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Importance of colostrum IgG antibodies level for prevention of infection with *Cryptosporidium parvum* in neonatal dairy calves

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ABSTRACT

Cryptosporidiosis is one of the most common zoonosis worldwide, causing intestinal infection to both humans and livestock. The purpose of this study was to assess whether the level of anti-*C. parvum* IgG antibodies transferred through colostrum from dams to newborn calves impacts the susceptibility to cryptosporidiosis. A number of 50 dams and their healthy newborns were included in the study. Colostrum samples were collected within 12 h after birth and anti-*C. parvum* IgG antibody levels were determined by single radial immunodiffusion. The health condition of the newborns was daily monitored, and fecal samples were collected at first diarrheic episode of a calf. In all dams, the *anti-C. parvum* IgG antibody concentration in colostrum varied between 570 and 4070 mg/dl; in dams who gave birth to calves with diarrhea and were *C. parvum*-positive, the antibody concentration in colostrum varied between 680 and 3680 mg/dl (Table 1). The point-biserial correlation showed a negative correlation between the levels of anti-*C. parvum* antibodies and manifestation of clinical cryptosporidiosis (r = -0.425). Our findings highlight the importance of IgG levels in colostrum received by neonatal calves during their first day of life for prevention of *C. parvum* infection.

1. Introduction

Cryptosporidiosis is caused by apicomplexan protozoans of the genus *Cryptosporidium* and is one of the most common agents causing intestinal infection to both humans and livestock worldwide (Chalmers and Katzer, 2013). The parasite infects the digestive tract of the major vertebrate groups, including humans, livestock (cattle, sheep, goats, horses and poultry), pets (dogs and cats), etc. (Chalmers and Katzer, 2013). In cattle, *Cryptosporidium* spp. infection was first reported in the early 1970s (Panciera et al., 1971). Of the four species commonly found in cattle (*C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*), only *C. parvum* is generally associated with clinical disease, especially in neonatal calves (Fayer, 2010; Sunnotel et al., 2006; Thompson, 2008). *C. ryanae* and *C. bovis* usually infect older calves and young stock, and *C. andersoni* mainly adults (Langkjær et al., 2007). Experimental infections have been carried out and it has been shown that infected animals shed oocysts with no clinical manifestations of the disease (Thompson, 2008). The importance of *C. parvum* as an etiological agent of gastroenteritis-like syndrome in dairy calves during their first weeks of life has been confirmed (Sunnotel et al., 2006). The main clinical signs are diarrhea, dehydration, weakness, anorexia, abdominal pain and mortality (Fayer, 2010).

Cryptosporidiosis has been studied in many countries and its prevalence ranges from 3.4 %–96.6 % in pre-weaned calves (Thomson et al., 2017). In Europe, it is considered the most important disease in neonatal calves (Torsein et al., 2011). In Greece, it is a common pathogen associated with neonatal calf diarrhea in dairy cattle farms (Panousis et al., 2007). It causes economic loss (Torsein et al., 2011) and has a negative impact on public health (Chalmers and Katzer, 2013; Fayer, 2010). The zoonotic nature of *C. parvum* has been confirmed by many studies and new subtypes with infectious potential in both humans and animals have been detected (Chalmers et al., 2011; Fayer, 2010).

Zoonotic infection occurs most-likely in individuals who have been long in contact with infected animals (Gait et al., 2008). One of the most important measures to control diarrhea in neonatal calves is colostrum management. The passive transfer of maternal antibodies to newborn calves may reduce the neonatal diarrhea caused by *C. parvum* (Furman-Fratczak et al., 2011).

The purpose of this study was to determine whether the level of

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specific anti-*C. parvum* IgG antibodies transferred from dams to neonatal calves through colostrum impacts the development of clinical manifestation of cryptosporidiosis among animals from farms with high prevalence of *C. parvum* infection.

2. Materials and methods

2.1. Animals and study design

This study was conducted during January 2018 and August 2018 in the prefecture of Xanthi, North Greece in five dairy farms with Holstein – Friesian cows. All farms had documented past records of *C. parvum* infection in neonatal calves. The first ten calves born within the investigation period in each farm and their dams were included in the study. To determine the level of specific IgG antibodies against *Cryptosporidium* in colostrum, the samples were collected within 12 h after birth. Antibody concentration was determined using the single radial immunodiffusion (SRID).

All neonatal calves suckled their dam in the first two hours postpartum. The health condition of calves was daily monitored during the first month of life and fecal samples were collected after diarrhea had been confirmed during this period. Diarrhea in calves is described as manure of looser consistency than normal that persists for 2 or more days, which color can be brown, grey, green or yellow and may contain mucus and blood. The fecal samples were analyzed using an immunochromatographic assay for the four major infectious causes of diarrhea in calves aged up to 30 days: *Rotavirus*, *Coronavirus*, *Escherichia coli* (K99) and *C. parvum.* All samples were transferred and examined at the Laboratory of Parasitology and Microbiology of Veterinary Faculty, University of Thessaly.

