

The Prevalence of *Cryptosporidium* Infection in Neonatal Diarrhoeic Sheep Lambs with Observation of Resolution using Paromomycin Treatment

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Abstract

This study was conducted between May and August 2016. A total of 45 fecal specimens obtained from 6-37 day old lamb were examined for the presence and prevalence of *Cryptosporidium* by rapid Immunochromatographic (ICT). The prevalence of the infection was found in 33 specimens out of 45 (73.33%). The infected lambs were treated by using Paromomycin sulphate (Parofo[®]) and retested for the presence of *Cryptosporidium* infection after 5 days of consecutive treatment. We report in this study the successful resolution of *Cryptosporidium* with Parofo[®].

Keywords: *Cryptosporidium*; Protozoan; Diarrheic; Neonatal; Resolution; Parofo-Paromomycin; Sulphate Immunochromatographic; BoviD4; Rotavirus; Coronavirus; *E. coli* K99; BoviD4; MZN; Aminoglycosides

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Introduction

Cryptosporidium parvum, a protozoan parasite, is considered to be an important agent in the etiology of the neonatal diarrhoea syndrome of calves, lambs and goat kids, causing considerable direct and indirect economic losses [1-5]. Moreover, the zoonotic potential of cryptosporidiosis makes it a public health concern. Of the seven *Cryptosporidium* species identified in sheep, two are predominant: *Cryptosporidium parvum* and *Cryptosporidium ubiquitum* [6]. Cryptosporidiosis occurs in lambs and kids at an early age of life (5-10 days of age), [7]. The most prominent clinical signs of ovine cryptosporidiosis are diarrhoea lasting 2 to 12 days and this is sometimes accompanied by anorexia, stiffness, hyperpnoea, slow gait and depression [8-10].

Cryptosporidiosis causes high morbidity and it has been recorded in various domestic young animals such as lambs, kids, foals, and calves, leading sometimes to mortality. Huge economic losses due to *Cryptosporidium* infection were recorded by different researchers [11-15]. There are different methods used for the detection of *Cryptosporidium* in faecal samples. Generally, microscopic examination is used for the detection of *Cryptosporidium* oocyst in faecal samples. The most procedure has been widely used, the Modified Ziehl-Nielsen (MZN) acid-fast

stain [16]. Different antigen detection methods such as ELISA, immunofluorescence, and genome detection methods such as PCR are used for the detection of the *Cryptosporidium* [17].

In recent years, the Immunochromatic Dipstick Test (ICT) has been used for the rapid diagnosis of *Cryptosporidium*. According to the manufacturer, these tests are rapid and sensitive enough but provide only qualitative results for the presence of the pathogen in faecal samples [18]. To control cryptosporidiosis is a big global challenge for veterinary as well as for human medicine. Different therapeutic agents up to thousands had been tested *in vivo* as well as *in vitro* conditions to treat cryptosporidiosis. Some agents were active against *Cryptosporidium* infection *in vitro* condition but showed poor efficacy or no efficacy during treatment therapy under field conditions [19]. More recently, Huvepharma Bulgaria has developed Parofo[®] (Paromomycin

in

sulphate). Paromomycin is a broad-spectrum antibiotic and belongs to the group of aminoglycosides. Paromomycin is poorly absorbed from gastro-intestinal tract and is not inactivated by organic material, remaining active form in the intestinal lumen. Two forms of Parofo[®] are produced by Huvepharma, water powder for use in drinking water or milk replacer. One gram of it contains 100 mg of paromomycin sulphate (=70 mg paromomycin as base). The second form is Parofo[®] 140 mg/ml OS, a solution used in drinking water, milk or milk replacer.

Previous studies have shown the efficacy of Paromomycin against *Cryptosporidium* [20,21]. Parofo[®] was first only registered for the treatment of diarrhoea caused by *Escherichia coli* but the recognized efficacy against *Cryptosporidium* leads Huvepharma to obtain an antiprotozoal registration (Parofo Crypto[®], UK, July 2018, following by several European countries). The aim of the present study was to determine the prevalence of *Cryptosporidium* infection in lambs using rapid Immunochromatographic Test (Bovi D4) manufactured by Bionote Inc, South Korea. The second aim was to investigate the effect of Parofo[®], water-soluble powder, in treating positive cases of *Cryptosporidium* infection in lambs.

