Drug combination therapy in control of cryptosporidiosis in Ludhiana district of Punjab

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Abstract The present report describes outbreak of cryptosporidiosis in neonatal cross bred cattle calves ageing 1–2 months in an organized dairy farm. The protozoan infection was confirmed by identifying bright red oocysts of Cryptosporidium spp. in the faecal samples after staining with modified acid Fast Zeihl–Neelsen stain. Metronidazole and furazolidone combination was able to induce clinically and parasitological recovery. This is believed to be the first report on the successful use of this drug combination against cryptosporidiosis.

Keywords Cow calves · Cryptosporidium spp. · Diagnostic · Outbreak · Therapeutic

Introduction

Bovine cryptosporidiosis caused Cryptosporidium species, an apicomplexan intracellular, extracytoplasmic protozoan parasite infects the microvillus epithelium of the gastrointestinal tract, leading to its erosion of a wide range of vertebrate hosts, including birds, fish, mammals, and reptiles (Xiao et al. 2004). The economic losses attributed to infection are mainly due to morbidity with diarrhoeic complications, dehydration and retardation of growth and to limited extent mortality (O’Donoghue 1995). The virulence of bovine cryptosporidiosis caused by C. Parvum is enhanced in young unweaned dairy calves having premature immune system resulting in scour, dehydration, and death (Moore et al. 2003; Trotz et al. 2007); however, weaned and adult animals can also become infected. The public health issues of Cryptosporidium species came into light in 1993 when a large population of human beings was affected in the world’s largest outbreak of water-borne disease recorded in Milwaukee, Wisconsin, USA (MacKenzie et al. 1994). Now it is well known that apart from the veterinary problems that Cryptosporidium can cause an important waterborne emerging zoonotic protozoan disease in immunocompromised humans (Fayer et al. 2000; Slifko et al. 2000) and cattle are thought to be involved in zoonotic transmission of infection (Smith and Rose 1998). In India, prevalence of cryptosporidiosis in bovines is reported to be quite high (Singh et al. 2006; Paul et al. 2008; Bhat et al. 2012). Though the aetiology of bovine neonatal enteritis also includes corona virus or rota virus or Salmonella or E. coli but many field and experimental studies have shown that cryptosporidia may act as a primary pathogen. Cryptosporidiosis has been classified by the World Health Organization (WHO) as a ‘reference pathogen’ reflecting water quality (Medema et al. 2006) given the resilience of oocysts in water as well as in the environment (King and Monis 2007), the relatively high cost and limited availability of chemotherapeutic compounds or regimens for treatment in animals and humans (Greif et al. 2001; Mead 2002; Armson et al. 2003; Zardi et al. 2005; Caccio and Pozio 2006; Randhawa et al. 2012) including its socioeconomic impact. In the present study we report an outbreak of cryptosporidiosis in cross bred cattle calves of 1–2 months age maintained at an organized dairy farm of village Latala in Ludhiana district which was characterised by history of persistent profuse watery diarrhoea since...
1–2 weeks and was unresponsive to conventional treatment with gut acting chemotherapeutic agents (antibiotics, sulphonamides) dewormers (fenbendazole, ivermectin) and antidiarrhoal drugs (furazolidone, atropine sulphate, quiniodochlor). The report describes the clinical efficacy of metronidazole and furazolidone combination against cryptosporidiosis which is believed to be the first report on use of this drug combination against cryptosporidiosis.

Materials and methods

The faecal samples were collected to check for developmental stages of parasites viz. eggs, ova or oocysts and for bacterial culture. Observations of Cryptosporidium oocysts were made under compound microscope at oil immersion in stained (modified Zeihl–Neelsen) faecal smears prepared directly and after floatation in zinc sulphate solution.

In direct faecal smear examination, a thin and transparent faecal smear was made by with the help of ear bud or applicator stick and air dried. The air dried smear was fixed in methanol for 2 min, air dried and stained by modified Ziehl–Neelsen staining method (OIE 2008).

In concentration methods, faecal samples were suspended in floatation medium (33 % zinc sulphate solution, sp. gr. 1.18) followed by centrifuging at 3000 rpm for 5 min. The supernatant was collected, smears were made, air dried and fixed in methanol followed by modified Ziehl–Neelsen staining.