2.2. Single radial immunodiffusion

Each dam's udder was washed and disinfected with cleaning towelette (MSD). Colostrum samples (100 ml) were individually collected and transferred into sterile tubes labelled with the dam's ear tag number. All samples were stored at − 20 °C until IgG analysis. SRID was performed according to the manufacturer's instructions to determine the specific anti-*C. parvum* IgG antibody concentration in colostrum (SRID kit from VMRD Inc. Pullman, WA). The SRID kit contains four reference standards (IgG concentration: 400, 800, 1.600 and 3.200 mg/dl) and 5 plates (12 wells per plate) with mono-specific antisera in buffered agarose. Colostrum samples were transported and further processed at room temperature and fat was removed through centrifugation. An amount of 3 μl of each reference standard was placed into the first wells and the same amount of colostrum sample in the remaining wells, by using a 3 μl precision pipette. The plates were covered and left right-side up at room temperature for 24 h. At 24 h, the diameter of each ring was read by direct measure on the plate and a standard curve was established. The diameters of the reference standards versus their concentrations were plotted on the graph paper provided in the kit. A standard curve was subsequently drawn and used for determination of *Cryptosporidium* IgG antibody concentrations in the colostrum samples.

2.3. Immunochromatographic assay

The fecal samples were individually collected from the rectum of each calf using sterile plastic gloves and placed into a clean plastic container, which was labeled with the calf's ear tag number. The sealed samples were stored in an insulated box packed with ice or frozen refrigerant packs. The samples were analyzed by using the immunochromatographic assay for *Rotavirus*, *Coronavirus*, *E. coli* (K99) and *C. parvum* in bovine feces. The analysis was performed using the commercially available dip–stick assay (BIO K156 – Tetra Calf Scours, BioX Diagnostics S.A, Belgium), according to the manufacturer's instructions. Approximately 0.1 g of feces from each sample was diluted with the reagent provided in the kit. The test strip was dipped into the homogeneous suspension and read after 3 and 10 min of contact. The presence of control line confirmed the validity of the test. Positive results were read as follows: red strip – Rotavirus-positive, blue strip – *E. coli* F5-positive, yellow strip – Corona virus-positive and green strip – *Cryptosporidium* positive. There were no mixed infections from the other pathogens tested.

2.4. Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics 19.0 software. One-way ANOVA was used to compare the mean anti-*C. parvum* IgG antibody concentrations detected in colostrum between farms. *t*-test was used to compare the mean anti-*C. parvum* IgG antibody concentration in the calves with positive and negative results for *C. parvum*. A statistically significant difference was considered if $p \leq 0.05$. A point biserial correlation was run to determine the relationship between the level of *C. parvum* IgG antibodies received by a calf and its susceptibility to cryptosporidiosis. The results were considered statistically significant if $p \leq 0.01$.

3. Results

Among the 50 calves included in the study, 18 (36 %) had diarrhea in the first 30 days of life. Of these, 13 (72 % of diarrheic calves) were positive for *C. parvum* without mixed infection with *E coli.*, Coronavirus and Rotavirus.

In all dams, the anti-*C. parvum* IgG antibody concentration in colostrum varied between 570 and 4050 mg/dl; in dams who gave birth to calves with diarrhea and were *C. parvum*-positive, the antibody concentration in colostrum varied between 680 and 3680 mg/dl (Table 1). No statistically significant difference in the level of anti-*C. parvum* IgG antibody concentration in colostrum was observed between farms ($p = 0.3165$) (Fig. 1A). The levels of anti-*C. parvum* IgG antibody concentration in colostrum received by calves with clinical signs of cryptosporidiosis (diarrhea) was significantly lower than in the colostrum received by calves that remained healthy ($p = 0.02$) (Fig. 1B). The point-biserial correlation showed a negative correlation between the levels of anti-*C. parvum* antibodies and the manifestation of clinical cryptosporidiosis ($r = -0.425$), which was statistically significant ($p = 0.002$, 2-tailed) (Fig. 2).

4. Discussion

Importance of colostrum in the passive transfer of immunity and protection of off-springs is well known, as antibodies do not pass through the cow's placenta to the fetus. It contains high concentrations of nutrients, hormones, cytokines, antibodies and a variety of maternal leukocytes that appear to support the growth and maturation of the calf. The neonate calves have a great ability to absorb immunoglobulins

Table 1
IgG antibody concentration in colostrums that calves received in each farm.