Materials and Methods

This study was conducted between May and August 2016.

Samples

A total of 45 faecal specimens were obtained from 45 lambs, from 6-37 days old. They were grouped into 5 groups. The first group is from 6-14 day old lamb, the second group is from 8-14 day old, the third group is from 18-37 day old; the fourth group is from 6-11 day old and the fifth group is from 6-9 day old. The faecal samples were detected by Bovi 4 Ag test Kit (Bionote). This kit detects *Cryptosporidium*, Rotavirus, Coronavirus and *E. coli* K 99. Faecal samples were processed according to the manufacture's recommendations. Briefly collect the sample from

Table 1: Diarrhoea test kit result, Group 1.

S. No.	Tag No.	D.O.B.	Result				Date
			Crypto	Rotavirus	<i>E. coli</i>	Giardiasis	
1	Y-3136	07-06-2016	Positive	Negative	Negative	Negative	20-06-2016
2	Y-3031	07-06-2016	Negative	Negative	Negative	Negative	20-06-2016
3	Y-3044	08-06-2016	Negative	Negative	Negative	Negative	20-06-2016
4	B-1053	08-06-2016	Positive	Negative	Negative	Negative	20-06-2016
5	Y-3072	10-06-2016	Positive	Negative	Negative	Negative	20-06-2016
6	B-1120	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
7	Y-3096	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
8	B-1119	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
9	Y-3088	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
10	Y-3094	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
11	B-1118	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016
12	Y-3114	13-06-2016	Positive	Negative	Negative	Negative	20-06-2016
13	B-1151	13-06-2016	Positive	Negative	Negative	Negative	20-06-2016
14	B-1156	14-06-2016	Positive	Negative	Negative	Negative	20-06-2016
15	Y-3141	14-06-2016	Negative	Negative	Negative	Negative	20-06-2016
16	B-1208	17-06-2016	Positive	Negative	Negative	Negative	20-06-2016

diarrhoeic lamb using the swab. Insert the swab into the sample tube containing assay diluent and mix the swab until the sample has been dissolved into the assay diluent. Squeeze the swab against the well of the tube and then discard it. Wait 30 seconds for sedimentation and take the supernatant with the disposable dropper provided. Add 4 drops into the sample hole with the disposable dropper and wait for 5-10 minutes.

Interpretation of the result

The appearance of red or purple color in the control line (C) indicates that the sample is negative for any of the 4 pathogens (*Cryptosporidium*, Rotavirus, Coronavirus and *E. coli* K99). The appearance of red or purple color in control line (C) and test line (T), indicates that the sample positive for *Cryptosporidium*, Rota, Corona and *E. coli* K99 or there is a mixed infection. The results are invalid when there is no purple colour in both control or test line, or it is only appearing in the test line.

Parofo[®] treatment

Parofo[®] (Paromomycin sulphate) manufactured by Huvepharma=70 mg Paromomycin as a base, is a water-soluble powder. Each of 33 *Cryptosporidium* positive cases was treated with Parofo[®]. Each lamb is given 5 gm Parofo[®]/10 Kg of live bodyweight for 5 consecutive days and retested for the presence of *Cryptosporidium* pathogen using the BoviD4 Ag Test Kit of Bionote.

Results

Cryptosporidium is detected in 33 faecal samples, while 12 samples were negative by BoviD4 Ag test. The prevalence of infection is 73.33%. Those gave negative *Cryptosporidium* results represented 26.67% **Tables 1-5**. Two of them were positive for Rotavirus infection. All the 33 positive animals gave negative results to *Cryptosporidium* after treatment with Parofo[®] for 5 days and they had responded to this treatment **Tables 6-10**.

Table 2: Diarrhoea test kit results, Group 2.

