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Mr. W. J. Bader
Managing Director
Hoechst Roussel Vet Pvt. Ltd.



Dear Sirs,

The 9th Issue of **“The Blue Cross Book”** is ready now and I am glad to hand over the same to you.

I am really pleased to note from the editor that the scientific articles / case reports published in **“The Blue Cross Book”**, are being cited now-a-days in other technical journals as reference work. This is really good news to us and this will give a boost to our editorial board members to put in more efforts to maintain the same.

As we have recently shifted our Head Office from Mumbai to Pune, I request you to send your articles / case reports to the following address :

Dr. A. K. Datta
Editor,
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Best Wishes,



Jochen Bader

Mr. K. K. Unni
Chairman
Hoechst Roussel Vet Pvt. Ltd.



Dear Sirs,

As informed earlier (ref. 8th issue, January 1996), the synergies between Hoechst and Roussel will provide greater number of New Molecules for the benefit of the veterinary profession, I take this opportunity to share our happiness of launching a new Amidine group of Ectoparasiticide, AMITRAZ (Taktic 5 % and 12.5 % EC) for canine and livestock sectors respectively. Being a different group of chemicals, Amitraz will be an appropriate **Rotational Partner** of our market leader product, Butox (deltamethrine). I am sure rotational uses of these two molecules will certainly prevent resistant development and also shall help effective tick and mange control in small and large animals in the country.

I hope this issue will give you happy reading with detailed information on the case reports, received from veterinarians practicing abroad.

Best Wishes,

A handwritten signature in black ink, appearing to read 'K. K. Unni'.

K. K. UNNI

Efficacy of a Combined Formulation of Fenbendazole (2.5%) and Oxyclozanide (7.5%) suspension in Cattle

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Introduction:

In cattle, sheep and goats, **Liver-fluke, Strongyle worm and Tape worm** infestations are very common and are of economic importance. While irrigated, low lying water-logged areas are ideal locations for **Liver-fluke** infestation. **Strongyles** and **Tape worms** have wider distribution, occurring also in non-irrigated areas. However, because of transportation of green fodder to far off places, parasitic infestations in general and *Fasiola sp* in particular, are disseminated to non-endemic areas also and as such in any location, mixed infestation of **Strongyles** and **Liver-flukes** are not uncommon.

Fenbendazole (FBZ) is a known effective anthelmintic against **Strongyle** worms and Oxyclozanide (OXZ) is in *Fasiola sp* but the effect of these drugs on the other group of parasites is limited. Thus in mixed infestations of **Strongyles** and **Liver-flukes**, it becomes necessary to deworm the animals twice, once with Fenbendazole and second time with Oxyclozanide. We have received personal communication stating that some doctors in the field have administered both the drugs on the same day simultaneously to animals having mixed infestations without any apparent side effects. It was therefore, decided to conduct a trial on cows of the

combined formulation of Fenbendazole and Oxyclozanide to treat mixed infestations of Strongyle worms and Liver-flukes.

Materials and Methods :

The trial was conducted on a herd of cows at "Shrikhari Gopal Society's Goshala, Doulat Nagar Borivli, Mumbai"

At the outset, faecal samples of all animals were tested by both qualitative (Sedimentation technique) and quantitative (Stoll's methods) to spot out positive animals and also to find the intensity of infestation (EPG).

Positive animals were administered orally with combined suspension of Fenbendazole 2.5% + Oxyclozanide 7.5% (Hoechst Roussel Vet Pvt. Ltd.) at the dose of 60 ml per adult cow weighing approximately 300 kg, giving an effective dose of 5 mg/kg of Fenbendazole and 15 mg/kg of Oxyclozanide.

The faecal samples of treated animals were examined twice, (7th Day and 14th Day) post treatment. The post-treatment EPG values were compared with pre-treatment EPG values of the same animals and also of the group. The overall efficacy of the treatment was worked out by the following formula :

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$$\% \text{Efficacy} = \frac{\text{Geometric Mean of Pre-Treatment EPG} - \text{Geometric mean of Post Treatment EPG}}{\text{Geometric Mean of Pre-Treatment EPG}} \times 100$$

The animals were observed daily for 14 days post-treatment for any clinical signs of abnormalities and also for any adverse reaction. The fortnightly averages of milk yield of cows at pre-treatment and post-treatment stages were compared.

Observations : (Ref. Table I and II)

Out of 23 animals screened, 13 were found positive for parasites. *Fasciola sp* infection was found in six, **Strongyle** worms in six

and *Monizia sp* in five animals. In case of *Moniezia* there was no means to assess the intensity of infestation since EPG has no correlation to worm burden in tapeworms.

These 13 animals were administered the suspension of FBZ + OXZ formulation as stated above.

Faecal examinations of all six animals having *Fasciola sp* infection, were negative on 7th

Table I : The pre and post-treatment of Parasitic Infestation and the EPG levels in cows

Sr.No.	Cow Number	Pre-Treatment EPG 31.3.97			7-Day Post Treatment EPG 16.4.97			14 Day Post Treatment EPG 23.4.97		
		F	S	M	F	S	M	F	S	M
1)	42	-	100	-	-	100*	-	-	-	-
2)	8	300	100	-	-	-	-	-	-	-
3)	12	1200	-	+ve	-	-	-	-	-	-
4)	13	1800	-	+ve	-	-	-	-	-	-
5)	5	100	-	-	-	-	-	-	-	-
6)	35	-	-	+ve	-	-	-	-	-	-
7)	51	-	-	+ve	-	-	-	-	-	-
8)	29	-	200	-	-	100*	-	-	-	-
9)	24	-	200	+ve	-	100*	-	-	-	-
10)	25	-	100	-	-	100*	-	-	-	-
11)	20	1200	-	-	-	-	-	-	-	-
12)	15	6000	-	-	-	-	-	-	-	-
13)	50	-	100	-	-	-	-	-	-	-
GEOMETRIC MEAN OF EPG		2855.87	154.92	-	0	115.47	-	-	-	-
%EFFICACY OF TREATMENT					100	25.46	-	100	100	-

Date of Treatment : 9.4.97 : F=*Fasciola sp* : S= Strongyle worms; M= *Moniezia sp.*;
***Indicate nonviable eggs.**

day and 14th day post-treatment. Thus the efficacy of treatment was 100% in this case.

As regards to **Strongyle** worm infestation, out of six treated only two were negative on 7th day and all became negative on 14th day post-treatment. But all the eggs detected on 7th day post-treatment were apparently having dead embryos.

No clinical symptoms suggestive of nontolerance or toxicity were found in any of the treated animals including pregnant ones in 3 to 6 month gestation.

The milk yield increased by an average of 650 ml per day per cow after treatment. This was mainly noticed in those with

Strongyle worm infestation since those with Liver-flukes infestation, were incidentally not in milking stage.

Discussion :

The combined formulation of FBZ (2.5%) + OXZ (7.5%) was found to be 100 % effective on *Fasciola sp* and **Strongyle** worms in cows. The formulation at the tried dosage was found to be safe and non toxic to animals even in pregnancy and lactation. The treatment resulted in substantial increase in the milk yield.

The combined formulation of FBZ (2.5%) and OXZ (7.5%) has an advantage of being

TABLE II - Effects Of Treatment on Cows

Sr. No.	Cow Number	Status of Cow	Fortnightly average of milk (Liter per day)			Clinical Side Effects
			Pretreat-ment Decrease	Post Treat-ment	Increase of Milk	
1)	42	Milking(S)	4.0	5.0	+1.0	Nil
2)	8	Milking (S+F)	4.0	4.5	+0.5	Nil
3)	12	Milking (F+M)	3.0	3.0	-	Nil
4)	13	5 Mth. Pregnant (F+M)	-	-	-	Nil
5)	5	5 Mth. Pregnant (F)	-	-	-	Nil
6)	35	Dry (M)	-	-	-	Nil
7)	51	Infertile (M)	-	-	-	Nil
8)	29	Milking (S)	5.0	6.0	+1.0	Nil
9)	24	3 Mth. Pregnant (S+M)	-	-	-	Nil
10)	25	Milking(S)	2.0	2.75	+0.75	Nil
11)	20	6 Mth. Pregnant (F)	-	-	-	Nil
12)	15	Dry (F)	-	-	-	Nil
13)	50	Infertile (S)	-	-	-	Nil
Total Milk yield per day			18.0	21.25	+3.25	
Average Milk per milking cow			3.6	4.25	+0.65	

In above "Status of Cow" the alphabets in bracket indicated the type of parasitic infestation present as follows (F=*Fasciola sp*; S= Strongyle worms; M=*Moniezia sp.*)

a single dose treatment in mixed infections of Liver-flukes and Strongyle worms. More trials are needed to find out whether there is synergic action if the two drugs are used together and if so, whether it is possible to reduce the dose rate of either of the drug or both, when used together, without affecting the total efficacy.

Conclusion :

- 1) A suspension containing Febendazole (2.5%) and Oxytoclozanide (7.5%) at the dose of 60 ml per adult cow (300 kg b.w.) as a single administration is 100 % effective in mixed infestations of *Fasciola sp* and Strongyle worms.

- 2) Nothing can be concluded on the efficacy of the formulation on *Moniezia sp* since the methods used in this trial are not useful in estimating tape worm infestations - the only method being sacrificing the animals and doing post-mortem examination is in this case.
- 3) The formulation at the dose used is safe and non toxic and well tolerated to cows even in pregnancy and lactation.
- 4) The treatment resulted in increase in the milk yield to the tune of 18%.

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Studies on Babesiosis in Calves and its Treatment with Berenil

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Bovine babesiosis is prevalent in most parts of the world especially in the tropics and subtropics. The infection with clinical manifestations of the disease has mainly been reported from adult cattle. The occurrence of babesiosis in young calves is rare due to age resistance (Riek, 1968). It has been demonstrated that calves upto the age of 9-12 months are naturally resistant to babesiosis as long as they are non-splenectomised. Hall (1960) reported that *Babesia bigemina* calfhood immunity is derived in the form of maternal antibodies. Leavy *et al.* (1982) reported presence of a factor in the blood of calves independent of antibodies providing resistance to severe babesiosis. The present investigation was undertaken to present the haematological changes and also the treatment response in calves affected with babesiosis.

