

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effect of *Cryptosporidium* in Association with *Eimeria* oocysts on Blood components of Naturally Infected Calves

Rahmatullah Rind

Department of Veterinary Microbiology,
Faculty of Animal Husbandry and Veterinary Sciences,
Sindh Agriculture University, Tando Jam, Pakistan

Abstract: An investigation on the blood parameters of fifteen Friesian young calves 3-4 weeks old at Aber Farm (UK), failed to demonstrate any differences in packed cell volume (PCV) and differential leucocyte counts (DLC) were seen between positive and negative cases, either when infected with pure or mixed infections of *Cryptosporidium* and *Eimeria* oocysts. The packed cell volume and differential leucocyte counts falling within normal range for uninfected calves.

Key words: *Cryptosporidium*, *Eimeria*, Oocysts, Blood components, Infected calves

Introduction

Cryptosporidium species are recognized alone or in combination with *Eimeria* species or other agents as the cause of diarrhoeal and non-diarrhoeal illness in murine, bovine, equine, caprine, ovine, feline, canine, porcine hosts, rabbits, galliform birds, reptiles and primates including man (Romaniuk *et al.*, 1993; Page *et al.*, 1994; Taylor *et al.*, 1994).

Since species of this genus are of zoonotic importance, infecting a very wide range of mammalian species, a great deal of work has been carried-out on the epidemiology, pathology, chemotherapy and immunobiology in naturally and experimentally infected animals worldwide (Lorenzo *et al.*, 1993; Taylor *et al.*, 1994; Xiao *et al.*, 1994).

A little information is known concerning the effects of *Cryptosporidium* oocysts on blood parameters in naturally infected children of Denmark (Molbak *et al.*, 1994) and lambs with *Eimeria* oocysts (Romaniuk *et al.*, 1993). An insignificant effect was observed on total white blood cell counts, but a slight increase in lymphocytes was seen compared with non-infected controls. Since no previous studies have been undertaken on cattle and since blood was being regularly removed from the cattle in connection with the immunological study, the present survey was carried-out with the aim of investigating the effects of cryptosporidiosis in association with coccidiosis on a range of blood parameters, such as packed cell volume (PCV) and differential leucocyte counts (DLC).

Materials and Methods

Detection of *cryptosporidium* oocysts from faeces of calves: Two techniques were used for the detection of oocysts from faecal samples:

Concentration technique: A concentration technique was used as suggested by Casemore (1987). This technique, which was slightly modified as compared to that described by Northcote (1988) is as follows:

1. One ml faecal suspension was mixed with 5ml of 10% formaline in a test tube.
2. The test tubes were then left for 1-4 hours to settle.
3. The supernatant was added to a clean test tube, using a Pasteur pipette and the remaining deposit discarded. The supernatant was then allowed to stand for 7 days.
4. The supernatant was carefully removed with a

Pasteur pipette and discarded. The deposit was used to make a faecal smear on a glass slide.

5. The smear was then stained.

Staining procedure: The modified Ziehl-Neelsen cold carbol fuchsin method was adopted and slight modifications were incorporated (Casemore, 1987).

1. Faecal smears were air dried and fixed in methanol for 3 minutes.
2. The smear made on the slide was then stained in cold carbol fuchsin for 10 minutes and then washed with tap water.
3. The smear was decolorized in 3% hydrochloric acid for 1 minute.
4. Methylene blue 1% counter stain was applied for 30 seconds, rinsed in tap water and air dried.
5. The slides were then examined under oil immersion objective.

Rapid staining technique: A rapid staining was used as recommended by Cross and Moorhead (1984).

Staining procedure:

1. Thin faecal smears (faecal suspension remaining from the concentration technique) were made on microscope slides and allowed to air dry and were then fixed over a gas or spirit lamp flame.
2. Any lumps on the slide were removed by scraping with the edge of another slide.
3. A few drops of 1% methylene blue and 1% borax (disodium tetraborate) were added and allowed to stain for 30-60 seconds.
4. The slide was then rinsed with tap water.
5. A few drops of 0.1% eosin solution were added and allowed to stain for 60 seconds.
6. The slide was then dipped in acetone for 1-2 minutes for dehydration.
7. Slides were examined under oil immersion X1000 magnification

Identification of *Cryptosporidium* oocysts: Since it is impossible to identify the different species of *Cryptosporidium* by simply examining their oocysts the oocysts counted have been grouped together as *Cryptosporidium* species. However, it is likely that they represent *Cryptosporidium parvum*. It is possible however, that *Cryptosporidium muris* could be also present.