Blood samples were collected using disodium salt of ethylene diamine tetraacetic acid (EDTA) @ 2 mg/ml as anticoagulant for estimation of hematological parameters (Coles 1980) and to check for any haemoproteozoon infection.

Results and discussion

The diarrhoeic faeces were yellow to pale in colour or sometimes mixed with blood clots, mucus and undigested milk clots. Affected calves were showing varying degree of anorexia and were emaciated with marked loss in body condition. From a total of 28 diarrhoeic calves, the rectal samples collected were examined by direct and salt (33 % ZnSO₄) flotation methods for helminthic eggs and ova but were found negative. However, at 40× objective, we were able to see oocysts resembling Cryptosporidium. The bacterial cultures of the faecal samples were also found negative. Haematological studies (Hb 8.62 ± 0.29 g%, TLC 10119 ± 561 cells/mm², and N: 40.38 ± 4.54 %, L: 59.37 ± 4.50 %) of the diarrhoeic calves were not suggestive of any significant alteration except for mild degree of anaemia. The calves were found negative for haemoproteozoa. The faecal smears (direct as well as prepared after zinc sulphate floatation) stained with modified acid fast staining demonstrated the bright red coloured oocysts of Cryptosporidium confirming cryptosporidiosis (Fig. 1). Based on these findings treatment of all calves was started with a combination of metronidazole 1,000 mg, furazolidone 500 mg, loperamide hydrochloride 7.5 mg (Marcogyl-LM*) orally twice daily for 5 days along with oral supplementation of ORS @ 1 pack/calf morning and evening. The affected calves responded well to the treatment and no more signs of diarrhoea were observed. After 4th day of treatment the consistency of faeces improved whereas marked clinical improvement was observed in the condition of calves (Table 1). After 1 week of treatment no oocysts were detected in the stained faecal smears of the recovered calves and no mortality was observed among the treated cow calves thus indicating efficacy of combination therapy. The controls (without treatment) were passing the oocysts.

Presently, there are neither consistently effective nor approved antimicrobial drugs for treatment of cryptosporidiosis in animals. More than 200 compounds such as lasalocid, paromomycin, decoquinate, bovine hyperimmune colostrum have been tried to combat cryptosporidiosis (Dubey et al. 1990 and O’Donoghue 1995) in experimental conditions but none of the results establishing their efficacy in natural infection had been published. Metronidazole is a nitroimidazole with antiprotozoals activity especially against Giardia and bovine genital trichomoniasis. Its antiprotozoal activity is due to the short lived intermediates or free radicals who produce damage by interacting with DNA and possibly other molecules (Adams 2001). It is absorbed well from the gastrointestinal tract and reaches high concentration in the tissues and therefore is active.
against both luminal and extraluminal protozoa (Finch and Snyder 1986). Furazolidone is a synthetic broad spectrum nitrofuran compound. It is active against a wide range of organisms including protozoa. It interferes with the DNA synthesis by causing breakage in the DNA strands. It is used in foals @ 4.4 mg/kg three times daily orally for the treatment of diarrhoea (Bryans et al. 1965). In the present study combination of metronidazole and furazolidone @ one bolus/calf twice daily orally (average body weight of calf was 30 kg) was highly effective in clinical improvement of the calves with faecal samples negative for Cryptosporidium oocysts after 7 days post treatment. In none of these cases subsequent diarrhoea was observed during observation period of 2 months post treatment.

For the control of cryptosporidiosis in an organized farm primarily focus include cleanliness of maternity pens, calf housing and feeding equipment, separation of dam and calf at birth, as well as early detection of anorexia, diarrhoea, and dehydration in neonatal calves (Harp and Goff 1998; Nydam and Mohammed 2005). Though clinical signs do not allow differentiating it from bovine neonate enteritis due to corona virus or rota virus or Salmonella or E. coli, immediate laboratory diagnosis is must to adopt specific treatment as recommended. It is therefore inferred that staining of faecal smears with modified Ziehl–Nelson stain is diagnostic for confirmation of cryptosporidiosis and combination of metronidazole and furazolidone @ one bolus/calf twice daily orally for 5 days is very effective in treatment of cryptosporidial diarrhoea.