Materials and Methods :

Six crossbred calves (ten to fifteen days old) were suffering with symptoms of anorexia, fever, jaundice and haemoglobinuria, at an organised farm in Ludhiana district. The calves were examined clinically and blood samples were collected for haematological examination i.e. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocytic Count (TEC) and Total Leucocytic Count (TLC) as per the methods of Jain (1986). The blood smear stained with Giemsa revealed the presence of *Babesia bigemina* (morphologically). The affected calves were

treated with one injection of diminazine compound (Berenil) @ 1.6 gm/100 kg body weight. The affected calves were also treated with vitamin B complex with liver extract injection and Cal-D-plus DS as haematinics for six days. Blood films of all affected calves were also examined after remission of temperature.

Results and Discussion

It is evident from the above case report that although young calves are generally considered to be resistant to natural infection of *Babesia*, its incidence is not uncommon. This fact is further supported by few reports of clinical babesiosis in calves less than one year of age. Rogers (1971) in his epidemiological studies of bovine babesiosis in Australia reported that calves younger than nine months of age were not completely resistant and mortalities do occur. The reports of incidence of babesiosis in Zebu calves has also been reported (Mallick *et al.*, 1980 and Kasaralikalikar *et al.*, 1996). However, this finding is in disagreement with those of Levine (1961), Hungerford (1971) and Seneviratna (1971) who opined that young bovine calves were resistant to *Babesia* infection. The present observation is in partial agreement with that of Soulsby (1968) who believed that young calves exposed to heavy infection of *Babesia* might suffer from mild or sub-clinical form of disease with low parasitaemia.

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In course of investigation into the transmitting vectors of the disease, *Boophilus microplus* was recovered from the herds and the cattle shed of the organized farm. Since the infection and its definitive host-cum-transmitting vectors (ticks) were available in the farm premises, it was probable that the calves suffered from tick borne *Babesia* infection. The time lapse between the occurrence of the disease and date of birth of the calves was sufficient to incubate the infection in them.

The mean values of Hb, PCV and TEC prior to treatment were 6.16 ± 0.1235 gm%, 15.75 ± 0.2718 % and 7.58 ± 0.1580 million/cmm respectively which significantly increased to 9.38 ± 0.2118 gm%, 25.90 ± 0.5260 % and 9.67 ± 0.2472 million/cmm respectively by tenth day of treatment. The mean TLC significantly decreased from pre-treatment value i.e. 9.065 ± 0.1897 thousand/cmm to post-treatment value i.e. 6.58 ± 0.1018 thousand/cmm. The clinical symptoms subsided and the blood smears which revealed *Babesia bigemina* before treatment did not reveal it after treatment.

In the present investigation, the mean values of Hb, PCV and TEC decreased significantly and these might be due to intravascular haemolysis of blood by the causative agent (Blood and Radostits, 1989). The affected calves were successfully treated with Berenil injection @ 1.6 gm/100 kg body weight. Berenil had already been used successfully by various workers (Samad *et al.*, 1987, and Blood and Radostits, 1989). All the affected calves recovered and haematological parameters took about 7-10 days to come back to normal. Hence it is concluded that possibility of clinical babesiosis should never be neglected in young calves having jaundice and haemoglobinuria.

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Thrombo-embolic Colic and its Management in Horses

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Thrombo-embolic colic has commonly been reported as a cause of recurrent mild abdominal pain in race horses due to migrating larvae of *Strongylus vulgaris* (Kope, 1987, Radosstits et. al., 1995). In India, such reports are lacking in the indigenous horses. The present communication describes two clinical cases of thrombo-embolic colic in horses, along with their successful management.

Case History :

Two indigenous horses, with the history of recurrent mild colic, hindleg incoordination and mild lameness, since a fortnight, were referred to the Referral Polyclinic, Indian Veterinary Research Institute, Izatnagar for diagnosis and treatment. This history further revealed that the treatment adopted by the field veterinarians with various NSAIDS, antibiotics and tonics remained futile.

Clinical Observations :

Clinical examination revealed almost normal temperature, and increased pulse and respiration rate. Frequent attacks of mild and intermittent colic, manifested by uneasiness, anxiety, looking at the flank (Fig. 1) and occasional patchy sweating over the head, neck and abdomen were evident. Conjunctiva was pale in one while injected in the other case. Abdominal auscultation revealed increased bordorygmi. Hind leg incoordination, ataxia (Fig.2), and mild larneness was also seen. Faeces were normal except for mucus in it. Hind limbs (left in



Figure 1 : Horse showing colicky signs.



Figure 2 : Horse showing colikia and hind leg in-coordination.

one and right in the other) were a bit dry and cool. The digital pulse was less marked in the affected limbs. Rectal examination revealed mildly distended loops of intestines, and an asymmetry of the iliac arteries.

Laboratory Examinations :

Faecal examination revealed normal consistency, greenish coloured mucus coated faeces and preponderance of *Strongylus* spp eggs with no blood in both the cases.

Haemogram was almost normal except for eosinophilia (18 and 21 percent) in both the cases.

Diagnosis :

On the basis of the clinical and laboratory examination, diagnosis was arrived at

* P. G. Scholar

thrombo-embolic colic. Positive rectal and coprological findings, and physical changes with respect to temperature and digital pulse, in the affected leg, differentiated these cases from simple lameness.

Treatment was attempted with Fenbendazole (Panacur, Hoechst Roussel Vet @ 10 mg/kg b.w. PO, daily; Acetyl salicylic acid (Dispirin, Reckitt & Colman) @ 15 mg/kg b.w. PO, daily and a combination of Analgin, Pitofenone hydrochloride and Fempiverinium bromide (Baralgan, Hoechst Roussel Vet) 20 ml intramuscularly, twice daily, all for five days.

Discussion :

Chronic recurrent colic, hind limb weakness and lameness; comparatively dry and cool hind limbs with diminished arterial pulse in the affected limbs; and an asymmetry of iliac arteries on rectal examination, observed in the present cases, were in accordance with the symptoms described by previous workers (Azzie, 1969; Maxie and Physick-Sheard, 1985; Radostits *et al.*, 1995). No doubt these findings are suggestive of thrombo-embolism, ultrasonography is more sensitive in detecting even mild embolism in iliac artery (Reef, 1987). The preponderance of Strongylid eggs in the faeces of the horses pointed towards its association with the development of thrombo-embolic colic. The migration of strongylid larvae to the vessels injuries, the intima of the vessels and causes, platelet aggregation with the development of thrombo-embolism (Rous, 1975; Robertson, 1982). The degree of abdominal pain depends upon the extent of the tissue injury. Mild colic, in these cases seems to be due to focal ischemia caused by vasoconstriction, induced by thromboxane (White, 1986). Ataxia, lameness and temperature variation, over the affected limbs, are the reflection of circulatory interference due to embolism. The blood picture was inconclusive except

for revealing eosinophilia. However, eosinophilia is commonly associated with ongoing parasitism or allergic response (Schalm *et al.*, 1975). Fenbendazole in higher doses by the fifth day further confirmed our clinical assumption. Therapeutic use of Acetyl salicylic acid was with the aim to reduce aggregation of platelet which occurs due to migrating larvae in the vessels (Rous, 1975). Colicky signs were managed with an analgesia detailed earlier (Baralgan.)

Conclusion :

Clinical cases of thrombo-embolic colic in non-descript horses managed successfully with Fenbendazole (Panacur), Baralgan and Aspirin are discussed herein.

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Efficacy of Salinomycin (Sacox 120 microgranulate) on a Broiler Poultry Farm

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Introduction :

Coccidiosis is a very common disease occurring in every place where poultry is raised. It is almost impossible to raise poultry on floor pen system without continuous preventive medication with one or the other anti-coccidial in the feed. Because of extensive use of these drugs clinical outbreaks of coccidiosis are, these days, rare but sub-clinical coccidiosis is reported to be very rampant (Narsapur *et. al.* 1995) which results in greater economic losses due to slow growth, higher FCR and immunosuppression.

The efficacy of an anti-coccidial should therefore be judged by its efficiency in preventing sub-clinical coccidiosis. This can be assessed in two ways : i) Performance factors (viz growth rate and FCR) ii) Parasitological methods (viz examination of droppings, litter and evaluation of lesion scores during growth period.)

Salinomycin (**Sacox 120 microgranulate**) is an ionophore anti-coccidial, widely used and reported to be having efficacy on all species of *Eimeria sp.* affecting poultry. It was decided to undertake a trial of **Sacox** in commercial broiler farm near Mumbai to find out its performance rate under the managerial conditions and biosecurity available here. A comparison has been made with DOT (which is commonly being used in this area.)

Materials and methods :

The trial was conducted from 18-2-97 to 31-3-1997 at Temphir Poultry Farm, Vasai.

A batch of 4920 day old chicks from the same hatchery (Venkateshwara) and same hatch was divided in two equal halves (Section A and B) and were raised separately in adjacent houses, on deep litter, under identical conditions of management, feed and vaccination, medication schedules. The feed prepared on the farm (Maize, soya, sunflower and ricepolish being ingredients) was used. In section A feed with DOT (125 ppm) was used while in section B feed with **Sacox** (60 ppm Salinomycine) was used from start to disposal. The liquidation started after 6th week and completed by 6th week + 4 Days. Hence all observations were made up to completion of 6th week.

Following observations were recorded :-

- 1) Average body weight and feed consumption every week
- 2) Droppings from 10 birds, each from both the sections collected at 3rd, 4th and 5th week and tested in laboratory.
- 3) Litter samples from 6 spots each (about 100 Grams from a spot) from both houses A and B collected for laboratory test at 3rd, 4th & 5th week.
- 4) Post-mortem examination on available mortality from both sections were done during 3rd to 5th week and lesion scores noted as per standard methods.

Laboratory methods :

- i) Droppings were first processed by sedimentation and then by salt floatation method.
- ii) Litter samples were first soaked in about 300 ml of water for 30 minutes and then

strained in to a flask through muslin cloth. The filtrate was allowed to stand for 30 minutes and the sediment obtained was further processed as mentioned in the previous step.

The oocysts detected were quantified and expressed as average number per low power field under the microscope (LPF).

Observations and discussion :

(Table I and II)

The group of birds on **Sacox** in general scored better than those on DOT in terms of body weight gain, average feed consumed and FCR. The body weight was higher by 57 grams and FCR lower by 0.139 points in **Sacox** group than in DOT group.

Mortality was also lower in **Sacox** group by 2.63%.

In DOT group, few birds showed clinical signs of coocidiosis (blood in droppings) in 5th week which was confirmed by finding oocysts microscopically. However, there was no mortality recorded.