Rahmatullah Rind: *Cryptosporidium*, *Eimeria*, Oocysts, Blood components

Table 1: The PCV values and incidence of oocysts of *Eimeria* (g⁻¹ of faeces) and *Cryptosporidium* (under 10 microscope fields) species in naturally infected calves during experimental period

Animal No.		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	PCV	41	39	35	38	41	36	32	32	38	39	39	34	38	48	38
	Oocysts	0	0	100	0	0	0	0	0	0	0	0	0	0	400	0
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(9)	(0)	(3)	(13)	(5)	(0)	(6)	(0)
2	PCV	42	37	35	34	37	33	34	31	37	38	38	34	35	43	41
	Oocysts	0	0	0	300	0	0	0	0	0	0	0	0	0	0	0
		(0)	(0)	(0)	(4)	(0)	(4)	(0)	(0)	(0)	(3)	(0)	(4)	(0)	(0)	(0)
3	PCV	38	37	35	34	35	31	31	35	30	32	29	25	28	31	36
	Oocysts	0	0	0	0	150	0	0	1150	400	0	0	0	0	0	0
		(0)	(0)	(0)	(5)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(5)	(3)	(0)	(0)
4	PCV	-	-	35	-	-	28	-	34	30	33	30	25	30	30	35
	Oocysts	-	-	0	250	100	500	0	7000	1000	3350	12000	5900	0	1350	800
		(0)	(-)	(0)	(0)	(3)	(0)	(3)	(0)	(4)	(4)	(0)	(0)	(0)	(0)	(0)
5	PCV	-	-	-	-	-	-	-	-	-	-	30	25	30	32	35
	Oocysts	-	-	-	-	-	-	-	-	-	-	200	100	200	50	100
		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(0)	(3)	(0)	(0)	(0)

{ } = Indicates number of oocysts of *Cryptosporidium* species under 10 microscope fields,

- = Not observed Oocysts = *Eimeria* oocysts in g⁻¹ of faeces.

Table 2: The DLC values and number of incidence of oocysts of *Cryptosporidium* (under 10 microscope fields) and *Eimeria* sp. (g⁻¹ faeces) in the faeces of calves under natural infections

Month	1					2					3					4					5									
	M	L	N	B	E	M	L	N	B	E	M	L	N	B	E	M	L	N	B	E	M	L	N	B	E					
Calf No.																														
18	6	51	30	2	11	5	60	20	2	13	5	67	25	2	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			0(0)				0(0)					0(0)						0(0)												
19	6	70	20	0	4	5	65	20	0	10	5	70	20	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			0(0)				0(0)					0(0)																		
20	9	55	24	2	10	5	70	20	0	5	5	75	17	1	2	4	74	18	1	2	-	-	-	-	-	-	-	-	-	
			0(100)				0(0)					0(0)						0(0)												
21	6	55	30	3	9	6	80	10	0	4	10	80	30	3	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			0(0)				0(300)					5(0)						0(250)												
22	7	80	7	2	4	5	70	20	0	5	5	70	20	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			0(0)				4(0)					0(150)						3(100)												
23	10	80	6	0	4	6	60	29	0	4	6	75	15	2	2	4	80	15	0	1	-	-	-	-	-	-	-	-	-	
			0(0)				0(0)					0(0)						0(500)												
24	5	60	30	0	5	5	65	32	0	3	2	80	17	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			0(0)				4(0)					0(0)						3(0)												
25	7	68	18	3	4	6	85	5	1	3	3	82	15	0	0	5	70	25	0	0	-	-	-	-	-	-	-	-	-	
			9(0)				0(0)					0(1150)						0(7000)												
26	10	60	27	0	4	4	74	10	2	10	5	74	15	0	4	2	67	25	1	5	-	-	-	-	-	-	-	-	-	
			0(0)				0(0)					0(400)						4(1000)												
27	7	65	30	3	4	4	75	15	0	6	5	75	15	2	3	5	80	10	1	4	-	-	-	-	-	-	-	-	-	
			3(0)				0(0)					0(0)						4(3350)												
28	4	65	20	0	11	4	76	15	0	5	3	77	17	0	3	5	70	25	0	0	4	75	15	1	5					
			13(0)				3(0)					0(0)						0(12000)					0(200)							
29	7	50	30	3	10	5	70	19	2	4	3	70	20	0	7	4	70	25	0	1	3	80	17	0	0					
			5(0)				0(0)					5(0)						0(5900)				3(100)								
30	7	55	30	0	5	6	74	15	0	5	10	60	25	0	5	4	80	30	0	6	3	60	35	0	2					
			0(0)				4(0)					3(0)						0(0)				0(200)								
31	7	64	24	2	5	6	60	27	0	7	3	80	12	0	5	4	73	20	0	3	5	64	30	0	1					
			6(400)				0(0)					0(0)						0(1350)				0(0)								
32	5	60	30	0	5	7	65	25	0	5	11	68	20	0	2	5	64	30	1	1	4	6030	2	4						
			0(0)				0(0)					0(0)						0(800)				0(100)								

