole being comparatively less and Albendazole the least effective.

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Article 14

Economic Implications of Bubaline Ketosis – A Clinical Study of Effects on Milk Yield

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ABSTRACT

Study outlines the effect of ketosis in 120 buffaloes. Seventy nine (65.83%) of 120 ketotic buffaloes showed 20-30 percent drop in milk yield, whereas 40-50 per cent drop in production was exhibited by 20 (16.66%) buffaloes. The average drop in daily milk yield was recorded to be 3.52 ± 0.16 liters (36.70%) and the recovery after treatment could restore only 25.30 per cent (1.73 ± 0.15 liters) milk per day.

INTRODUCTION

Bovine ketosis is of substantial economic significance and has been found to be responsible for decline in milk production even two weeks before its clinical form, (Lucey et al, 1986). Major economic losses have been attributed to the loss of milk yield and failure of the animals to return to the fullest production potential even after recovery in clear cut cases of ketosis (Waage, 1989; Lean et al, 1994). The present study puts on record the drop in milk production on the onset of disease and the regain in its productivity after recovery.

MATERIALS AND METHODS

One hundred twenty clinically ketotic buffaloes from various localities of Chattisgarh, were included in the study for assessing the drop in milk yield. Twenty four of them were followed beyond recovery to assess resumption of yield.

RESULTS AND DISCUSSION

Seventy nine (65.83%), twenty one (17.50%) and twenty (16.66) buffaloes suffering from clinical ketosis showed 20-30%, 30-40% and 40-50% drop in milk yield respectively (Table 1).

Table 1
Effects of clinical ketosis on milk yield of buffalo

Animals		Drop in milk yield (%)
Number	Percentage	
79	65.83	20 – 30
21	17.50	30 – 40
20	16.66	40 – 50
120	100	Total

A drop of 3.52 ± 0.16 liters milk/animal/day (36.70%) was thus noticed (Table 2). Average daily milk yield recorded before illness in healthy buffaloes was 9.59 ± 0.88 liters, that dropped to 6.07 ± 0.91 liters per day in ketotic buffaloes. Decline of 22–60 percent in milk production in bovine clinical ketosis has also been placed on record by Dohoo et al, (1984), Swan and Tripathy (1987) and Mir and Malik (2003).

Table 2
Lactation kinetics during bubaline ketosis

Milk yield per buffalo (L/day)		Mean \pm S.E. (S.D.)
N = 120*	Before ketosis	9.59 ± 0.88 (2.15)
	During ketosis	6.07 ± 0.91 (2.24)
	Drop	3.52 ± 0.16 (0.40)
	Percent drop	36.70
N=24	Regain after treatment	1.73 ± 0.15 (0.72)
	Percent regain at clinical recovery	25.30

*120 animals divided into six lactational phases

The fall in milk yield varied in degree at various stages of lactation. The decline ranged from 25.31 percent in 0 to 1 month of lactation to 49.77 percent at 5–6 month postpartum with an increasing trend (Table 2). Contrary to the present findings, Andersson (1988) demonstrated that the losses were marked in early as compared to late lactation stages in cows. Complete restoration of milk yield could not be achieved in present study also. An average of 1.73 ± 0.15 liters milk/animal/ day (25.30%) (Table 3) was regained in animals after recovery with various therapeutic regimens. Huge economic losses, because of drop in milk yield and failure of animal to return to full production potential have been recorded (Waage, 1989). In ketosis the capacity of the animal to supply the lactogenic precursors to mammary gland is reduced than the capacity of the gland to produce due to homeorhetic drive for production (Lean et al, 1992). Moreover, elevated blood ketones also result in decreased milk production (Andersson and Lundstrom, 1985). According to Radostitis et al, (2006) the decline in milk production in ketosis was not proportionate to reduction in energy status at early stages of lactation because of excessive hormonal stimuli.