In the **Sacox** group few birds revealed oocysts of *Emeria sp* in the droppings during 5th week but since this was not accompanied by symptoms and the oocysts output was also very low, this finding could be termed as leakage of oocysts. The leakage of oocysts is a normal feature of ionophores (Edgar 1993).

The lower mortality in **Sacox** group might be due to better immunocompetence afforded by better control of sub-clinical coccidiosis by **Sacox**.

Conclusion :

Sacox was found to be effective in controlling coccidiosis and enhancing body weight and improving the feed conversion than DOT.

Table I : Sacox Vs DOT Trial conducted at TEMPHIR Poultry Farm Vasai from 18-2-97 to 31-3-1997 on Cobb Commercial broiler chicks from Venkateshwara Hatcheries received on 18-2-97. Total 4920 chicks divided in two sections A&B

Section	No. Chicks	Study Details	Age in Weeks						Total	Comparison A&B
			1st	2nd	3rd	4th	5th	6th		
A (on DOT)	2448	Mortality %	1.92	0.57	0.49	0.69	0.21	0	3.88	
		Cum. Feed Kg.	300	850	1800	3050	4650	6400	6400	
		Cum. Feed/bird in Kg.							2.72	
		Av. Body wt. Gms.	100	280	400	560	890	1163	1163	
		F C R	1.249	1.272	1.894	2.309	2.22	2.338	2.338	
B (on Sacox)	2472	Mortality%	0.61	0.12	0.2	0.04	0.2	.08	1.25	-2.63
		Cum. Feed Kg.	300	850	1800	3000	4600	6550	6550	
		Cum. Feed/bird Kg.							2.683	-0.037
		Av. Body Wt. Gms.	100	280	420	610	970	1220		+57
		F C R	1.221	1.237	1.750	2.009	1.941	2.199	2.199	-0.139

Cum.: Cummulative, Av.: Average,

Table II : Showing results of parasitological examination after Sacox (Section B) & DOT (Section A) treatment to poultry broilers. (Cobb commercial broiler chicks)

Type of sample	Details of examination	Section A (DOT)			Remarks	Section B Sacox			Remarks
		Week				Week			
		3rd	4th	5th		3rd	4th	5th	
Droppings	No. Examined	10	10	10	*Blood in droppings 2 Birds Oocyst output = 350-400/LPF in 2 Birds 0-2/LPF in 2 Birds 0-1/LPF in 5 Birds	10	10	10	**No Blood in droppings oocyst output =15-30/LPF in 1 Bird 2-3/LPF in 3 Birds
	No. +ve for Oocysts of <i>Eimeria sp</i>	Nil	Nil	9*		Nil	Nil	4**	
Litter	No. Examined	6	6	6		6	6	6	
	No. +ve for Oocysts of <i>Eimeria sp</i>	Nil	Nil	Nil		Nil	Nil	Nil	
Post-mortem Examination for lesion score	No. examined	— Total 10 —				— Total 5 —			
	Lesion score	Nil				Nil			

LPF : Low Power Microscopic Field

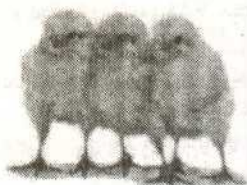
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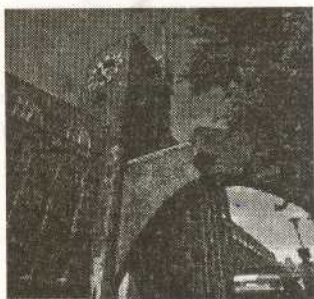
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Antibacterial Efficacy of Enrofloxacin against *Pasteurella multocida* P52, *P.haemolytica*, *Escherichia coli* and *Salmonella* Sp.

D. S. Khatpe, A. K. Datta, S. N. Singh

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Introduction :

Enrofloxacin, a fluroquinolone group of latest antimicrobial agent was developed exclusively for veterinary use. It was identified in 1983 by M/s. Bayer Laboratories, Germany through their extensive research efforts. A series of laboratory and clinical investigations proved its efficacy in regards to its spectrum of activity, stability, coverage for different clinical indications in different species of animals.

The present paper describes the antibiotic sensitivity pattern of four groups of bacteria, which are pathogenic to animals. The pathogens tested were pure virulent strains of *Pasteurella multocida* (P52), *P.haemolytica*, *Escherichia coli*, *Salmonella* sp. The antibiotics were taken into account, were - Tetracycline, Terramycin, Ampicillin, Chloramphenicol, Gentamycin, Streptomycin, Norfloxacin, Enrofloxacin and other antibacterial agents like Sulphadiazine and Furazolidone.

Materials & Methods :

18-24 hours young, pure and virulent cultures were grown on suitable growth medium. The purity and pathogenicity of cultures (*Pasteurella* sp.) were pretested by in-vitro culture method and in-vivo pathogenicity tests (mice).

Inoculum was transferred in Trpticase soya broth and incubated at 37°C for 2 to 5 hours to obtain moderate turbidity.

Broth culture was then diluted with sterile saline to match the density of Mcfarland standard no.1. This culture was then evenly

inoculated on agar plates and the antibiotic discs were implanted as per usual procedures.

Observations :

After incubating at 37°C for 18 - 24 hours the readings were taken and inhibited zones were measured. The details of findings are tabulated in tables I and II.

It was observed that *P.multocida* P52 was highly resistant to Tetracycline, Terramycin, Streptomycin and Sulphadiazine while it was equally sensitive to Chloramphenicol, Gentamycin, Norfloxacin and Enrofloxacin. Enrofloxacin was highly effective at the concentration of 10µg. The similar efficacy of Enrofloxacin i.e.10µg was also noticed with *Salmonella* sp.

Against *P.haemolytica*, Enrofloxacin was found highly effective at MIC 5µg while other antibiotics taken into account for this experiment did not show any inhibition. The same MIC range of 5µg was found in *E.coli* also.

Conclusions :

Enrofloxacin inhibits the growth of *P.multocida* P52 & *P. haemolytica* at MIC of 10µg and 5µg respectively. Whereas in *E.coli* and *Salmonella* sp it showed inhibition at 10µg level.

The drug is therefore, effective in treating the diseases caused by these above mentioned pathogens in livestock and poultry. The MIC level noted here, may be taken as a guidance in determining the dosage level for treatment with the drug.

TABLE I : Showing Comparative Antibiogram of Known Cultures of Bacteria with *P.multocida* P52, *P.haemolytica*, *E.coli* and *Salmonella* sp.

Antibiotics	Concentration *(µg)	Zones of Inhibition (mm)			
		<i>P. multocida</i> P52	<i>P. haemolytica</i>	<i>E. coli</i>	<i>Salmonella</i> sp.
Tetracyclin	30	Nil	11	17	18
Gentamycin	10	22	15	16	15
Ampicillin	10	17	Nil	19	16
Streptomycin	10	10	12	16	18
Chloramphenicol	30	20	20	19	19
Norfloracin	10	24	24	19	19
Terramycin	10	Nil	10	12	16
Sulphadiazine	300	Nil	Nil	15	14
Furazolidone	50	Nil	12	12	10
Enrofloxacin	10	24	25	19	19

* As per the standard Antibiotic sensitivity disc supplied by Hi-media Laboratory Ltd., Mumbai

TABLE II : Showing Inhibition Zones of Four Cultures of Bacteria at different concentration of Enrofloxacin (Floxin)

Concentration of Enrofloxacin (µg)	Zones of inhibition (mm)			
	<i>Pmultocida</i> P52	<i>Phaemolytica</i>	<i>E.coli</i>	<i>Salmonella</i> sp
1	10	10	10	10
2	11	10	11	10
3	11	10	11	10
4	12	10	11	10
5	15	18	16	10
10	24	25	19	19
25	25	27	20	19
50	27	27	20	20
100	28	30	22	21

A note on Animal Cloning

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* Hoechst Roussel Vet Pvt. Ltd., Neeta Park, Yerawada, Pune

In the land mark paper published in Nature (March 1997), Dr. Ian Wilmut and his colleagues from Roslin Institute Edinburg (Scotland U.K.), reported successful cloning of an mammal from a somatic cell. They have created from a cell of an adult ewe's mammary gland, a lamb "DOLLY" which is a genetic carbon copy or a clone of the donor of the somatic cell.

'Cloning', which is a procedure of producing animals of identical genetic make up, is the latest and most revolutionary development in the field of animal genetic and reproduction. In nature, cloning occurs in few cases where monozygous twins are born due to natural splitting of embryo at very early stage of development. Induced monozygous twinning was first achieved in 1952 by scientists in Pennsylvania (USA) who raised a live frog from an embryonic cell.

Presently, embryo twinning, embryo splitting to produce cloned twins, triplets is commonly practiced in cattle, sheep, rabbits and pigs and has been achieved in rhesus monkeys also.

Dr. Alan Trounson and coworkers of Monash University Clayton Australia, have mass produced cattle embryo clones (New Scientist Dt. 13.3.97 and Times of India 14.3.97) The technique involved, growing the embryo invitro to blastocyst stage and separating the cells. Each of these cells were then fused with ova from which nucleus had been removed, thereby creating new embryos. The process was repeated on new embryos, again and again, to create a whole line of cloned embryos, a total of 470 till

date.

Although none of these cloned embryos have been successfully implanted in surrogate animals so far, this research is considered a great step towards mass production of identical farm animals.

Thus all successful efforts in cloning till date have been from embryonic cells and in some cases using 'Nucleus transfer technique' as well. What is therefore new in Dr. Wilmut's work is - as per Dr Neal First of Wisconsin USA-" not cloning mammals but it's cloning from cells that are not embryonic". In 1980, Dr Robert Mc Kinnel and his team of University of Minnesota USA had come very close to this achievement, but it was in amphibians. They raised frogs up to the tadpole stage from the red blood cells of an adult frog and inexplicably, the clones did not survive beyond tadpole stage in life.

Dr Wilmut's work can be understood in following steps :-

- a. A fully differentiated somatic cell isolated from udder of a pregnant ewe was transformed in to an embryonic cell capable of growing in to an adult animal. This is a major breakthrough for the science. This was achieved by Dr. Wilmut by maintaining the somatic cells for one week in a medium with very low concentration of nutrients. At this stage the cells stopped multiplication and fell in to a stage resembling deep hibernation.
- b. Nucleus of an unfertilized ovum (Oocyte) of a sheep was removed,

leaving cytoplasm intact, using a very thin pipette.