{ } = Indicates number of *Eimeria* oocysts and figures outside () indicates *Cryptosporidium* sp. oocysts, - = Indicates not observed, M = Monocytes, L = Lymphocytes, N = Neutrophils B = Basophils E = Eosinophils

Detection of *Eimeria* oocysts from faeces of calves:

Faecal samples collected for *Cryptosporidium* oocysts per rectum from naturally infected calves were also used for *Eimeria* oocysts, using the modified McMaster method. A simple solution was prepared by emulsifying a small sample (3g faeces) of the faeces in (45ml) tap water (MAFF, 1986). This was then strained through a 0.15 mm aperture nylon mesh. The test tubes were filled with faecal solution and then centrifuged at 1500rpm for 15 minutes. The debris was removed, this was repeated for several times. The saturated NaCl was then added to the tubes containing sediment. The oocysts of mixed species were then counted. While counting the number of oocysts g⁻¹ of faeces, the two chambers of a McMaster slide were filled using a pasteur pipette. All the oocysts within the ruled area, a centimetre square of each chamber, were counted using a microscope with

X100 eyepiece and X10 objective. In calculating the numbers of oocysts g⁻¹ of faeces, the mean number of oocysts from the two chambers of the McMaster slide was multiplied by 100. This factor was derived from the following formula:

$$\frac{x \times 45 \times 1}{0.15 \times 3}$$

- x = Mean number of oocysts from two chambers
- 0.15 = Volume of sample in cm²
- 45 = Total volume of sample ie., 3g faeces + 42ml water
- 1/3 = Correction factor for 1g of faeces.

Collection of blood samples: Blood samples were

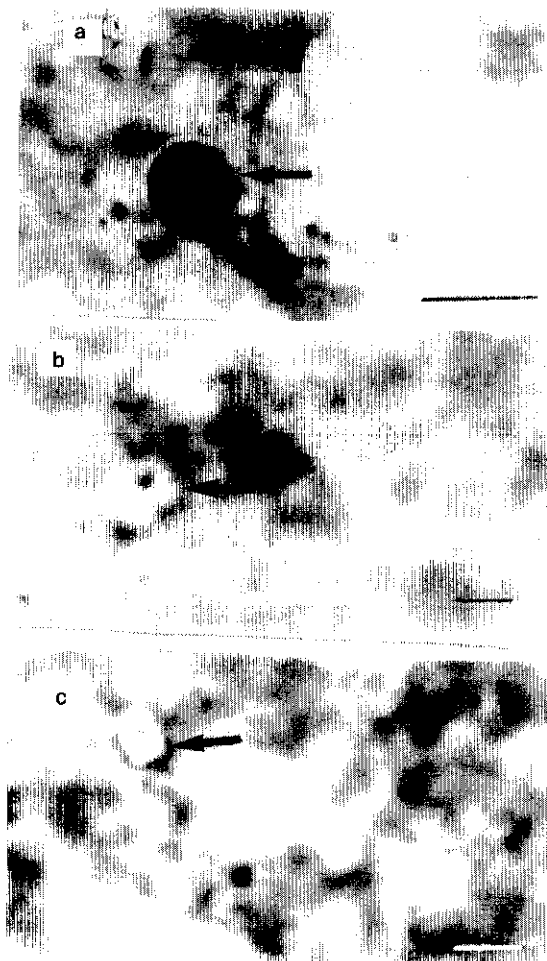


Fig. 1: Staining characteristics of *Cryptosporidium* oocysts. a: *Cryptosporidium* oocyst (+) in a faecal smear stained by the modified Ziehl-Neelsen method X 2520. b: *Cryptosporidium* oocyst (+) in a faecal smear stained by the rapid staining technique X 1600. c: *Cryptosporidium* oocyst (+) in a faecal smear of calf stained by the rapid staining technique X 1000. Scale bars represent 5µm.

collected at monthly intervals from the same group of calves at the same time as faecal samples were being collected. Before collection the jugular vein was shaved and cleaned. A needle was pricked into the vein and blood was collected into heparinized tubes and processed for haematological studies immediately on arrival in the laboratory.

