Research Article

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Investigation and Analysis on the Pathogen of Calf Diarrhea in Henan Province

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ABSTRACT

In order to understand the prevalence of various pathogens of calf diarrhea in Henan Province, *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV, BRV antigens or antibodies were detected in feces and blood samples of 132 calves with diarrhea under 6 months in Henan Province by ELISA method. The results showed that the average positive rates of *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV were 28.03%, 6.82%, 6.06%, 5.03%, 3.79% and 2.27%, respectively. The prevalence of *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV exists in most areas of Henan Province, especially in central, southern and northern Henan. In addition to the single infection types of C. parvum, BVDV, E. coli K99, MAP, BCoV and BRV, there are also many kinds of mixed infection types. The proportion of single infection and mixed infection is 17:3. There are many types of mixed infection and the situation is complicated. The infection of MAP in diarrhea calves in Henan Province was reported for the first time. The results showed that there were a variety of single and mixed infection of calf diarrhea in Henan Province, and the single and mixed infection of *C. parvum* was more serious, which provided a reference for comprehensive prevention and control of calf diarrhea in Henan Province.

ABBREVIATIONS: CP: *Cryptosporidium Parvum*; EC: *Escherichia Coli*; MAP: *Mycobacterium Paratuberculosis*

INTRODUCTION

Calf diarrhea is a digestive tract disease of gastrointestinal dysfunction that often occurs in newborn young cattle, with dyspepsia, diarrhea, dysentery and other main symptoms [1], resulting in massive dehydration of calves, imbalance of water and salt metabolism of the body, auto poisoning and other metabolic syndrome [2], which is easy to cause poor growth and development, prolonged growth cycle, secondary infection and serious death of sick calves. It is also known as the "newborn calf killer" [3], which has caused huge economic losses to the rancher. There are many factors causing diarrhea in calves, which can be divided into infectious factors and non-infectious factors. Among the infectious factors, of *Cryptosporidium parvum* (*C. parvum*), of bovine viral diarrhea virus (BVDV), of bovine rotavirus (BRV), of bovine coronavirus (BCoV), *Escherichia coli* K99 (*E. coli* K99), which are widely studied and reported [4,5,6].

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Mycobacterium avium subsp. of *Mycobacterium paratuberculosis*. (*MAP*) is a pathogen causing chronic enteritis in ruminants, widely distributed all over the world, and causing significant economic losses [7], but there are few reports about calf *MAP*.

In order to understand the epidemic situation of the pathogen of calf diarrhea in Henan Province, the ELISA method was used to detect the prevalence of *C. parvum*, BRV, BCoV, *E. coli* K99, BVDV, *MAP* antigen or antibody in calf feces and blood samples. The test results were statistically analyzed by region and infection type, so as to provide reference for the prevention and control of calf diarrhea in Henan Province.

MATERIALS AND METHODS

From 2018 to 2019, 132 faeces and corresponding blood samples of calves with obvious diarrhea symptoms were collected from large-scale farms in central, northern, southern, eastern and western Henan province (54 in central Henan, 36 in northern Henan, 22 in southern Henan, 11 in eastern Henan and 9 in western Henan).

Detection of *C. parvum* in fecal samples by ELISA (IDEXX, USA, P0063-1), detection of *MAP* in blood samples by ELISA (IDEXX, USA, P071305), detection of BVDV in blood samples by ELISA (IDEXX, USA, 99-43830), detection of BRV, BCoV *E. coli* K99 in fecal samples by triple antigen ELISA(IDEXX, USA, P0065-1), following the steps provided by the manufacturer. The detection results of 132 blood and fecal samples were counted, and the individual and mixed infections of six pathogens were analyzed.

RESULTS AND ANALYSIS

ELISA Test Results

The pathogen infection of *C. parvum*, BRV, BCoV, *E. coli* K99, BVDV and *MAP* in feces and blood samples of diarrhea calves from southern Henan, northern Henan, central Henan, western Henan and eastern Henan were detected by ELISA method. the positive infection rates were 28.03%, 6.82%, 6.06%, 5.03%, 3.79% and 2.27%, respectively. The detection of pathogens in each area is shown in Figure 1.