- c. The quiescent donor cell (Step a) was placed in contact with empty oocyte (step b) and gentle pulses of electricity applied which resulted in the fusion of donor cell with empty oocyte. Further pulses triggered bio-chemical activity 'jump starting' the process of cell division.
- d. About six days later the fused cell (Embryo clone) was implanted in the uterus of a surrogate ewe which after full gestation gave birth to "DOLLY" which is genetically identical to the donor of the somatic cell.

Dolly has been described variously as 'Carbon copy' 'Identical twin of it's own mother' 'parthenogenetically produced mammal' and so on.

This project was in progress for more than 10 years and out of 277 tries, only 29

embryos could be produced which survived more than six days and could be implanted. Among these, except Dolly the rest died at some stage.

Possible applications of cloning are far reaching. Viz.

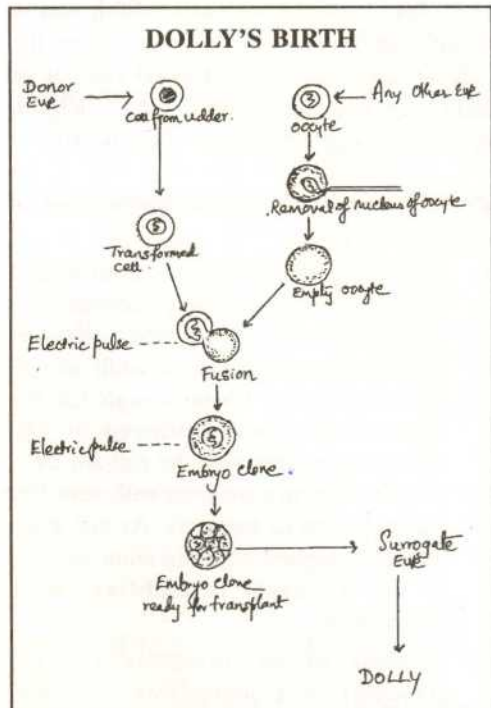
- i. Cloning champion animals
- ii. Cloning high yielding cattle, sheep, pigs poultry etc and increasing their population in shorter time
- iii. Propagating flocks / herds of transgenic animals of value in organ transplantation surgery and medicine in humans.
- iv. Protecting and propagating endangered species of animals.
- v. Better understanding of the mechanism of gene switch off and switch on.

M/S P.P.L. Theurapeutics Edinburgh, who collaborated with Dr. Wilmut, have decided to continue the research projects on cloning in two specific areas :

- a. To raise a number of transgenic pigs whose organs are suitable for transplantation in to human beings
- b. To raise flocks of transgenic sheep which are genetically engineered to produce in milk certain proteins which from potential treatment for cystic fibrosis in humans.

Source material :

- Nature (March 1997)
 New Scientist (March 1997)
 Times of India (14.3.97)
 Time vol. 149 (10) (10.3.97)



Transmissible Spongiform Encephalopathies In Animals (TSE's)

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Abstract :

Available published literature on TSE's of animals is reviewed with particular emphasis on features such as geographical distribution, clinical and histological hallmarks, methods of confirmative diagnosis and concepts of infectivity of the causal prions. There is, as yet, no convincing proof of transfer across species of the different mammalian prion diseases, nor there is striking evidence that the recently emerged atypical or variant form of CJD has a food-borne or other form of link with BSE.

Introduction :

In the recent UK media, there was a spate of sensational reports on Bovine Spongiform Encephalopathy (BSE) in their dairy and beef herds, and some of these focussed on a putative link between the causal agent and the atypical or variant form of CJD occurring in relatively younger age groups of humans. This allegation, as yet unproven, has served to rekindle scientific interest in TSE's in general. TSE's are prion diseases, which constitute a distinct group of neurodegenerative disorders, and are characterized by an unusually long incubation period (ICP), variable clinical course associated with tell-tale clinical signs and a classical pattern of neurohistology restricted to the central nervous system (CNS). The casual agents, designated as prions, are not yet fully characterized. Available data suggest that they are mostly composed of an abnormal or isoform of a

cellular membrane protein designated as prion protein (PrP^c). Specific diagnostic tests are available to demonstrate the prion isoforms in infected brains.

Animal Prion Diseases :

At least five disease entities have been documented. These are scrapie of sheep, goats and moufflon, Chronic Wasting Disease (CWD) of mule deer and Rocky Mountain Elk, Transmissible Mink Encephalopathy (TME), Bovine Spongiform Encephalopathy (BSE) and Transmissible Feline Encephalopathy (TFE). In addition, isolated cases of a BSE-like syndrome have also occurred in captive bovids and felines in the UK and Australia and in ostriches in Germany (Wyatt, 1991; Bradley and Mathew, 1992).

1. SCRAPIE :

It is the oldest of prion diseases, the earliest record of its occurrence in Britain being in 1732 (McGowan, 1992). Between 1878 and 1900, atleast 17 countries had frequently reported the disease. Australia, which had recognized it earlier reported freedom from it subsequently. On the other hand, Japan which was free for several decades was invaded in 1990.

Scrapie is the most intensively studied prion disease and several aspects of scrapie research have shed considerable light on multiple parameters such as the

characteristic of the agent, its unique interaction with host genes (Sip and sine in sheep and mice), concepts of infectivity and pathogenesis, histological hallmarks so valuable in diagnosis, transmissibility to diverse mammalian hosts and the delineation of the fine structure of the so-called Scrapie - Associated Fibrils (SAF's) in detergent - extracts of infected tissues. Several strains of scrapie prions with distinct characteristics as incubation period and specific lesion profile have been identified. Moreover, scrapie has served as the prototype for the study of maternal, horizontal and iatrogenic transmissions.

2. BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

BSE was first clinically suspected in April 1985 in a British dairy herd. It was identified and accurately defined as a prion disease in November 1986 (Wells *et al.* 1987). It occurred as a typical adult syndrome with a peak incidence in those aged 4-5 years. The course was progressive over several weeks and the outcome fatal. The source of BSE has been traced to the incorporation in cattle feeds of scrapie prion - contaminated meat and bone meal (MBM) that was inadequately rendered. It was postulated that initially the scrapie agent in contaminated feed might have infected some cattle and undergone mutation and that subsequently, the mutant prion was maintained by successive passages in an epidemic form through the extensive use of infected cattle offals. It has been estimated that 45 per cent of all rendered material in U.K. was bovine-derived. There was a remarkable exponential increase in the incidence of BSE in

National herds from the middle of 1989 to end of 1992. The ban on feeding ruminant - derived protein imposed in July 1988 resulted in progressive decline in its incidence in 1994. there was also a concomitant reduction in incidences in 2-year and 3-year olds in 1991 and 1992 respectively and a much lowered incidence in 4- and 5-year olds in 1994. By October 1994, only 450 cases per week were reported. This amounted to a 61% reduction of the number reported in 1991. In 1996, the number plummeted to 300.

BSE was also recognized in N. Ireland (123 cases), Switzerland (200), Portugal (31), Germany (4) and Italy and Oman (2 each) and Canada, Denmark and Falkland Isles (one each). All these countries except Switzerland and France had imported British cattle.

It was only after the emergence of BSE that a similar spongiform encephalopathy was recognized in 10 captive bovids and felines in eight zoological gardens in the U.K. The bovid species were Nyala, Gemshot, Arabian Ornyx, Eland and Greater Kudu. All cases except those in Greater Kudu were regarded as food-borne, the alleged source being contaminated ruminant-derived protein. In the Greater Kudu, there was convincing evidence of maternal transmission.

Feline cases occurred from 1990 onwards, and by end of 1995, 69 cases were confirmed. The felines were Puma (*Felis concolor*), Ocelot (*F. pardatus*) and Cheetah (*Actinomyx jubatus*). Feline Spongiform Encephalopathy was also reported in 1984 in N. Ireland and Guernsey.

3. CHRONIC WASTING DISEASE (CWD) OF MULE DEER

The disease in mule deer (*Odocoileus herminus hermimous*) was first recognized in fort Collins, Colorado, U.S.A. in 1967 and ten years later in Wyoming (Williams and Young, 1992). Up to 1981, over 100 cases were reported. A few cases were also recognized in Black-tailed deer (*Odocoileus hermionus columbirnus*). The mean age of involvement was 3-4 years, the youngest case was 18 months old and the oldest, 9 years. Cases were also frequently reported in the same localities in Rocky Mountain ELK (*Cervus elapus nelsoni*). There was evidence of unlimited free movement of mule deer and ELK herds between the two facilities. However, no clear proof existed that CWD in these species had initially originated from scrapie of sheep reported 2 decades earlier.

4. TRANSMISSIBLE MINK ENCEPHALOPATHY (TME)

It was first reported in Wisconsin in farmed mink (*Mustela vision*) in 1947 (Kimberlein, 1992). Untreated abattoir waste, chiefly ovine-derived, was incriminated as the most likely source. However, there is as yet, no experimental proof for this assumption. For instance, mink are not orally susceptible to scrapie prion. It is possible that some strains of scrapie got adapted to mink.

The disease re-emerged in Wisconsin in 1961 and 1985 with morbidity rates of 10 to 30 per cent and mortalities of 100 per cent. In the period, 1963-1965 sporadic cases were reported in Ontario, Finland, E.Germany, and Russia with a

strong circumstantial evidence of food-borne transmission.

In experimental infection studies in mink, scrapie strains behaved rather erratically. Of 21 strains tested (14 mouse adapted and 1 and 6 strains from naturally infected goat and sheep respectively) in 65 mink, only one developed a TME - like syndrome after an incubation period of 22 months. In contrast, 2 scrapie strains maintained in sheep induced TME in intracerebrally but not orally inoculated mink after 11-12 months.

Mink encephalopathy agent, however, induces clinical disease in mink after I/c, I/m and oral inoculation of infected brain suspensions. It also infects ferret, skunk, racoon, hamster, rhesus, macaque and squirrel, monkeys, sheep, goats and cattle but not mice.

Clinical Signs :

The commonly observed signs are behavioural changes, locomotor ataxia, a crouching, hypermetric gait, hyperexcitability and hyperaesthesia to sound and touch. Other less common symptoms are polyuria and polydipsia. Behavioural changes consisted of timidity alternating with bouts of aggressiveness and grinding of teeth.

Species-specific changes are skin pruritus in scrapie and TME, tremors, shivering, going-in-circles, curling of tails and impaired vision or total blindness in TME. Self-mutilation inflicted through bites is an additional feature in the mink disease. Hypersalivation is marked in BSE and FSE.