Processing and determination of packed cell volume (PCV):

1. Before filling the capillary tubes, the blood sample in the vacutainer tube was mixed gently, care was taken to avoid the break-down of red blood cells.
2. The capillary tube were filled two thirds with blood.
3. The tubes were sealed with haematocristseal.
4. The tubes were placed into Hawksley Microhaematocrit centrifuge machine and centrifuged at 1100rpm for 15 minutes.
4. The packed cell volume (PCV) was measured, using a Hawksley Microhaematocrit reader.

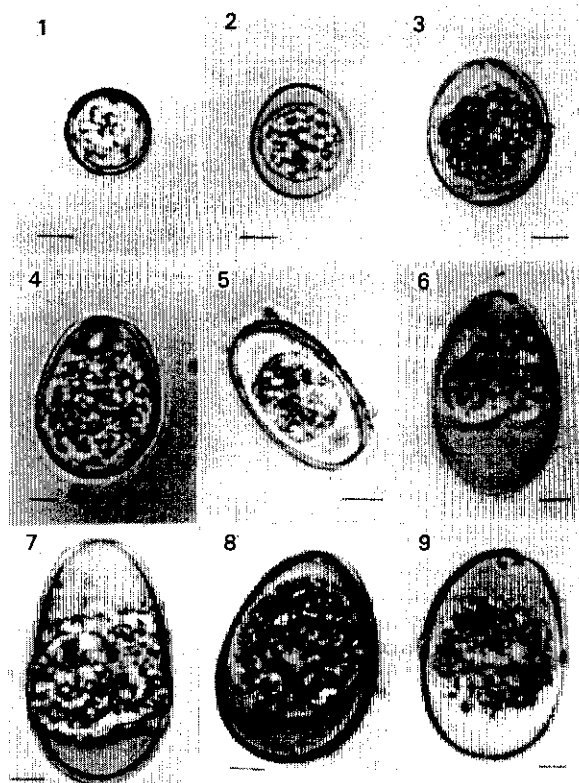


Fig. 2: Photographs of unsporulated oocysts of *Eimeria* species isolated from faecal samples by the Clayton Lane method x 250 (Bright field P.C.).

- | | |
|--------------------------------------|---------------------------|
| 1. <i>Eimeria subspherica</i> | 2. <i>E. zuernii</i> |
| 3. <i>E. ellipsoidal/alabamensis</i> | |
| 4. <i>E. bovis</i> | 5. <i>E. cylindrica</i> |
| 6. <i>E. canadensis</i> | 7. <i>E. auburnensis</i> |
| 8. <i>E. wyomingensis</i> | 9. <i>E. brasiliensis</i> |
- Scale bars represent 5µm

Processing and determination of differential leucocyte count (DLC):

1. The new unused slides were placed in 95% alcohol and left over night and then cleaned with muslin cloth and dried.
2. A drop of blood was placed on the slide and smear made with the edge of another slide.
3. The slides were allowed to dry and then fixed in 70% alcohol.
4. After fixing in alcohol, the slides were allowed to air dry.
5. The slides were stained with Wright's stain (1% wright's stain in equal parts of glycerol and methanol) for 10 minutes and the stain was removed off gently with distilled water until the slide appeared pink to the naked eye.
6. Slides were allowed to air dry and examined under oil immersion objective, 100 cells were counted and the percentage of different WBC was counted.

Results and Discussion

The results regarding PCV and DLC values obtained were

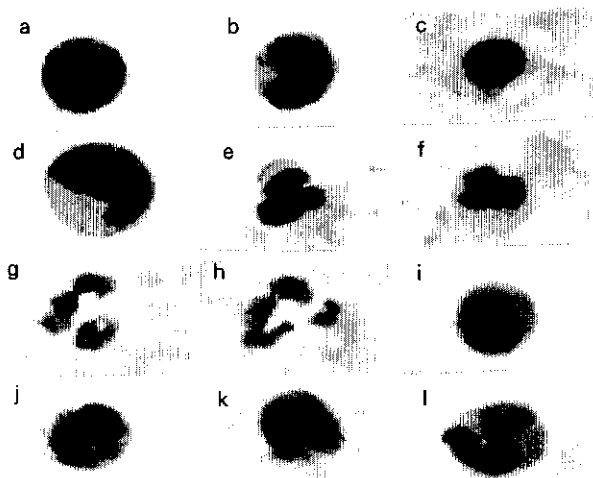


Fig. 3: The morphological differences of blood cells of cattle stained by wright's methods x 1750. Lymphocytes: a, Large lymphocyte b, Large lymphocyte with low nuclear chromatin c, Small lymphocyte d, Monocyte, a large cell in the blood with vacuolated cytoplasm and chromatin with rake-like appearance. Neutrophils: e-f, Bend neutrophils g-h, segmented neutrophils. Eosinophils and Basophils: i-k, Eosinophils with bilobed nucleus in peripheral blood containing acidophilic granules, l, Basophil with numerous acidophilic granules which also overlie the bilobed nucleus

compared with the number of oocysts of *Cryptosporidium* and *Eimeria* spp. and their effect on PCV values investigated.