S. No.	Tag No.	D.O.B.	Result				Date
			Crypto	Rotavirus	<i>E. coli</i>	Giardiasis	
1	B-1076	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016
2	B-1142	06-13-2016	Positive	Negative	Negative	Negative	6/21/2016
3	Y-3053	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016
4	B-1067	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016
5	Y-3038	06-07-2016	Positive	Negative	Negative	Negative	6/21/2016
6	B-1005	06-06-2016	Negative	Negative	Negative	Negative	6/21/2016
7	B-1107	06-12-2016	Positive	Negative	Negative	Negative	6/21/2016
8	B-1108	06-12-2016	Positive	Negative	Negative	Negative	6/21/2016
9	Y-3057	06-10-2016	Negative	Negative	Negative	Negative	6/21/2016
10	Y-3067	06-10-2016	Positive	Negative	Negative	Negative	6/21/2016

Table 3: Diarrhoea test kit results, Group 3.

S. No.	Tag No.	D.O.B.	Result				Date
			Crypto	Rotavirus	<i>E. coli</i>	Giardiasis	
1	G-979	04-06-2016	Positive	Negative	Negative	Negative	22-06-2016
2	O-451	02-06-2016	Positive	Negative	Negative	Negative	22-06-2016
3	G-916	01-06-2016	Positive	Negative	Negative	Negative	22-06-2016
4	G-848	24-05-2016	Negative	Positive	Negative	Negative	22-06-2016
5	O-203	16-05-2016	Negative	Positive	Negative	Negative	22-06-2016
6	O-1988	28-05-2016	Negative	Negative	Negative	Negative	22-06-2016
7	O-500	04-06-2016	Negative	Negative	Negative	Negative	22-06-2016
8	No Tag	25-05-2016	Negative	Negative	Negative	Negative	22-06-2016
9	G-855	25-05-2016	Negative	Negative	Negative	Negative	22-06-2016

Table 4: Diarrhoea Kit test results, Group 4.

S. No.	Tag No.	D.O.B.	Result				Date
			Crypto	Rotavirus	<i>E. coli</i>	Giardiasis	
1	G-1404	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016
2	O-1417	7/26/2016	Positive	Negative	Negative	Negative	08-06-2016
3	O-1450	7/30/2016	Positive	Negative	Negative	Negative	08-06-2016
4	O-1447	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016
5	O-1416	7/31/2016	Negative	Negative	Negative	Negative	08-06-2016

Table 5: Diarrhoea Kit test results, Group 5.

1	O-1464	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016
2	O-1438	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016
3	O-1469	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016
4	O-1942	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016
5	G-1415	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016

Table 6: Diarrhoea Test kit Results after Parofor treatment Group 1.

S. No.	Tag No.	D.O.B.	Result				Date	Treatment results
			Crypto	Rotavirus	<i>E. coli</i>	Giardiasis		
1	Y-3136	07-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
2	Y-3031	07-06-2016	Negative	Negative	Negative	Negative	20-06-2016	Not treated
3	Y-3044	08-06-2016	Negative	Negative	Negative	Negative	20-06-2016	Not treated
4	B-1053	08-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
5	Y-3072	10-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
6	B-1120	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
7	Y-3096	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
8	B-1119	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
9	Y-3088	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative

10	Y-3094	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
11	B-1118	12-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
12	Y-3114	13-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
13	B-1151	13-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
14	B-1156	14-06-2016	Positive	Negative	Negative	Negative	20-06-2016	Negative
15	Y-3141	14-06-2016	Negative	Negative	Negative	Negative	20-06-2016	Not treated
16	B-1208	17-06-2018	positive	Negative	Negative	Negative	20-06-2016	Negative

Table 7: Diarrhoea test kit results after Parofor treatment, Group 2.