Histology :

The triple hallmarks are spongiosis, astrogliosis and neuronal loss. Vacuolation is

a dominant feature in grey matter neuropil and in the perikarya and neurites of neurons. Vacuolation is a specific change in spongiosis. Multiple vacuoles are common. The distribution of lesions varies in the different encephalopathies but medulla, midbrain and cerebral cortex are frequently involved.

Infectivity Concepts :

Three theories have been advanced to explain infectivity and the mechanism of replication of the causal agent. None has received universal acceptance.

Prion Theory : Prusiner (1989) who advocated this theory was of the view that the prion isoform designated as PrP^{sc} is infectious and that it replicates by an unknown mechanism. In infected tissues the isoform is generated from the normal cellular prion protein (PrP^c) as a post-translational event. Limited proteolysis converts PrP^{sc} into a smaller proteaseresistant moiety of an apparent M.Wt of 27-30 KD_a. However, this model has failed to explain the emergence of several distinct genotypes of TSE agents which have been distinguished on the basis of differences in ICP and lesion profile.

Virino Theory : This is based on the unproven assumption that a small nucleic acid exists in the core of PrP^{sc} molecule and that is acquired during the morphogenesis of PrP^{sc} in the target cell. This segment of genome is thought to confer on the prion its strain characteristics. Infectivity is assumed to be the result of interaction between the host-coded PrP^c and this putative genome. An analogy exists between virinos and plant viroids. The latter do not encode proteins but are infectious. The main opposition to the virino theory lies in the fact that to date no nucleic acid has been demonstrated in purified PrP^{sc} fractions. However, recently,

Narang (1993) isolated a single band of ssDNA of about 1.2 kb from nuclear contents of mitochondria or tubulofilamentous particles in infected hamster brains. The ssDNA was synthesized into a double stranded DNA, cloned and sequenced. The author advanced the hypothesis that prion infectivity is associated with an abnormal D-loop fragment in the mitochondrial genome. In concert with the mitochondrial genomic fragment a protein compound such as PrP^{sc} is thought to be converted into an "informational hybrid" comprising an infectious, host-coded protein component in tune with the virino hypothesis on one hand and it also accommodates the recently postulated "Unified theory" as well.

Unified Theory : The proponents of the 'unified therapy' concede that as yet, undetected nucleic acid core exists in PrP^{sc} molecule as in conventional viruses and it is protected by the prion protein shell. The former has been referred to as 'Coprion' which confers the replication advantage on the protein shell designated "apoprion" (Weissmann, 1991)

Transmissibility :

Scrapie prion is the model of varied transmission potential. Transmission occurs *in utero* and there are high levels of infectivity in the placenta and genital secretions at parturition. This facilitates horizontal spread from the dam to offspring. Iatrogenic transmission through the use of formalinised louping ill vaccine raised sheep brains was reported earlier (Gordon, 1946). Vertical and lateral transmissions are regarded as a theoretical possibility in BSE and TME. Maternal transmission has possibly occurred in the BSE-like syndrome identified in Greater kudu in the London Zoological Gardens.

Experimentally, scrapie and BSE have been transmitted to a variety of mammalian species by oral and I/c routes. Similarly, CWD agent has been known to be transmissible to a wide range of mammals but not to mice and hamsters. The transmission potential of TME has been already referred to.

Diagnosis :

Apart from suspicious clinical signs and distinctive lesion profiles, the detection of prion protein (PrP^{sc}) fibril aggregates in extracts of infected brains is another procedure useful in confirmative diagnosis. The aggregated fibrils of PrP^{sc} referred to as Scrapie - Associated Fibrils (SAF's) are immuno-reactive to PrP^{sc} - specific antiserum. There is no immunoreaction of this serum with the normal cellular prion protein (PrP^c).

Yet another useful procedure is the demonstration of PrP^{sc} in detergent-treated extracts are first purified by differential centrifugation and proteinase K-treatment. Polyclonal antiserum against sheep or mouse PrP^{sc} raised in the rabbit is the antibody source.

Zoonotic Considerations :

TSE's of animals are known to be host-specific. There are no documented reports of proven natural infections across the species barrier. A link between scrapie and CJD has never been clearly established. Sporadic CJD has occurred in Japan and Australia without any evidence of scrapie. Also there are reports of CJD in U.K. and India in strict vegetarians. The postulated link between BSE and CJD through ingestion of infected beef is, therefore, yet to be convincingly proven.

It is possible that animal spongiform

encephalopathies had a common evolutionary source in scrapie. Human spongiform encephalopathies such as CJD and Kudu may have evolved independently of scrapie. The unravelling of the genomic component in PrP isoform molecules might lead to studies on phylogenetic relationships between the different prions.

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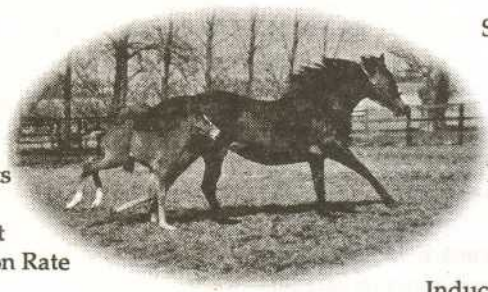
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***Cryptosporidium* And Cryptosporidiosis : A Review**

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Introduction :

Untill comparatively recently, *Cryptosporidium* was considered to be a harmless and a rare coccidium parasitising the epithelial cells of the digestive and respiratory tracts of some of the vertebrate animals. This view is no longer tenable. It is now recognised that *Cryptosporidium*, a form being harmless, produces disease in animals and man alike. Very often the latter acquires the infection from the former, hence making it a zoonosis. This realization naturally led to global research efforts on this organism (Tzipori, 1988; Dubey *et al*, 1991). In contrast, we, in India, are hardly aware of the organism and its role in inducing disease in man and animals.

Cryptosporidiosis in domestic animals :

Veterinary interest in *Cryptosporidium* started in 1971 when it was incriminated in a case of severe diarrhoea in a young calf. This report was soon followed by numerous other reports from a variety of domestic animals from all over the world. These included laboratory animals like mice, rats, rabbits and guinea pigs and domestic animals like cat, dog, pig, equines, sheep, cattle and buffalo. In addition, it has also been reported from a variety of birds including fowl, turkey, duck, domestic goose, quail and pheasant (for details see Fayer and Ungar, 1986; Tzipore, 1988; and Dubey *et al*, 1991).

Cryptosporidium harbours only the digestive tract (and not the respiratory organs) in mammals. It is the lower small intestine which is most severely affected in very young

animals especially in neonates (in calves 1-15 days old) resulting in severe diarrhoea which may occasionally end in death. It has been suggested (Current, 1985; snf Tzipori, 1988) that the malabsorption caused by villus atrophy resulting from accelerated loss of epithelium was the basis of *Cryptosporidium* induced diarrhoea. According to Tzipori (1988) *Cryptosporidiosis* has a marked effect on the membrane bound digestive enzymes and the diarrhoea results from brush border maldigestion and malabsorption. Similar findings have also been reported from germfree calves and pigs which also confirmed that *Cryptosporidium* is an enteric pathogen in its own right and does not require concomitant infection of a virus or a bacterium or a fungus as believed by some workers. Current (1985) recorded that as a rule a large number of calves get infected in a herd but only a few suffer from diarrhoea. He, therefore, argued that the isolates of *Cryptosporidium* may not be uniformly pathogenic but, vary in their virulence. Similar differences have also been pointed out by Tzipori (1988).

Unlike mammals, *Cryptosporidium* arborws both intestinal and respiratory tracts in poultry. However, the intestinal *Cryptosporidium* has not been associated with noticeable disease, but several outbreaks of respiratory cryptosporidiosis had been associated with high morbidity and mortality in broiler chickens and turkeys. For these, both (*C. meleagridis*) and (*C. baileyi*) have been incriminated, the latter being the more virulent of the two (Lindsay and Blangburn, 1991).

Respiratory cryptosporidiosis leads to hypertrophy, hyperplasia and deciliation of the epithelium resulting in its loss. The clinical signs associated with respiratory cryptosporidiosis are depression, difficulty in breathing, gurgling respiration, coughing, sneezing and oculonasal discharges. These symptoms are more pronounced in young birds than older ones (Fayer and Ungar, 1986; Tzipore, 1988; and Lindsay and Blangburn, 1991).

Cryptosporidiosis in man :

Tzipori (1988) and Ungar (1991) have provided in the detailed histological account of *Cryptosporidium* infection in man. According to them, it was first reported from two human cases in 1976. However, the medical importance of *Cryptosporidium* suddenly enhanced in early 1980's when it was associated with severe diarrhoea in 21 patients suffering from AIDs. The first case of cryptosporidiosis in immunocompetent adult was reported in 1980 who had severe watery, but self-limiting, diarrhoea and vomiting. Around this time, it was also established that human cryptosporidiosis is a zoonosis as human isolates could easily be transmitted to mice and lambs. Zoonosis nature of human cryptosporidiosis was reconfirmed by Rees *et al.* (1982) who provided the evidence of human infection from infected calves.

Human infection with *Cryptosporidium* has been reported from 18 countries including India. It will suffice to mention below only the salient features of this infection in man:

- 1) It occurs both in developed and developing countries alike where it is equally prevalent in urban and rural areas.

- 2) The age range for *Cryptosporidium* infection in humans is from 3 day old child to 95 years old man. However, the children especially in the age range of 2 years and above, are most susceptible and have higher prevalence.
- 3) In addition to the geographical variability, *Cryptosporidium* infection also shows seasonal differences with prevalence being higher in warmer and more humid months.

It is necessary to point out here that the clinical manifestations of cryptosporidiosis in humans differ in their intensity and duration in immunocompetent and in immunodeficient persons. In the former, the disease is a short term, often cholera like, diarrhoeal illness which is self-limiting.

In the latter, especially in patients suffering from AIDS, the diarrhoea due to *Cryptosporidium* progressively becomes worse and may be a major contributory factor in the death of patients. Along with gastrointestinal tract, a few reports indicate respiratory involvement also (Tzipori, 1988, and Ungar, 1991).