### References

Adams R (2001) Veterinary pharmacology and therapeutics, 8th edn. Iowa State University Press, Ames, pp 993–995

Armson A, Thompson RC, Reynoldson JA (2003) A review of chemotherapeutic approaches to the treatment of cryptosporidiosis. Expert Rev Anti Infect Ther 1:297–305

Bhat SA, Jyal PD, Singla LD (2012) Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. Asian J Anim Vet Adv 7:512–520

Bryans JT, Moore BO, Crowe MW (1965) Safety and efficacy of furoxone in the treatment of equine salmonellosis. Vet Med Small Anim Clin 60:626–633

Caccio SM, Pozio E (2006) Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. Expert Rev Anti Infect Ther 4:429–443

Coles EH (1980) Veterinary clinical pathology, 3rd edn. W. B. Saunders Company, London

Dubey JP, Speer CA, Fayer R (1990) Cryptosporidiosis of man and animals. CRC Press, Boston

Fayer R, Morgan U, Upton SJ (2000) Epidemiology of Cryptosporidium: transmission, detection and identification. Int J Parasitol 30:1305–1322

Finch RG, Snyder IS (1986) Antiprotozoal drugs. In: Craig CR, Stitzel RE (eds) Modern pharmacology, 2nd edn. Little, Brown, Boston, pp 729–740

Greif G, Harder A, Haberkorn A (2001) Chemotherapeutic approaches to protozoa: coccidia—current level of knowledge and outlook. Parasitol Res 87:973–975

Harp JA, Goff JP (1998) Strategies for the control of Cryptosporidium parvum infection in calves. J Dairy Sci 81:289–294

King BJ, Monis PT (2007) Critical processes affecting Cryptosporidium oocyst survival in the environment. Parasitology 134:309–323

MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP (1994) A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. N Engl J Med 331:161–167

Mead JR (2002) Cryptosporidiosis and the challenges of chemotherapy. Drug Resist Updates 5:47–57

Medema G, Teunis P, Blokker MD, Deere A, Davison A, Charles P, Loret JF (eds) Modern pharmacology, 2nd edn. Little, Brown, Boston, pp 729–740

Nydam DV, Mohammed HO (2005) Quantitative risk assessment of Cryptosporidium species infection in dairy calves. J Dairy Sci 88:3932–3943

O’Donoghue PJ (1995) Cryptosporidium and cryptosporidiosis in man and animals. Int J Parasitol 25:139–195

OIE (2008) Cryptosporidiosis. Terrestrial Manual chapter 2.9.4: 1192–15

Paul S, Chandra D, Ray DD, Tewari AK, Rao JR, Banerjee PS, Baidya S, Raima OK (2008) Prevalence and molecular characterization of bovine Cryptosporidium isolates in India. Vet Parasitol 153:143–146
Randhawa SS, Zahid UN, Randhawa Swaran S, Juyal PD, Singla LD, Uppal SK (2012) Therapeutic management of cryptosporidiosis in cross bred dairy calves. Indian Vet J 89:17–19
Singh BB, Sharma R, Kumar H, Banga HS, Aulakh RS, Gill JPS (2006) Prevalence of Cryptosporidium parvum infection in Punjab and its association with diarrhoea in neonatal dairy calves. Vet Parasitol 140:162–165
Slifko TR, Smith HV, Rose JB (2000) Emerging parasite zoonoses associated with water and food. Int J Parasitol 30:1379–1393
Smith HV, Rose JB (1998) Water borne cryptosporidiosis current status. Parasitol Today 14:14–22
Trotz WLA, Martin SW, Leslie KE, Duffield T, Nydam DV, Peregrine AS (2007) Calf-level risk factors for neonatal diarrhoea and shedding of Cryptosporidium parvum in Ontario dairy calves. Prev Vet Med 82:12–28
Xiao L, Fayer R, Ryan U, Upton SJ (2004) Cryptosporidium taxonomy: recent advances and implications for public health. Clin Microbiol Rev 17:72–97
Zardi EM, Picardi A, Afeltra A (2005) Treatment of cryptosporidiosis in immunocompromised hosts. Chemotherapy 51:193–196