Colostrum sample	Farm A	Farm B	Farm C	Farm D	Farm E
1	2450	3480	**750**	**680**	650
2	**1650**	1750	860	1950	570
3	2850	**1810**	**900**	2340	1930
4	3350	2760	2160	3310	**3680**
5	**3460**	3280	3460	**890**	3970
6	3260	3100	**1890**	2890	4050
7	2860	**2970**	**1280**	3210	3890
8	2100	3150	3760	3490	3850
9	**2370**	**850**	3350	1870	3690
10	3210	1750	2890	1960	3980

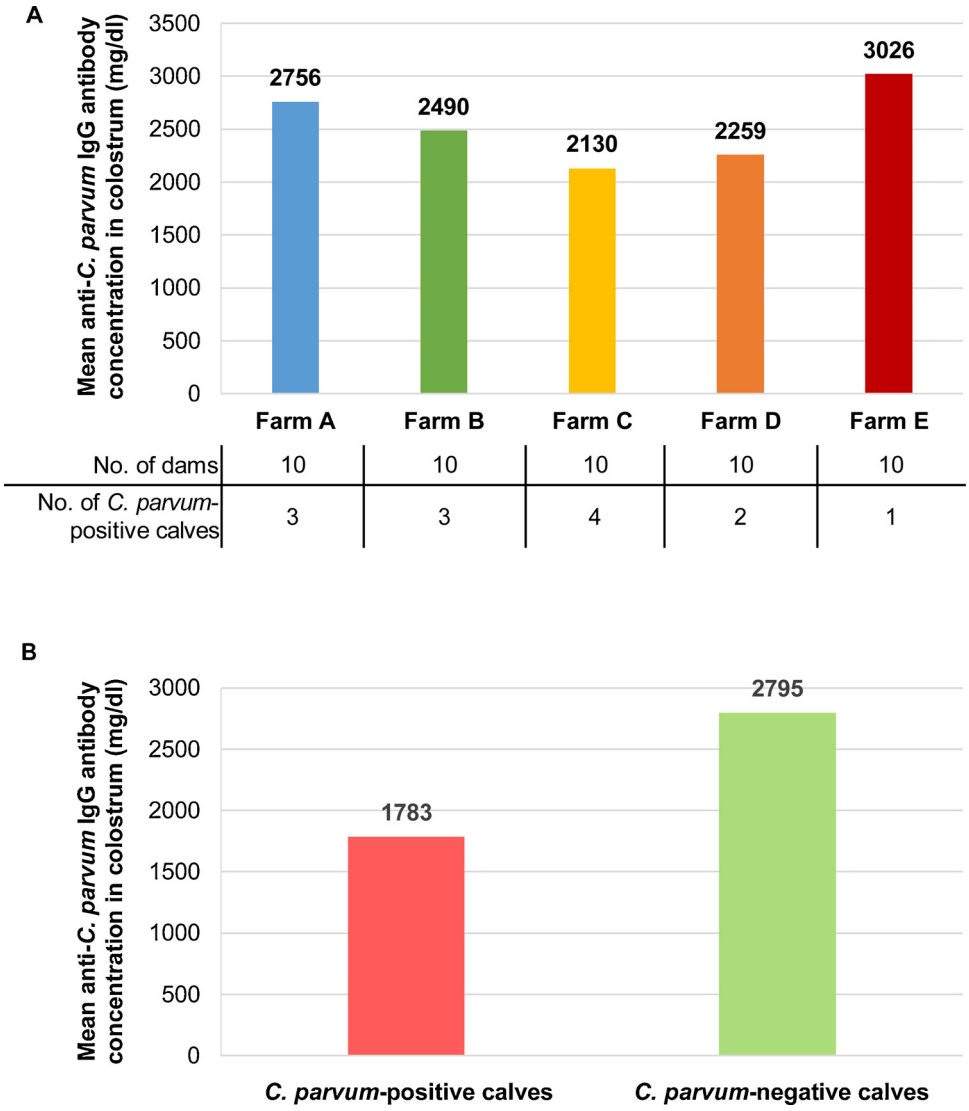

Fig. 1. A. Mean anti-C. parvum IgG antibody concentration in colostrum observed in each farm. (B) The levels of anti-C. parvum IgG antibody concentration in colostrum received by calves with clinical signs of cryptosporidiosis (diarrhea) compared to the colostrum received by calves that remained healthy.

and growth factors from the gut (e.g. IgA, IgM, IgG, IGF-1, lactoferrin, and lysozyme) (Costa et al., 2017). Quality of colostrums is always a crucial factor which ensures that there is sufficient amount of antibodies for absorption by the calves.