Status of *Cryptosporidium* in Indian Subcontinent :

Dubey and Pande (1963) were first to report the occurrence of what they claimed as "Cryptosporidial / coccidial bodies " from an Indian jungle cat (*Felis chaus*). Their description leaves us in no doubt that they had mistaken the sporocysts of *Sarcocystis* for *Cryptosporidium*. Likewise Pande *et al* (1972) reported a new genus and a new species of Cryptosporidid which they named *Hoareosporidium pellerdil* from the small intestine of a dog. Once again the description of the parasite, its line drawings and above

all its location in the lamina propria of the small intestine of the dog, makes it, without doubt, the sporocyst of *Sarcocystis*. These mistakes should not be viewed harshly because at that point of time the knowledge regarding the coccidial nature of the life-cycle of *Sarcocystis* was not known. Similar mistakes had also been made by other workers elsewhere (Fayer and Ungar, 1986).

The first authentic report of cryptosporidiosis from Indian subcontinent came from Bangladesh. Rehman *et al* (1984) reported 14% of 208 (1 to 28 days old) bovine calves with diarrhoea examined by them were positive for *Cryptosporidium* while only 1% of 202 calves of the same age but without diarrhoea were positive. The most of the positive cases were detected during warm and humid season between August and September. This study also revealed the zoonotic nature of *Cryptosporidium*. It showed that all the 88 animal attendants had diarrhoea during August and September and 7 of them had cryptosporidian oocysts in their faeces and additional 7 positive cases were found among the 77 family members with diarrhoea. These positive cases were associated with two *Cryptosporidium* positive attendants.

In yet another paper from Bangladesh Shahid *et al* (1985) reported 4.3% of the 578 human stool samples collected between January to May 1984 positive for *Cryptosporidium*. 18 of 25 patients with cryptosporidiosis were children below 2 years old. The author concluded that *Cryptosporidium* was the sole pathogen to cause mild, transient and easily managed form of diarrhoea particularly among children.

In the same year, Mathan *et al* (1985) made a comprehensive study on cryptosporidiosis in southern India. they examined stool samples from 2 groups of children at Vellore.

Group-I had 582 children, aged 3 years or less (mostly 5 months or younger), with acute diarrhoea and group -II had 418 children, age matched with group-I, but without any diarrhoea. *Cryptosporidium* oocysts were present in 13.1% of the patients of group-I and 9.8% of the group-II. *Cryptosporidium* was detected during all seasons of the year, but was more frequent in rainy months and low in cooler months. A comparison between breast-fed children and bottle-fed children showed no difference in prevailing in rural south India where animals were kept adjacent to or inside houses. Thus babies and their mothers were regularly exposed to high infection doses of oocysts.

Cryptosporidium has also been reported subsequently from children from other parts of the country viz. Bangalore, Bombay, Chandigarh, Calcutta, Madurai, Wardha and a few other places (Saraswathi *et al*.1988; Singh *et al*. 1988; Prasad *et al* 1990; Parija 1990; Das *et al*. 1993; and Ganguli, 1993). These studies were usually based on the microscopic demonstration of oocysts of *Cryptosporidium* in the faeces.

Prasad *et al*. (1989), perhaps for the first time, reported the presence of *Cryptosporidium* in domestic animals in India when they showed that 15.75% of 112 bovine calves (28 days old) had this infection accompanied by diarrhoea. Slightly later, Dubey *et al*. (1992) not only found cryptosporidian oocysts in the faeces of 8 of the 45 bovine calves but also in 10 of the 60 buffalo calves examined in the same area (Bareilly, U.P.) However, the occurrence of *Cryptosporidium* in birds has not been reported from India so far.

Treatment :

A distinction must be made between immunocompetent and immunocompromised

patients vis-a-vis their treatment for cryptosporidiosis. The disease in the former is self-limiting, hence may require no therapeutic intervention apart from oral or intravenous hydration and parenteral nutrition. In the latter, on the other hand, the disease is severe and often life threatening and, therefore, very much in need of treatment. Nearly 95 drugs had been tested for their efficacy in humans and animals but with indifferent results (Fayer *et al.* 1991.) This includes spiramycin which is claimed to be beneficial (Tzipori, 1988).

A large number of anticoccidial drugs have also been tried against cryptosporidiosis in animals again with equivocal results. However, Arprinocid, Lasalocid, Cylosporin A, Alborixin and Maduramycin have been shown to hold promise (Fayer *et al.*, 1991).

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Case Report :

Successful Bovine Surgery for Benign Tumour of weighing of 36.7Kgs.

K. M. Desai, P. R. Pandey, J. M. Desai, P. K. Kanzaria,
Surat District Co-op Milk Producers Union Ltd. (SUMUL), Surat

Introduction

We were called for surgery for chronic case with a huge swelling at the brisket region between fore-legs on 18.10.1995.

A mass of benign tumour was hanging at the same site which was painful to the animal, and this swelling touched to land which caused wound (i.e. maggot wound). The Animal could not walk much, more-over her milk production had dropped down from 15 lits. to 3 lits. per day.

Materials & Methods :

We took the decision that the mass had to be removed from the body because of maggot wound at the lower region of the mass and painful condition to the animal.

The brisket region was shaved properly and 2ml Xylazin injection given intramuscularly followed by local infiltration anesthesia.

An elliptical incision was given on healthy skin and after ligating the major blood vessels, the growth of benign tumour was detached which was having a weight of 36.7 Kgs. This operation was completed after 6 hrs. of time.

A continuous suture was applied after the removal of the complete mass and Tr. idine was applied properly followed by Tetracycline powder (Hoechst) and the Himax Ointment along with the suture line. The treatment was continued as follows :

Inj. Lactaid plus 900 ml. i/v.

Inj. Betenesol 8 ml. i/v.

Inj. Terramycin LA 70 ml., i/m. and then

Inj. Avil 10 ml. (i/m.) plus

Inj. Diclofenac 20 ml. (i/m.)

Daily for 4 days

The antibiotics-level was monitored for 3 weeks with giving Inj. Terramycin-LA.



before operation



after operation

Dressing has been carefully done with Tr. Iodine and Himax ointment for 4 weeks.

Results & Discussions :

The operated site healed nicely and the animal came back to her normal movement and there was no pain and stress,

Then the animal was given Receptal 5 ml. through i/m route which helped the animal to come into heat as followed by AI resulted pregnancy. The animal was delivered on 18.10.1996. (Exactly just after 1 year of operation) and now a days the same animal gives 16 lits. milk/day.

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Case Report :

Successful post-operative treatment with Floxidin 10% injection in teat surgery.

P. B. Patel and R. M. Patel

Livestock Research Station, Gujarat Agricultural University, Sardar Krushinagar.

A kankrej cow, furious in nature, recently parturated was brought to hospital with deep lacerations on right hind teat due to sharp mechanical injury by farm equipment. Milk mixed with blood was coming out from deep irregular lacerations.

The animal was restrained in lateral recumbancy by deep sedation using xylazin 2ml i/m along with ring block by 2% Lignocuin hydrochloride.

The teat mucosa was sutured with continuous suture using absorabable suture material. The submucosa, musciosa and skin were sutured by non absorbable suture. A polythelene intramammary tube was fixed in to the teat canal.

Post operative treatment was carried out with inj. Floxidin 10%, 20 ml i/m for 5 days. 5 ml Floxidin 10% Inj. was infused intramammary after milking in morning & evening for 5 days. Local dressing was also carried out daily. On 3rd post-operative day the polythelene tube was removed and on the 7th post-operative day the skin sutures were removed.

There was uneventful recovery with normal milk. No abnormal changes in milk quality were seen during treatment and after treatment. The animal regained normal production within 2 weeks.

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Animal	Body weight kg	Dose
Sheeps & Goats Dogs	30	6 ml
	10	20 ml for 3 consecutive days

Panacur Suspension 10%

Animal	Body weight kg	Dose
Cattle Horses	400	20 ml
	400	30 ml

Presentation

Panacur Suspension 2.5% : 60 ml. 450 ml & 1 litre Plastic Bottles

Panacur Suspension 10% : 450 ml Plastic Bottles

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Case Report :

Perineal Hernia in a Donkey

M. T. Nassef

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Assiut University, Assiut, Egypt

Perineal hernia is most common in aged male dogs, (Silbersiepe *et al.*, 1958; Bolz, 1970; and Christoph, 1975). In the equine species, this type of hernia is not recorded.. This report describes a case of perineal hernia in a she donkey.

History of the case :

A she donkey was admitted with a large swelling of the size of a child's head located between the base of the tail and tuber ischii (Fig.1). The anus and vulva were deviated laterally by the pressure of the swelling which was reducible.

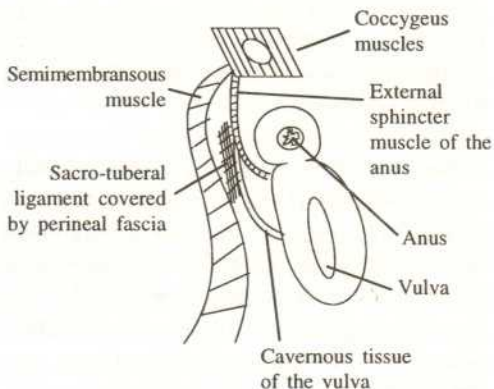
Infrequent difficult urination was accompanied with straining and the urine was voided in jets. The reducibility of the swelling in this area led to the diagnosis of perineal hernia and the content was suspected to be the urinary bladder bent on itself. Operation was the only choice to solve this problem.

Pre-operative preparation :

The animal was sedated with intramuscular injection of Combelen (Bayer®) 1% in a dose of 1 ml per 100 kg. of body weight and intravenous injection of 10% chloral hydrate deep narcosis in a dose of 12 gm per 100 kg. of body weight. The animal was casted and the operation was performed on lateral recumbency. The area for operation was prepared for aseptic surgery.

Operative techniques :

a. Digramatic surgical anatomy :



b. Operation :

A skin incision of about 15 cm long was carefully done over the swelling and parallel to the vulva on its left side. Dissection of the underlying structures (Recto-vaginal Cul-d-sac) revealed the presence of the urinary bladder filled with urine. The bladder was replaced in its normal position and the urine was evacuated by a catheter. It was noticed that there was no rupture of the muscles in this area but the perineal fascia was ruptured and the sacro-tuberal ligament was deviated forming a pouch like recess. This pouch was formed between the deviated ligament and the surrounding structures.

Suturing using No. 2 chromic catgut was done including sacro-tuberal ligament, perineal fascia and external sphincter muscle of the anus in the pattern of

simple continuous suture. The subcutaneous tissue was sutured with No. 0 chromic catgut in the form of simple continuous pattern, while the skin was sutured with No. 2 silk thread in the form of interlocked stitches. The skin incision was smeared with collodion to avoid wound infection.