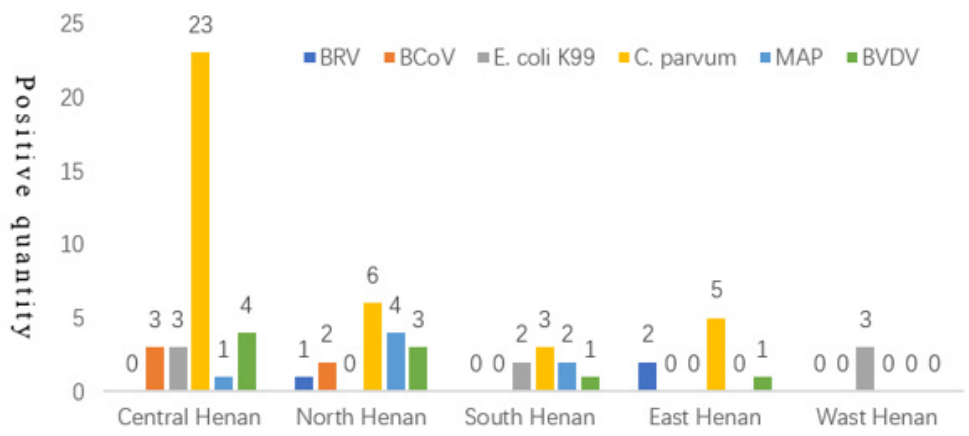

Figure 1: Distribution of calf diarrhoea pathogens in Henan Province.

A total of 37 *C. parvum* antigen positive samples were detected in feces, with an average positive rate of 28.03%. *C. parvum* infection was found in central, southern, eastern and northern Henan, with the highest positive rate of 45.45% in eastern Henan and 42.59% in central Henan. A total of 9 blood BVDV antigen positive samples were detected, with an average positive rate of 6.82%. There was an epidemic of BVDV in central, southern, eastern and northern Henan, with the highest positive infection rate of 9.09% in eastern Henan, followed by 8.33% in northern Henan. A total of 7 MAP antibody positive samples were detected in blood, with an average positive rate of 5.03%. There was MAP infection in central, northern and southern Henan, with the highest positive infection rate of 11.11% in northern Henan, followed by 9.09% in southern Henan. A total of 8 *E. coli* K99 antigen positive samples were detected in feces, with an average positive rate of 6.06%. *E. coli* K99 infection existed in central, southern and western Henan, with the highest positive infection rate of 33.33% (3/9) in western Henan, followed by 9.09% (2/22) in southern Henan. A total of 5 BCoV antigen positive samples were detected in feces, with an average positive rate of 3.79%. There was BCoV infection in central and northern Henan, and the positive infection rates in central and northern Henan were 5.56%. A total of 3 stool samples with positive BRV antigen were detected, with an average positive rate of 2.27%. There was an epidemic of BRV in eastern and northern Henan, with the highest positive infection rate of 18.18% (2/11) in eastern Henan, followed by 2.8% (1/36) in northern Henan.

Analysis of Single and Mixed Infection

The single infection and mixed infection of six pathogens *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV were statistically analyzed in the feces and blood samples of calves with diarrhea. The results are shown in Table 1. There is a single infection of six pathogens *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV, among which the single infection rate of *C. parvum* is the highest, reaching 28.03%, followed by BVDV, 6.82%. In terms of mixed infection, there are five mixed infection types: BVDV / *C. parvum*, *MAP* / *C. parvum*, BRV / *C. parvum*, *E. coli* K99 / *C. parvum* and BVDV / *E. coli* K99. Among them, the mixed infection types of BVDV / C. parvum, *MAP* / *C. parvum* and *E. coli* K99 / *C. parvum* are more serious, and the infection rates are 15.79%, 15.79% and 10.53%, respectively. The above results showed that the main causes of diarrhea in calves in Henan Province were single infection and mixed infection of six pathogens, *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV. There were many types of mixed infection, and the situation was more complicated. The situation of single infection and mixed infection of *C. parvum* was more serious.

Table 1: Statistical Analysis of single infection type and mixed infection type.

Type	Detection Quantity	Positive Quantity	Positive Rate
BVDV	132	6	4.55%
MAP	132	4	3.03%
C. parvum	132	29	21.97%
BRV	132	2	1.52%
BCoV	132	5	3.79%
E. coli K99	132	5	3.79%
BVDV / *C. parvum*	132	2	1.52%
MAP / *C. parvum*	132	3	2.27%
BRV / *C. parvum*	132	1	0.76%
E. coli K99 / *C. parvum*	132	2	1.52%
BVDV / *E. coli* K99	132	1	0.76%