These results show (Table 1) that the number of oocysts (infection) and thus intensity of infection in natural conditions had no effect on the PCV values. Thus while animal 28 had a PCV ranging from 29 to 30% when no oocysts were recorded and a PCV of 38 and 39% when oocysts were 3 and 13 respectively, yet many animals showed high PCV (43%) in the complete absence of infection. It has been concluded from the present survey that thus both, *Cryptosporidium* (Fig. 1) and *Eimeria* (Fig. 2) species at these levels of infection had no effect on PCV values in naturally infected calves under two months of age.

The differential leucocytic counts recorded were within the normal range with and without infection (Table 2 and Fig. 3). This indicates that *Cryptosporidium* in association with *Eimeria* infection at these levels have no effect on DLC.

No differences in packed cell volume (PCV) and differential leucocyte counts (DLC) were seen between positive and negative cases, either when infected with

pure, or mixed infections with *Cryptosporidium* and or *Eimeria* oocysts. Molbak *et al.* (1994) also failed to demonstrate any differences in the haematological values of children and lambs suffering with severe cryptosporidiosis and coccidiosis respectively. Only a little variation in increase of lymphocytes (mean 74%, range 49 to 98% in infected and 27 to 97% in control) occurred. They also showed that cryptosporidiosis had no effect on DLC of infected children. While no effects of infection with *Cryptosporidium* spp. were evident on the blood parameters of calves, even in the presence of *Eimeria* species, this could be purely a feature of subclinical disease. No evidence of acute cryptosporidiosis or even with association of coccidiosis was seen in any of our animals. This does not mean that acute infection will not produce an effect. The conclusion of a negative effect is only applicable to these levels of natural infections seen in these calves.

References

- Casmore D.P., 1987. The antibody response to *Cryptosporidium*: development of serological test and its use in a study of immunologically normal persons. *J. Infect.*, 14: 125-134.
- Cross R.F. and P.D. Moorhead, 1984. Rapid staining technique for *Cryptosporidium*. *Modern Vet. Practice*, 65: 307.
- Lorenzo L.M.J., E.M. Ares and M.de-I. Villacorta, 1993. Detection of oocysts and IgG antibodies to *Cryptosporidium parvum* in asymptomatic adult cattle. *Vet. Parasitol.*, 47: 9-15.
- MAFF., 1986. Ministry of Agriculture, Fisheries and Food. *Manual Veterinary Parasitological Laboratory Techniques*. Reference book 418. HMSO., London.
- Molbak K., I.A. Lisse, N. Hojlyng and P. Aaby, 1994. Severe cryptosporidiosis in children with normal T-cell subsets. *Parasite Immunol.*, 16: 275-277.
- Northcote A.J., 1988. Investigation of *Cryptosporidium* in sheep and other mammalian species. B.Sc. Dissertation, University of North Wales, Bangor, Uk.
- Page E.V., B.G. Co, E.H. Caradanz, A. Raymudo, E. Lagamayo, E. Lavadia, E.Rigor and C.A. Hart, 1994. Non-bacterial diarrhoea in children in the Philippines. *Annals of Trop. Med. and Parasitol.*, 88: 53-58.
- Romaniuk K., M. Michalski, R. Sokol and M. Szelagiewicz, 1993. Wplyw inwazji *Eimeria* species i nicieni u jagniat na poziom niektórych wskaźników ematologicznych i biochemicznych krwi. *Medycyna weterynaryjna*, 49: 273-275.
- Taylor M.A., J. Catchpole, C.C. Norton and J.A. Green, 1994. Variation in oocyst out put associated with *Cryptosporidium baileyi* infections in children. *Vet. Parasitol.*, 53: 7-14.
- Xiao L. R.P. Herd and K.E. McCture, 1994. Preparturient rise in the excretion of *Giardia* sp. cysts and *Cryptosporidium parvum* oocysts as source of infection for lambs. *J. Parasitol.*, 80: 55-59.