A Case Report of Fungal Diarrhea in a Preweaned Calf in Iran

Saeed Azimpour,1 and Hadi Pourtaghi2,*

1Department of Clinical Sciences, Babol Branch, Islamic Azad University, Babol, IR Iran
2Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, IR Iran

*Corresponding author: Hadi Pourtaghi, Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, IR Iran. Tel: +98-9122763975, E-mail: hadi.pourtaghi@gmail.com

Published online 2016 May 1.
Int J Enteric Pathog. 2016 May; 4(2):e34199.
doi: 10.17795/ijep34199.

1. Introduction

Diarrhea is the most common cause of death in neonatal calves (1, 2). The highest risk period for diarrhea is from birth until about 1 month of age. There are a variety of causes of diarrhea in young calves (2), most of which are infectious agents (3-5). Diarrhea is often caused by more than one infectious agent acting together (3, 6-8). The most important agents of diarrhea in young calves include bacteria (Escherichia coli, Salmonella spp, Clostridium perfringens type C), viruses (Rotavirus, Coronavirus, bovine viral diarrhea [BVD] virus), and protozoa (Cryptosporidium, Eimeria spp, Giardia spp.). Because fungi are part of the normal microbial flora of the digestive system (2, 8), only limited attention has been paid to their role in calves’ diarrhea.

In recent years, there have been some reports indicating the possibility of a relation between fungi and diarrhea in human patients, particularly children (9-11). The presence of no other pathological microorganisms, failure of antibacterial therapy, and an isolation of yeasts in the feces indicate the possibility of involvement of the fungi in diarrhea in pediatric patients. Mycological investigation revealed that Candida albicans, Cryptococcus neoformans, Candida krusei, and other Candida species may play a role in calves’ diarrhea when it is not responsive to antibiotics (12).

2. Case Presentation

A 15-day-old female calf was referred to the veterinary faculty’s clinic in the Karaj branch of Islamic Azad university for dysentery treatment. The calf had had dysentery for the last 10 days despite good appetite. The calf had been treated with oxytetracycline tabulations for 5 days. No other clinical signs were seen. Fecal and blood samples were taken from diarrheic calves by rectal swab and plain Venoven, respectively. In the laboratory, a lot of fungal hyphae and red blood cells were seen under direct microscopy (Figures 1 and 2). The fecal sample was examined for Clostridium parvum and Coccidia. There were no Eimeria oocysts. Fecal bacterial culturing (for Salmonella spp., E. coli, and C perfringens) and reverse transcription polymerase chain reaction (RT-PCR for BVD) were carried out. To detect BVD, first, RNA extraction was performed using a Cinnagen Viral kit, which is capable of simultaneous isolation of viral DNA and RNA (Cat No: PR921733). Then, cDNA was synthesized through the following protocol: 8 µL of RNA, 1 µL of random hexamer (Cinnagen), and 3.5 µL of dH2O were denatured at 65°C for 5 minutes and cooled on ice. The following was added to each reaction tube as the template: 1 µL of M-MuLV reverse transcriptase (Cinnagen), 2 µL of 10x reaction buffer, 2 µL of dNTP (Cinnagen), 0.5 µL of ribonuclease inhibitor (Vivantis), and 2 µL dH2O, to give a final reaction volume of 20 µL. This was then incubated for 60 minutes and 10 minutes at 42°C and 70°C, re-
respectively. RT-PCR was carried out using the following protocol: PCR amplification of cDNA was carried out in a total volume of 25 mL containing 3 µL of cDNA, 12.5 µL of PCR Master Mix (Cinnagen), 1 µL of each primer (2.5 mM each; forward, 5’-ATGCCCTTAGTAGGACTAGCA-3’ and reverse, 5’-TCAACTCCATGTGCCATGTAC-3’) (13), and 7.5 µL of dH2O. The reaction mix was subjected to 35 cycles of 95°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute using a thermocycler. For visualization, 10 µL of the PCR products were submitted to electrophoresis in a 2% agarose gel in TBE (pH 8.4) under constant voltage (90 V) for approximately 45 minutes. The gel was stained with ethidium bromide (0.5 µg/mL) and visualized under ultraviolet (UV) light. The expected product size for the positive control was 288 bp, but no product was seen in the examined sample (Figure 3).