DISCUSSION

In recent years, with the rapid development of cattle industry, calf diarrhea has been paid more and more attention by researchers at home and abroad, and a large number of investigations on the prevalence of pathogens related to bovine diarrhea have been carried out. Dai et al. [8] used the rapid detection kit to detect 55 calf diarrhea samples in Yangling City, China. It was found that the overall infection rate was Cryptosporidium 35.7%, BRV 21.4% *E. coli* K99 21.4%, BVDV 0.0%, respectively. Wang et al. [9] isolated and identified *E. coli* of calf diarrhea in Hebei area. The results showed that more than 90% of, *E. coli* K99 was the main pathogenic bacteria. Song [10] isolated and identified E. coli from calves in some areas of Xinjiang and found that *E. coli* K99 was one of the main pathogens causing diarrhea and death of newborn calves in cattle farms. Zhang [11] used RT-PCR method to detect BRV in the feces of 91 calves with diarrhea in some areas of Xinjiang and found that the positive rate of rotavirus in each cattle farm was 23.08% 90.91%. Zhao [12] used PCR method to investigate the pathogen of 147 calf diarrhea fecal samples collected in Henan Province. It was found that the positive rates of *C. parvum*, BRV and BCoV were 14.97%, 12.24% and 4.76%, respectively. The positive rate of *E. coli* K99 was 2.04%.

Lee et al. [13] detected a total of 14 pathogens in 207cases of diarrhea calves in Korea by PCR. The most common pathogens were BRV (34.8%), *E. coli* (22.0%), BVDV (8.5%), BCoV (7.9%) and *Cryptosporidium* (7.3%). Yong et al. [14] used PCR method to investigate the prevalence of 11 intestinal pathogens in the feces of diarrhea calves from cattle farms in the Midwest of the United States, and multiple logical regression model was used to analyze the correlation between diarrhea and the detection of various pathogens. The results showed that more than 50% of the fecal samples of diarrhea calves contained a variety of pathogens. Statistical analysis showed that, BRV A group, BCoV, *E. coli* K99 and *C. parvum* were significantly correlated with calf diarrhea. Among them, *C. parvum* and BRV are the most common intestinal pathogens of calf diarrhea, with a high detection rate of 33.7% and 27.1%, respectively.

In order to provide reference for comprehensive prevention and control of calf diarrhea in Henan Province, and to enrich the pathogenic epidemiological data of calf diarrhea in Henan Province, fecal and blood samples of 132 calves with obvious diarrhea symptoms were collected from 12 cities of Henan Province, and the related pathogenic antigens or antibodies were detected by ELISA method. The results showed that *C. parvum*, BVDV, *E. coli* K99, *MAP*, BCoV and BRV infection existed in most areas of Henan Province, and the average positive rates were 28.03%, 6.82%, 6.06%, 5.03%, 3.79% and 2.27%, respectively. *C. parvum*, BVDV, *E. coli* K99 and *MAP* infection were more serious, mainly single infection, and there were many types of mixed infection. The proportion of single infection and mixed infection was 17:3. Among them, the positive infection rate of *C. parvum* is higher than that of Zhao [12] in Henan, and lower than that of Yong [14] in the Midwest of the United States, which may be due to geographical factors, sampling quantity and different detection methods [15]. *C. parvum* is dominant in single infection and mixed infection, and it is easy for other pathogens to cause mixed infection, which may be due to the decrease of immunity of calves caused by *C. parvum*, which makes calves susceptible to other pathogens. It was found that the positive rate of *C. parvum* was the highest in single infection and mixed infection. So far, no specific drug has been developed to treat animal *C. parvum* [16], which also increases the difficulty of prevention and control of calf diarrhea in Henan Province.

MAP mainly affects domestic ruminants such as goats, sheep and cattle, causing chronic enteritis, which is one of the main pathogens of diarrhea in adult cattle [17] and causes serious economic losses to the dairy industry all over the world [18]. In the United States, the annual loss of the cattle industry affected by *MAP* is estimated to be about $1.5 billion [19]. *MAP* infects a wide range of hosts, infecting wild animals as well as domestic ruminants. However, there are few studies on calves. In this experiment, the prevalence of *MAP* antibodies in the blood of calves with diarrhea in Henan Province was reported for the first time, and the total antibody positive rate was 5.03%. Therefore, attention and detection of calf *MAP* should be strengthened.

CONCLUSION

Sum up the above, there are multiple pathogenic infections in calf diarrhea in Henan Province, with a single pathogen as the main infection, and *C. parvum* alone and mixed infection are serious. So, the attention of calf diarrhea in Henan Province should be strengthened, at the same time, the pathogen detection and

surveillance of calf diarrhea should be strengthened, and the scope of pathogen detection should be expanded. The infected calves should be isolated and eliminated in time, and gradually establish and maintain disease-free herds, to achieve healthy breeding of dairy cows in Henan Province.

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