S. No.	Tag No.	D.O.B.	Result					Date	Treatment results
			Cipro	Rotavirus	E. coli	Giardiasis			
1	B-1076	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
2	B-1142	06-13-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
3	Y-3053	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
4	B-1067	06-09-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
5	Y-3038	06-07-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
6	B-1005	06-06-2016	Negative	Negative	Negative	Negative	6/21/2016	Not treated	
7	B-1107	06-12-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
8	B-1108	06-12-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	
9	Y-3057	06-10-2016	Negative	Negative	Negative	Negative	6/21/2016	Not treated	
10	Y-3067	06-10-2016	Positive	Negative	Negative	Negative	6/21/2016	Negative	

Table 8: Diarrhoea Test kit Result after Parofor treatment, Group 3.

S. No.	Tag No.	D.O.B.	Result					Date	Treatment results
			Crypto	Rota Virus	E. coli	Giardiasis			
1	G-979	04-06-2016	Positive	Negative	Negative	Negative	22-06-2016	Negative	
2	O-451	02-06-2016	Positive	Negative	Negative	Negative	22-06-2016	Negative	
3	G-916	01-06-2016	Positive	Negative	Negative	Negative	22-06-2016	Negative	
4	G-848	24-05-2016	Negative	Positive	Negative	Negative	22-06-2016	Not treated	
5	O-203	16-05-2016	Negative	Positive	Negative	Negative	22-06-2016	Not treated	
6	O-1988	28-05-2016	Negative	Negative	Negative	Negative	22-06-2016	Not treated	
7	O-500	04-06-2016	Negative	Negative	Negative	Negative	22-06-2016	Not treated	
8	No Tag	25-05-2016	Negative	Negative	Negative	Negative	22-06-2016	Not treated	
9	G-855	25-05-2016	Negative	Negative	Negative	Negative	22-06-2016	Not treated	

Table 9: Diarrhoea test kit after Parofor treatment, Group 4.

S. No.	Tag No.	D.O.B.	Result				Date	Treatment Result
			Crypto	Rota Virus	E. coli	Giardiasis		
1	G-1404	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
2	O-1417	7/26/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
3	O-1450	7/30/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
4	O-1447	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
5	O-1416	7/31/2016	Negative	Negative	Negative	Negative	08-06-2016	Not treated

Table 10: Diarrhoea test kit after Parofor treatment, Group 4.

1	O-1464	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
2	O-1438	7/28/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
3	O-1469	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
4	O-1942	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative
5	G-1415	7/31/2016	Positive	Negative	Negative	Negative	08-06-2016	Negative

Discussion and Conclusion

In recent years, the number of commercially diagnostic

immunoassays for the presence of the antigen to *Cryptosporidium* spp. increased rapidly, focusing especially on speed, ease and sufficient sensitivity of testing. However, these tests are used as screening tests, provide qualitative results and are suitable only

for detecting the presence/absence of infection in large groups of animals. BoviD4, a rapid Immunochromatographic Test is used in this study to detect the prevalence of *Cryptosporidium* in lambs. The test is simple, rapid with high specificity (99%) and high sensitivity (98.2%) versus PCR as claimed by the manufacturer (Bionote, Inc). Observations and investigations on the presence of *Cryptosporidium* infection in neonatal lambs were studied and the prevalence rate of infection was 73.33%. In the present study, all the diarrhoeic lambs were 6-37 days old. The chemotherapy of the cryptosporidiosis remains a major challenge. The main goal of modern and parasitic chemotherapy must bring the drug as directly to target pathogen as possible and minimize potential side effects.

Also, to study the efficacy of the therapeutic agent *in vitro* as well as *in vivo* conditions (under field condition). Different therapeutics were used but, up to now, only two molecules have shown satisfactory results to control Cryptosporidiosis: Halofuginone and Paromomycin. In this study, Parofo[®] showed good efficacy

during treatment therapy under field conditions and proof as a convenient and promising therapeutic agent for *Cryptosporidium* infection.

In conclusion, Immunochromatographic Test (ICT) has been used as a simple, rapid and sensitive qualitative test to confirm the presence and prevalence of *Cryptosporidium*. Parofo[®] efficacy was studied and it showed a convenient, promising therapeutic drug for *Cryptosporidium* infection.