Table 3
Lactation stage wise drop in milk yield in clinically ketotic buffaloes

Stage of lactation (Months)	Average Milk yield per Animal (L/day)		Daily loss/Animal (L/Day)	Decrease in milk yield (%)
	Before illness	During illness		
0 – 1	11.22	8.38	2.84	25.31
1 – 2	12.25	8.63	3.62	29.55
2 – 3	10.57	6.86	3.71	35.10
3 – 4	9.11	5.22	3.89	42.70
4 – 5	7.88	4.06	3.82	48.48
5 – 6	6.53	3.28	3.25	49.77
Mean \pm S. E.	9.59 \pm 0.88	6.07 \pm 0.91	3.52 \pm 0.16	36.70

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Article 15

Shigellosis Outbreak in Poultry and its Management with Ciprofloxacin and A Homeopathic Drug *Aconitum napellus*

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ABSTRACT

*An outbreak of dysenteric diarrhoea was encountered in 2-3 months old birds in an organised poultry farm at Srinagar. Microscopic examination of cloacal swabs and morbid material revealed infection with *Shigella flexneri*. Antibiogram of the isolates showed sensitivity to ciprofloxacin, amikacin and gentamicin but resistance to ampicillin, cloxacillin, norfloxacin, penicillin, and chlortetracycline.*

*Administration of homeopathic medicine *Aconitum napellus* coupled with ciprofloxacin controlled the disease successfully.*

INTRODUCTION

Shigellosis is an important cause of various diseases of livestock resulting in high morbidity and mortality.

Perusal of literature reveals very scanty information on incidence of Shigellosis in poultry.

Present communication records an outbreak of shigellosis and its successful treatment using ciprofloxacin along with homeopathic medicine *Aconitum napellus* in an organised poultry farm.

MATERIALS AND METHODS

Four carcasses from an organized poultry farm were presented at Poultry Disease Investigation Laboratory, Hariparabath for post-mortem examination. All carcasses were

having pale combs and wattles with soiled. Gross and microscopic examination of gut contents was conducted to rule out any sort of parasitism.

On spot inspection of the affected flock revealed morbidity of 25%. Birds manifested signs of dullness, depression, dysentery and heat intolerance. Cloacal swabs and pieces of intestine, spleen and liver were aseptically collected and subjected to culture tests at Institute of Animal Health and Biological Products, Zakura. The procedure outlined by Edwards and Ewing (1972) and Cruickshank et al, (1975) were adopted to identify bacterial growth on the basis of their cultural, morphological and biochemical characteristics. Disc diffusion method as described by Cruickshank et al, (1975) was followed to study drug sensitivity pattern of the isolates.

Homeopathic medicine *Aconitum napellus* (Monkshood) @ 5 drops/lit administered in cool hours of the day in drinking water sufficient to consume in two hours was followed by 500mg ciprofloxacin/lit for three days.

RESULTS AND DISCUSSION

Bacteriological examination of cloacal swabs and morbid material revealed infection with *Shigella*. The cultures were confirmed as *Shigella flexneri* on the basis of standard morphological and biochemical tests as described by Buchanan and Gibbon (1994). *Shigella flexneri* has been reported earlier by Bhatia and Phathak (1978) in parakeets. Calnek et al, (1991) and Tuttura et al, (1996) also reported *Shigella* species in poultry. Anti-biogram of isolates showed sensitivity to ciprofloxacin, amikacin and gentamicin with a maximum zone of inhibition for ciprofloxacin and minimum for gentamicin and resistance to ampicillin, cloxacillin, norfloxacin, penicillin, enrofloxacin and chloramphenicol. This sensitivity profile is in accordance with observations of Verma et al, (2002) in owls. Clinical signs of dullness, depression, dysenteric diarrhoea observed during present trial were in conformity with the observations of Verma et al, (2002) in owls.

Post-mortem examination revealed haemorrhagic intestinal mucosa and necrotic foci both in liver and spleen. Necropsy findings of severe enteritis with slight haemorrhages on intestinal mucosa are in accordance with reports of Gove Hambidge (2004). Pfaff (1905) isolated *Shigella pfaffi* from canary birds that had died of acute infection exhibiting signs of severe enteritis, diarrhoea with necrotic foci on liver and spleen. Verma et al, (2002) isolated *Shigella dysenteriae* from owls manifesting symptoms of severe enteritis, diarrhoea and pyrexia.

Following the combination treatment, 80% of the birds recovered and mortality was controlled within three days after cessation of treatment. In homeopathic system of medicine *Aconitum napellus* is indicated in the inflammation of bowels, pyrexia and dysentery (Edward 2002& Shah et al, 2003).

In present trial *Aconitum napellus* might have activated immune system to combat heat intolerance, inflammation of bowels and dysentery more effectively when administered with ciprofloxacin at the initial stage of ailment.

Aconitum napellus may prove helpful in reducing exposure of poultry birds to high and prolonged doses of antibiotics in treatment of dysenteric gut infections.

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