Figure 1. Direct Microscopic Examination With Pseudo-Hyphae of C. albicans (100x)

Figure 2. Smear Prepared From Stool, Stained With Malachite Green

Conkey sorbitol agar plates were used for bacterial culture. Colony morphology, physical characteristics of the bacteria, and biochemical tests were used to characterize and identify the isolated bacteria. MacConkey sorbitol agar was used to distinguish non-pathogenic E. coli from enterohemorrhagic E. coli. The Rota-Corona K99/ELISA Trikit (Pourquier, France) was used to detect rotavirus, coronavirus, and enterotoxigenic E. coli.

All bacterial and viral tests were negative. A lot of pseudo-hyphae of C. albicans were seen under direct microscopy. Streaking culture of stool was carried out on Sabouraud dextrose agar (SDA) including chloramphenicol to detect fungal infections. A pure yeast colonies was observed in SDA (Figure 4). To detect the type of colonies, an API 20C test kit was used, and C. albicans was confirmed. Antibiotic treatment was stopped, and the calf was fed with low amounts of milk more times per day. After a week, the severity of was diarrhea reduced.

3. Discussion

There are some difficulties in the interpretation of laboratory results in of calves’ diarrhea because some fecal bacterial isolates such, as E. coli or C. perfringens, are normal intestinal flora in most calves with infectious diarrhea (14). Clinicians use the findings from clinical signs, stool exams and stool culture, and the age of onset of diarrhea to determine the most likely cause of the diarrhea problem.

Yeasts and molds are sometimes associated with lesions in the stomach or intestines of scouring calves, but there is limited information about their role in calf diarrhea. In an epidemiological study, yeasts were found in the gastrointestinal tract of 34.8% of the examined samples, with Candida glabrata emerging as the most preva-
lent species (69.5%) in preweaned calves suffering from diarrhoea (12). Mycological investigation revealed that C. albicans, C. neoformans, C. krusei and other Candida species may play a role in calves’ diarrhea when it is unresponsive to antibiotics (15).

In this study, C. albicans was isolated in a 15-day-old dysenteric calf. These organisms are not likely the primary cause of diarrhea in calves, but like children, they are possibly opportunistic pathogens that proliferate and invade intestinal mucosa following antibiotic therapy (16). The Candida concentration in children’s stool has a positive association with recent antibiotic use and malnourishment (16, 17). The mechanism by which Candida causes diarrhea is not yet clear (11). In humans, fungal infections occur as a result of non-invasive proliferation and lead to diarrhoea and sometimes systemic disease, especially in immunocompromised persons (11). Candida and its species are the predominant fungal species in the normal person’s alimentary tract, but in some conditions, they may cause enteritis and acute diarrhea in children (11, 18, 19).

Culture of stool is not useful in normal persons because Candida spp. are part of the normal flora of the alimentary tract (20). However, the presence of an increased number of yeast cells with or without the mycelial form under direct microscopic examination suggests non-invasive fungal infection of the intestine (20).

Acknowledgments

We want to thank Islamic Azad University, Karaj branch for material support.