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References

- 1 Dubey JP, Fayer CA (1990) Cryptosporidiosis of man and animals. CRC Press, Boca Raton, Florida 2009.
- 2 Current WL (1985) Cryptosporidiosis. J A Vet Med Assoc 187: 1334-1338.
- 3 Foreyt WJ (1990) Coccidiosis and cryptosporidiosis in sheep and goats. Vet Clin North Am Food Anim Pract 6: 655-670.
- 4 Snodgrass DR, Angus KW, Gray EW (1984) Experimental cryptosporidiosis in germfree lambs. J Comp Pathol 94: 141-152.
- 5 Xiao L, Herd RP, Rings DM (1993) Diagnosis of *Cryptosporidium* on a sheep farm with neonatal diarrhoea by immunofluorescence assay. Vet Parasitol 47: 17-23.
- 6 Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 124: 80-89.
- 7 Radostits O, Gay C, Hinchciff K, Constable P (2008) Diseases associated with protozoa. 10th Ed In: Veterinary Medicine: A Textbook of cattle, horse, sheep, pigs and goats. Saunders Elsevier, pp: 1483-1540.
- 8 Angus KW, Appleyard WT, Menzies JD, Campbell I, Sherwood D (1982) An outbreak of diarrhoea associated with cryptosporidiosis in naturally reared lambs. Vet Rec 110: 129-130.
- 9 de Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE (1999) A review of importance of cryptosporidiosis in farm animals. Int J Parasitol 29: 1269-1287.
- 10 Tizpori S, Angus KW, Clerihew LW, Campbell I (1981) Diarrhoea due to *Cryptosporidium* infection in artificially reared lambs. J Clin Microbiol 14: 100-105.
- 11 Degerli S, Celiksoz A, Kalkan K, Ozcelik S (2005) Prevalence of *Cryptosporidium spp.* and *Giardia spp.* in cow and calves in Sivas. Turk J Vet Anim Sci 29: 995-999.
- 12 Nasir A, Avais M, Khan MS, Ahmed N (2009) Prevalence of *Cryptosporidium parvum* infection in (Pakistan) and its association with diarrhoea in dairy calves. Int J Agri Biol 11: 221-224.
- 13 Prakash S, Prabu K, Palanivel KM (2009) Prevalence of cryptosporidiosis in dairy calves in Chennai. J Vet Anl Sci 5: 41-46.
- 14 Potter I, Esbroeck VM (2010) Negative staining of Heine foe the detection of *Cryptosporidium spp.* A fast-simple technique. Open Para J 4: 1-4.
- 15 Ayinmode FB, Fagbemi BO (2011) Cross-reactivity of some *Cryptosporidium* species with *Cryptosporidium parvum* coproantigen Commercial Elisa kit. Nig Vet J 10: 1-4.
- 16 Fayer R, Ungar BLP (1986) *Cryptosporidium spp* and Cryptosporidiosis. Microbial Rev 50: 458- 483.
- 17 Areeshi MY, Beeching NJ, Hart CA (2007) Cryptosporidiosis in Saudi Arabia and neighbouring countries. Ann Saudi Med 27: 325-332.
- 18 Agnamey P, Sarfati C, Pinel C, Rabodoniriina M, Kapel N, et al. (2011) Evaluation of four commercial rapid immunochromatographic assays for detection of *Cryptosporidium* antigen in stool samples: a blind multicentre trial. J Clin Microbiol 49: 1605-1607.
- 19 Shahiduzzaman M, Dausgchies A (2012) Therapy and prevention of cryptosporidiosis in Animals. Vet Parasitol 188: 203-214.
- 20 Viu M, Quílez J, Sánchez-Acedo C, del Cacho E, López-Bernad F, et al. (2000) Field trial on the therapeutic efficacy of paromomycin on natural *Cryptosporidium parvum* infections in lambs. Vet Parasitol 90: 163-170.
- 21 Paraud C, Chartier C (2012) Cryptosporidiosis in small ruminants. Small Ruminant Research 103: 93-97