Post-operative care :

A prophylactic dose of antitetanus serum (3000 IU) was subcutaneously injected. Penicillin (1,20,000 IU) and 2 gm of streptomycin were intramuscularly injected for five successive days.

Follow up of the case was continued daily for 10 days until the removal of the skin stitches. The animal became normal with regular urination, (Fig. 2). The periodical observation of the animal indicated that she became pregnant 6 months post-operation and the delivery occurred without any complications.

Comment :

Although perineal hernia is common in dogs and cats and is rarely recorded in equines and bovines, yet in the present study it was recorded in a she donkey. The etiology of perineal hernia in dogs and cats is referred to as either weakness of the sacro-coccygeal muscles and the muscle *levator ani* in male dogs than in females. In addition, perineal hernia is caused by prolapse of the pelvic fat between the muscle *levator ani* and coccygeal muscles.

This case of perineal hernia may be caused by either direct trauma with a blunt object like wedge in the ground, severe kick from another animal or a horn gore from horned animal, which lead to rupture of the deep tissues except the skin.



Fig.1 : Showing a large swelling between the base of the tail and tuber ischii.



Fig. 2 : Showing the animal one month after operation.

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Case Report :

Treatment of Poultry Helminths with Fenbendazole (Panacur)

C.S. Hayat, N. Badar, A. Magbool and B. Hayat

Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan

In Pakistan, Helminth infestations namely *Ascaridia galli*, *Railletina spp.*, *Heterakis gallinarum* and *Syngamus trachea* are causing tremendous economic losses to the poultry industry due to hot humid climate, poor hygienic and managerial conditions (Hassan, 1966).

Fenbendazole (Panacur) is a broad spectrum anthelmintic which possesses high activity against adult and immature stages of wide range of helminth parasites of domestic, wild and zoo animals. Since only a few reports are available on its use against poultry helminths (Lawrence, 1983, Kirsch, 1984 and Yazwinski *et al.*, 1986.)

The study was conducted in two parts. Each treatment group in both experiments was kept separately in clean dry litter of rice straw and in new isolation units. Food and water were available *ad lib* at all time and care was taken to avoid adventitious infection once treatment has commenced.

In the first experiment Tetramisole was used to treat 80 chickens aged between 5-6 months. This flock known to be naturally infected with *A. galli*, *H. gallinae* and *Railletina spp.* The 75 percent of these birds were unthrifty and weak. Before commencement of treatment 20 birds died and their postmortem examination showed large number of *A. galli*, *H. gallinae* and *Railletina spp.* in the gut. Examination of droppings by concentration / floatation method (Sloss, 1976) showed an egg count of 400,000 - 1000, 000 ascarid eggs per

gram of faeces. E.P.G. of droppings was determined by Mc-master egg counting technique (Coles, 1967).

These 80 birds were divided randomly into four groups. The first group was treated with 30 mg/kg Fenbendazole, the second with 40 mg/kg and the third with 50 mg/kg orally mixed in feed for 3 days/. The fourth group acted as controls. The droppings were examined twice weekly for 2 weeks after administration of the drug post-mortom examination was carried out on 20 birds, 5 from each after the last treatment.

In a second experiment, 30 broiler birds aged 5-6 weeks naturally infected with *Syngamus trachea* showing characteristic "Grapes" and worms in their trachea, were randomly divided into two groups i.e. A and B. Birds in group A, were treated with Fenbendazole at the rate of 50 mg / kg body weight for 3 days while the birds in group B acted as untreated control. All the birds were slaughtered on the 15th day after the last treatment and their trachias were examined for the worms.

In the first treatment, the birds tolerated the drug well and no side effects were observed. The faecal egg counts dropped to less than 400 egg in birds receiving 30 mg / kg and to less than 50 egg in those receiving 40 mg/kg by the 3rd day of treatment. At post-mortem examination none of the birds which had received 40 mg or 50 mg/kg contained any worms. While only 2 of those which had received 30 mg/kg still had a few *Railletina*

tape worms. All birds in the untreated control group had many worms in their intestines with mixed infection in most cases.

The treated birds in the second experiment also showed a dramatic recovery by 7 day after the last treatment. At post-mortem examination the treated birds had cleared all the worms from their tracheas except for one bird in which a few remained. Birds in the untreated control group still had many worms in their tracheas and 3 birds died after the beginning of the trials from asphyxiation due to the presence of large number of tracheal worms.

In the present study Fenbendazole (Panacur) seems to be very effective anthelmintic with a wide spectrum of activity. The results of the present study are closely related to many other workers. Kirsch (1984), Lawrence (1985), Yazwinski et al., (1986) and

Kovalenko (1988) reported that 50 mg/kg cure 90 percent.

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Case Report :

Polioencephalacia in a Kid

R. K. Tanwar

Department of Veterinary Medicine

College of Veterinary and Animal Science, Bikaner, Rajasthan

A 2 months old male caprine kid on a diet of goat's milk and roughage (*Zizyphus nummularia*) was presented in a lateral recumbency. The kid was severely depressed and was unable to rise. The rectal temperature was 36°C. Auscultation of lung revealed increased vesicular sound. Pulse was weak and impalpable. there was decrease in the intensity of the heart sounds. The pupils were dilated and eyes were dry and staring. Pupillary light reflex was absent. The haemogram was normal and faeces were negative for helminth eggs. There was less than 1 coccidia/low power microscopic field. Serum calcium level was 4.2 mg/dl. Drug administration included, 5g sulphamezathine orally and 15 ml of 25% calcium borogluconate intravenously. After half an hour of treatment, the kid became alert and stood up and started eating roughage. The kid was sent home in the evening.

Next morning, the kid had tremors, convulsions and marked opisthotonus. It was blind and lacked a menace response. Pupillary responses were normal, but nystagmus and dorsomedial strabismus were noticed. Manual opening of the jaws precipitated opisthotonus and extensor rigidity. The kid was grinding its teeth and paddling in lateral recumbency. Rectal temperature was 39°C. Increased vesicular sounds were audible on auscultation of lungs.

Based on clinical symptoms, polioencephalomalacia was diagnosed and the kid was treated with 100 mg of Thiamine

hydrochloride intravenously. One hour later, the kid was up, eating and apparently normal. An additional 100 mg of Thiamine hydrochloride was administered intramuscularly for two more days, at which time the kid was still clinically normal.

Discussion :

The actual physiological cause of drop in serum calcium level is not understood but it may be due to under feeding of goat's milk. The ruminants meet its Thiamine requirements via intraruminal microbial synthesis. Thiamine deficiency may result when tissue Thiamine concentrations are inadequate because of decrease rumen synthesis, increased Thiamine destruction from Thiaminase of rumen bacteria (Smith, 1979). In this case both hypocalcemia and polioencephalomalacia occurred concurrently and recovered completely after treatment.

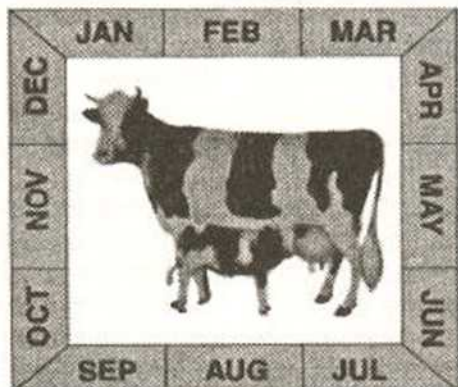
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Case Report :

Myiasis in Dogs and its Chemotherapy

C. S. Hayat, A. Maqbool, N. Badar, and B. Hayat.

Faculty of Veterinary Science, University of Agriculture, Faisalabad (Pakistan)

A total 58 dogs brought to the out door clinic of Department of clinical Medicine and surgery, University of Agriculture, Faisalabad for treatment of myiasis during 1995-96. The month season, breed, age, and sex wise prevalence was recorded. The identification of larvae was made by per key described by Soulsby, 1968.

The larvae of *Chrysomya bezziana* fly was identified to be the cause of myiasis in all the cases of dogs studied. This is in agreement with the findings of Nachiappan (1971). It was noted that most cases occurred during the month of February, March, April, May i.e. during spring and early summer season. These results are closely related to the findings of Soulsby (1968) and Thilagar *et al.* (1989). They reported that *Chrysomya bezziana* were more abundant during spring and early summer.

Among breed the most cases were of Alsatian (65.5%), followed by Doberman (15.5%), Bull Terrier (8.6%), German Shepherd (5.2%) and local breed (5.2%). It was also reported that incidence of myiasis was more in males than females (41.4%), during two years study period. This may be due to bitches biting and house soiling behavior (Campbell, 1975), which may lead to self mutilation of minor injuries and complicate the wound nature. In all the cases of myiasis, maggots or larvae were removed by applying oil of Turpentine and Chloroform and local dressing was done with Ivermectin cream. All cases were recovered within a period of 7-15 days. Ivermectin

(Ivotek) a product of Star Laboratories Limited at the rate of 0.2 mg per kg body weight was injected by subcutaneous route to 20 dogs. This product proved to be very effective against maggots. Similar results were also recorded by Campbell & Benz (1984), Campbell (1985), Spradberry *et al.* (1985), Bhattacharya & Laha (1995).

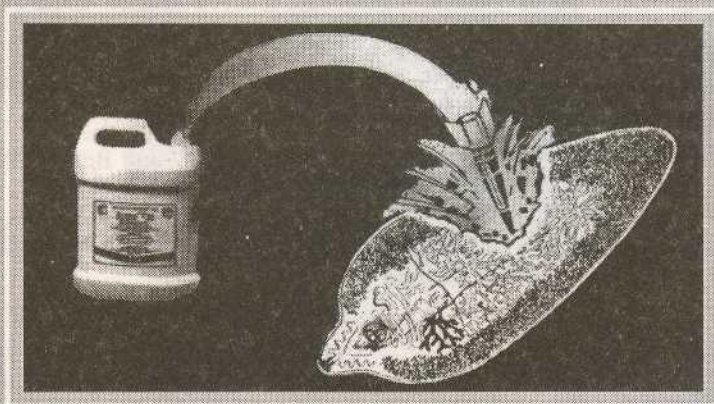
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Case Report :

Necrotic Enteritis In Poultry In Chhatisgarh Region

Neelu Gupta

Department of Veterinary Pathology, College of Veterinary Sciences & Animal Husbandry, Anjora (M.P.)

Necrotic enteritis is a relatively new disease, recognised since 1960. Most of the studies on this disease have been carried out in U.K. and Australia. In India, the disease is relatively less important but has been known to cause a few deaths in large poultry farms.