References

1. Hur T, Jung Y, Choe C, Cho Y, Kang S, Lee H, et al. The dairy calf mortality: the causes of calf death during ten years at a large dairy farm in Korea. Korean J Vet Res. 2013;53(2):103–8. doi: 10.4145/kjvr.2013.53.2.103.
2. Izzo MM, Kirkland PD, Mohler VL, Perkins NR, Gunn AA, House JK. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. Aust Vet J. 2018;95(5):467–73. doi: 10.1111/avj.12884.
3. Cho Y, Yoon K. An overview of calf diarrhoea—Infectious etiology, diagnosis, and intervention. Journal of veterinary science. 2014;15(1):1-7.
4. Rings DM. Clostridial disease associated with neurologic signs: tetanus, botulism, and enterotoxemia. Vet Clin North Am Food Anim Pract. 2004;20(2):379–91. doi: 10.1016/j.cvfa.2004.02.006. [PubMed: 15201231] vii-viii.
5. Schultze B, Gross HJ, Bressmer R, Herrer G. The S protein of bovine coronavirus is a hemagglutinin recognizing 9-O-acetylated sialic acid as a receptor determinant. J Virol. 1991;65(11):6326–7. [PubMed: 1920610].
6. Bartels CJ, Holzhauer M, Jorritsma R, Swart WA, Lam TJ. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev Vet Med. 2010;93(2-3):162–9. doi: 10.1016/j.prevetmed.2009.09.020. [PubMed: 19819774]
7. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123–40. doi: 10.1038/nrmicro818. [PubMed: 15040260] viii.
8. Barker IK, Van Dreumel AA, Palmer N. The Alimentary System. 1993:1–318. doi: 10.1016/b978-0-12-391606-8.00009-0. viii.
9. Levine J, Dykoski RK, Janoff EN. Candida-Associated Diarrhea: A Syndrome in Search of Credibility. Clinical Infectious Diseases. 1995;21(4):881–6. doi: 10.1093/clinids/21.4.881.
10. Elad D, Brenner J, Markovics A, Yakobson B, Shlomovitz S, Rasan J. Yeasts in the gastrointestinal tract of preweaned calves and possible involvement of Candida glabrata in neonatal calf diarrhea. Mycopathologia. 1998;144(1):7–14.
11. Talwar P, Chakrabarti A, Chawla A, Mehta S, Walia BN, Kumar L, et al. Fungal diarrhoea: association of different fungi and seasonal variation in their incidence. Mycopathologia. 1990;110(2):101–5. [PubMed: 2368849].
12. Elad D, Brenner J, Markovics A, Yakobson B, Shlomovitz S, Rasan J, Mycopathologia. 1998;144(1):7–14. doi: 10.1023/a:1006860418915.
13. Vlcek S, Herring AJ, Herring JA, Nettleton PF, Lowings JP, Paton DJ. Fivestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch Virol. 1994;136(3-4):309–21. [PubMed: 8036216].
14. Radostits OM, Gay CC, Hinchcliff KW, Constable PD, Jacobs DE, Ikede BO, et al. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 2007.
15. Nayal M, Salama A, Elsify A, Allam M, Ballal S, Eldesoky I, et al. Prevalence of yeasts in diarrheic calves with molecular identification of Candida albicans. 3rd Annual International Conference on Advances in Veterinary Science Research. Bangkok, Thailand.

16. Ponnuvel KM, Rajkumar R, Menon T, Sankaranarayanan VS. Role of Candida in indirect pathogenesis of antibiotic associated diarrhoea in infants. Mycopathologia. 1996;135(3):145-7. [PubMed: 9066955].

17. Gracey M, Stone DE. Microbial contamination of the gut: another feature of malnutrition. Am J Clin Nutr. 1973;26(1):1170-4. [PubMed: 420121].

18. Chaudhury A, Nath G, Shukla B, Panda S, Singh TB. Diarrhoea associated with Candida spp.: incidence and seasonal variation. J Diarrhoeal Dis Res. 1996;14(2):110-2. [PubMed: 8870405].

19. Ahmed T, Ali M, Ullah MM, Choudhury IA, Haque ME, Salam MA, et al. Mortality in severely malnourished children with diarrhoea and use of a standardised management protocol. The Lancet. 1999;353(9168):1919-22. doi: 10.1016/S0140-6736(98)07499-6.

20. Warren NG, Shadomy HJ. Yeasts of medical importance. Manual of clinical microbiology. 5th ed. American Society for Microbiology, Washington, DC. 1991:817-29.