Previous studies have shown the existence of *C. parvum*-specific antigenic epitopes on oocysts, which trigger the production of *C. parvum*-antibodies that are detected in serum and colostrum (Jenkins et al., 1998). Arsenopoulos et al. (2017) referred that colostrum

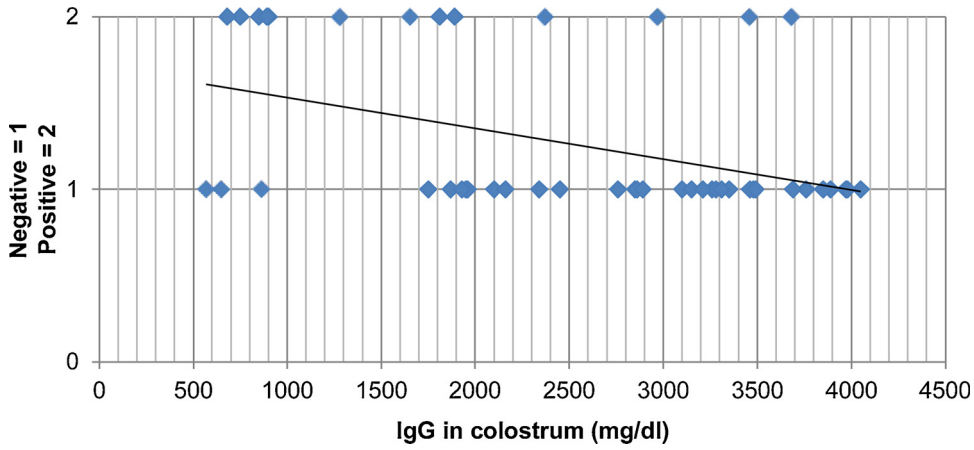

Fig. 2. Correlation between the levels of anti-*C. parvum* antibodies and the manifestation of clinical cryptosporidiosis.

management and season of the year influence both the number of *Cryptosporidium spp*. oocysts seeding and the fecal consistency. The antibodies transferred through colostrum offered partial protection against low dose of *C. parvum*-infection (Jenkins et al., 1998).

Factors that contribute to the quality of colostrum are the breed and the age of the cow, the season of the year, the appropriate management of cow during the dry period, quality of nutrition and the length of the period are significant. Furthermore, stress and extreme environmental conditions should always be avoided in order to keep a high quality colostrum.

Cryptosporidiosis can cause high rates of morbidity and mortality in calves (Thompson, 2008). Calves acquire *C. parvum*-infection shortly after birth and are at a great risk of infection until the age of 1 month (Santín et al., 2008).

Humans working in close contact with calves are also at risk of *C. parvum* infection (Siwila et al., 2007; Panousis et al., 2007). In addition, massive infections can also be caused by water contamination with domestic or wild animal feces. It is well known that bovine cryptosporidiosis has a serious financial impact on the infected farms. The exact cost should be calculated in each circumstance separately, but surely the cost of treatment and management of enteritis, reduced feed conversion and production efficiency and losses due to animal death should be included in this (Thomson et al., 2017). Preventive care measures should be taken by individuals who are in close contact with infected animals and special attention should be given to the waste management in farms.

Many studies have been carried out to detect *C. parvum* by using ELISA, and it has been shown that this is the most common species found in calves until the age of one month (Langkjær et al., 2007). In our study, SRID method was used to quantify *C. parvum* IgG antibody concentration in colostrum and the immunochromatographic assay for detection of *C. parvum* bovine feces. The use of immunochromatographic assay for diagnosis of *C. parvum* infection in individual animals or for screening of animal groups could provide practitioners and researchers a quick and accurate method for detection of infection in young calves at a low cost (Trotz-Williams et al., 2005).

Our findings highlight the importance of IgG levels in colostrum fed to neonatal calves during their first day of life for prevention of *C. parvum* infection. The results show a negative correlation between the passive transfer of anti-*C. parvum* IgG antibody through colostrum during the first day of life and the appearance of cryptosporidiosis during the first 30 days of life in calves. These findings suggest that newborn calves are partially protected by colostral antibodies during their first day of life. Similar results have been published by Wang et al. (2003). Our results highlight the importance of colostrum management in prevention of neonatal diarrhea caused by *Cryptosporidium* spp. in calves and are in line with results from previous studies. Preventive colostrum management leads to protection of calves against cryptosporidiosis through passive immunity (Arsenopoulos et al., 2017; Furman-Fratczak et al., 2011). Moreover, a linear relationship between IgG absorption efficiency and IgG density in colostrum has been demonstrated (Stott and Fellah, 1983).

We recommend preventive measures against Cryptosporidiosis to be taken in neonatal calves who have been fed with colostrum containing lower levels of anti-*C. parvum* IgG antibodies than the mean level of the farm. These measures include suitable colostrum management and improvement of the immunological status of dams and calves through optimized housing and hygiene conditions, adequate nutrition, elimination of stress factors and other infectious agents, and preventative medication.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.104904.

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