In this communication the incidence and pathology of necrotic enteritis in birds from Golden Poultry Farm, Chhatisgarh is reported.

Material for present study consisted of 260 birds out of 1500 birds in a flock. These were showing the clinical signs of general weakness, poor appetite, enteritis, diarrhoea and dehydration.

Necropsy revealed emaciation, dehydration, enteritis. Small intestine showed patches of necrosis yellowish in colour. After removing the necrotic material, bleeding surface was seen.

Histologically changes were characterised by necrotic enteritis viz hyperemia of jejunal mucosa with coagulative necrosis, infiltration of erythrocytes, lymphocytes and few macrophages in submucosa. Spleen showed hyperplasia of lymphocytic follicle depletion of lymphocytes from malpighian corpuscles. Liver showed congestion and degeneration of hepatocytes.

Technology Development :

*We are prone, especially in this fast-moving world,
to believe that every new technology displaces
the old technology, that television will replace radio,
that electronic news will displace print journalism,
that the automobile will displace the human foot,
and that television will displace the book,
but each of these new technologies has simply given
a new role to the earlier technologies.
The development of technology is not displacive
- it is cumulative.*

Cloning of Sheep

Dr.Ian Wilmut and team of scientists from Roslin Institute, Edinburgh Scotland, U.K. created from an adult somatic cell of a sheep, a cloned sheep named 'Dolly'.

- Nature, March 1997

(A separate note on this epoch making discovery is published elsewhere in this journal).

Antisense therapy to fight Cancer

Scientists at Royal Hospital, U.K. have developed a pioneering method of treatment for Non-hodgkin Lymphoma, an incurable cancer of man.

In Non-hodgkin Lymphoma, Protein BCL - ∞ which interferes with apoptosis (process of programmed cell suicide) is over produced thereby resulting in uninhibited replication of cancer cells. In this new therapy, the production of BCL - ∞ is blocked by the use of synthesized DNA strands (antisense strands) which bind with RNA carrying BCL - ∞ protein codes.

This principle could lead to development of therapy in other types of cancer in man and animals.

- Times of India (P:13) 21.04.97

New approaches in Cancer treatment

M. D. Anderson, Cancer Centre, Houston and Onyx pharmaceuticals, Richmond California reported new approach to cancer treatment which involves use of adenovirus.genitically engineered to carry gene P53. This gene stops the multiplication of those cells whose genes are damaged. Thus it stops multiplication of cancer cells. In conjunction with chemotherapy, the new treatment has shown some good effects.

- Times of India (P:14) 14.05.97

IBD in Penguins

Researchers from Australia have detected IBD antibodies in penguins at three sites 40 to 60 km from Australia's Mawson Station between Dec. '95 to Feb'96. This is the first evidence that IBD virus has reached Antarctica and raises concern for the conservation of avian Wild life in Antarctica.

- Nature; May 1997

Chaga's Disease is 4000 yrs. Old

Trypanosana cruzi causing Chagas disease has been detected in 4000 year old mummies in Southern Peru and Northern Chile Scientists from Columbia detected the parasite in skeleton and abdominal organs of mummies using genetic identification techniques.

- Lancet; May 1997

Myostatin

Different species of animals produce their own 'myostatin' which regulates the development of muscle tissue in them. Dr.Se-Jin-Lee and his colleagues at John Hopkins School of Medicine, USA have produced a strain of mice in which gene that codes myostatin has been deleted(knocked off) and such mice grew with extra ordinarily well developed Musculature. These findings could lead to development of strains of beef cattle, sheep, broiler poultry, which give better carcass yield.

- Time vol 149 (19): 45; 12.05.97

Record Milk yield

A Holstein cow in Wisconsin, USA set a milk production record averaging 174 pounds per day for 365 days with a peak day of 211 pounds. Average production of dairy cows in USA is steadily rising from 42.7 pounds to 45.2 pounds per day.

- Times of India (P.12) 23.05.97

News

Value added food products in India

A study titled "Food and Agriculture Integrated Development Action (FAIDA)" in India conducted jointly by McKinsey Company and Confederation of Indian Industry says that consumption of value added food products is expected to treble to Rs.2,25,000 crores in the next decade (larger than entire manufacturing industry at present). The largest opportunities are in wheat, poultry and dairy products. A dramatic growth in food processing industry is expected by 2005 with investment of nearly 1,40,000 crores.

Mr.Jaipal Reddy, Hon.Minister, Information and Broadcasting Govt.Of India released this study on 23.05.97

- Times of India (P. 15) 25.05.97

Bleak future on Food Front

Noted Agriculture Scientist, Dr.M.S. Swaminathan in his foundation day address to National Academy of Agriculture Science, New Delhi on 06.06.97, said that per capita world grain harvested, declined from 415 kgs in 1965 to 360 kgs in 1996 as the effect of green revolution has started to taper off in many countries. No dramatic technological breakthroughs are on the horizon to facilitate another quantum jump in food production. Global fish production has been stagnant since 1990.

In India if the population increase is unchecked, it would outstrip food production capacity by 21st century leading to famines and starvation deaths.

- Indian Express (Pune Adv., P. 5) 07.06.97

Duckweed based Technology to treat waste water

Sulabh International, in collaboration with All India Institute of Hygiene and Public Health, Calcutta have developed a low cost technology to treat waste water using duckweed. This small, free floating, fastgrowing aquatic plant has the ability to break down BOD, COD, Suspended solids, toxic and heavy metals and pathogens in the water. The weed can also be a source of nutrient to poultry and animals.

- Times of India (P. 5) 23.04.97

Guidelines to Contributors

THE BLUE CROSS BOOK is published biannually. The contributions to the journal are accepted in the form of invited review articles, research articles, short communications, clinical studies, preliminary communications, letters to the Editor and other information pertaining to animal health and production. The decision of the Editorial Board will be final regarding acceptance of the article for publication. The manuscript should be typed on one side of the paper with double spacing except for abstracts, footnotes and references for which single spacing be used. The words to be printed in Italics should be underlined. The manuscript should be arranged in the following order :

Title : e.g. CLINICAL TRIAL OF BUTOX IN DOGS

Names/s of author/s : e.g. BADOLE P.C., NEMADE P.K. and KARKHANIS R.A.

Place of work : e.g. Department of Pharmacology
Bombay Veterinary College, Mumbai

Abstract : Not more than 200 words.

Materials and Methods :

Results and Discussions :

References : **For Periodicals** : Name and initials of author/s, year of publication in parenthesis, title of article, abbreviated title of journal, volume number, first and last page number/s.

: **For Books** : Name/s of author, year of publication in parenthesis, title of the book, edition, name of publication and page number/s.

Tables and Figures : Tables be numbered in Roman numerals, each table having a clear title. Figures should be good quality and numbered in Arabic numbers.

Abstracts and subheadings are not necessary for clinical articles and short communications. These should not exceed three typed pages. For clinical articles history, observation, tentative and confirmatory diagnosis, line of treatment and follow up on the case should be given.

Authors are requested to indicate that the paper has not been published elsewhere.

All manuscripts should be mailed to the following address :

DR. A. K. DATTA, Editor, **THE BLUE CROSS BOOK**, Neeta Park, Air Port Road, Yerawada, Pune : 411 006.

Readers' Column

1. Dr. K. K. Ameer Shaikh,

Vety Asst. Surgeon, Kavasntti Island, Lakshadweep

I like this issue because the articles are of excellent quality and it is very helpful for doctors working in remote areas. I extend my sincere compliments and best wishes to the BCB publisher.

2. Dr. B. S. Karada,

Vet. Physician Surgeon, Indore

I like this issue because of interesting clinical case reports like "Canine Neosporosis - A Potential cause of posterior paralysis in Indian dogs; Efficacy of Enrofloxacin in Bovine Mastitis - Clinical Study". Your BCB issues will encourage the veterinary doctors and refresh the professional knowledge.

3. Dr. A. Bhanu Murty,

Chief Dist. Veterinary Officer, Orissa

I like this issue because of its contents like "Efficacy of Enrofloxacin in Bovine Mastitis - Clinical Study; Self Medicating Anthelmintic Delivery Devices for Ruminants" and other important clinical articles - most important for field veterinarians for application in the field to achieve the goal of milk production of the country.

4. Dr. Ashok Kumar Jha,

Laheriasarai, Darbhanga

I like this issue because of an interesting case of "Chronic Udder Odema in a Primigravid Jersey Crossbred Cow". I was glad to go through it because I had lost a same type of case a year before due to lack of such type of knowledge. **"Many many thanks to the Blue Cross Book and Mr. W. J. Bader"**.

5. Dr. S. D. Choudhary,

Mobile V. A. S., Assam

It contains information about rabbit farming which may create a new era in production of meat. Nothing to dislike because it is informative. As mentioned in the chapter, clinical studies on coccidiosis on rabbit farming is carried out in Assam, it is not so. Rabbit farming is carried out in Meghalaya.

Readers' Column

6. Dr. V. J. Shidhaye,
Pune

I like this issue because of a good account of "Enrofloxacin medication" through four articles. Other articles are also of good technical quality.

7. Dr. Arvind Sharma,
Gurdaspur

It is informative and presentation of the articles in the 6th, 7th and 8th issue are excellent.

8. Dr. Jitesh Chakraborty,
West Bengal

It contains articles which will be of great benefit to the field veterinarians. "News", "Abstracts" and "Readers Column" are also very nice and useful. Please keep a page on the availability of your biological products.

9. Dr. B. Suresh Subramanian,
Madras

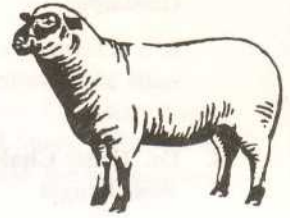
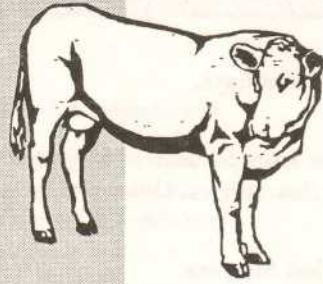
It is highly field oriented and bridges the gap between a laboratory and field.

10. Prof. R. C. Pathak,
Mathura

I like this issue because articles are both from clinical and infectious diseases. However, the articles from prominent scientists and research workers are less in number.

11. Dr. Niranjana Panda,
Bhubaneswar

This is an excellent booklet to update the professional knowledge of practicing vets.



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