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Dominance of *Enterocytozoon bieneusi* genotype J in dairy calves in Xinjiang, Northwest China

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ABSTRACT

Enterocytozoon bieneusi is the most common microsporidia species in humans and has a variety of animal hosts. To assess the prevalence and molecular characteristics of *E. bieneusi* in dairy calves in the Xinjiang Uyghur Autonomous Region of China, 514 fecal samples were collected from 15 farms and examined by polymerase chain reaction based on the internal transcribed spacer of the ribosomal RNA gene of *E. bieneusi*. The overall prevalence of *E. bieneusi* in calves was 16.5% (85/514). No significant difference in prevalence was observed between pre- and post-weaned calves. Sequence analysis of ITS nucleotide sequences identified six known genotypes (BEB4, CC4, D, I, J, and EbpC), five of these previously detected in humans. Genotype J was the most prevalent genotype (57/85) and was identified on 11 farms. The high prevalence of zoonotic *E. bieneusi* genotypes in dairy calves suggests they are a potential source of zoonotic infection in humans.

1. Introduction

Microsporidia are a large and diverse group of unicellular eukaryotes that have diverse vertebrates and invertebrate hosts [1]. The phylum microsporidia contains approximately 150 genera and 1300 species; 14 species in eight different genera have been documented in human infections [2]. Within the four major human-pathogenic microsporidian species, *E. bieneusi* is the most prevalent, causing >90% cases of human microsporidiosis [3]. *E. bieneusi* is distributed worldwide and has a broad range of hosts, including humans and domestic and wild birds and animals. It is frequently detected in immunocompromised hosts, notably AIDS patients and children, caused by chronic diarrhea and wasting syndrome [1,3].

E. bieneusi is mainly transmitted fecal orally, and has been commonly identified in water sources and domestic and wild animals, indicating that the pathogen may be waterborne and is potentially zoonotic [4]. Genotyping of *E. bieneusi*, based on the polymorphisms of the internal transcribed spacer (ITS) of the rRNA gene, has identified >200 genotypes [5]. Using phylogenetic analysis, all the ITS genotypes of *E. bieneusi* are divided into nine different groups [5]. Group 1 is frequently found in both humans and animals, and is therefore considered to have zoonotic potential [6]. To date, >40 genotypes of *E. bieneusi* have been identified in cattle [7–10]. Among them, genotypes Peru6 (syn. PtEbI, PtEbVII), EbpA (syn. F), I (syn. BEB2, CebE), J (syn. BEB1, CebB, PtEbX), Type IV (syn. K, Peru2, PtEbIII, BEB5, BEB5-var), D (syn. PigITS9, WL8, Peru9, PtEbVI, CEbC), BEB4 (syn. CHN1), BEB6 (syn. SH5), CHN3, CHN4, CS-4, O and EbpC (syn. E, WL13 , Peru4, WL17) have been identified in cattle and humans [3,7–12], indicating the possible role of cattle in the transmission of *E. bieneusi* to humans.

Although fewer studies have been conducted on the distribution and public health potentials of *E. bieneusi* genotypes in cattle, water buffalos, and yaks in China [7–8,10,12–15], the knowledge of the presence of this pathogen in cattle remains incomplete. The Xinjiang Uyghur Autonomous region (hereafter referred to as Xinjiang) is the largest Chinese administrative division in the northwest of China, spanning over 1.6 million km^2 (0.64 million square miles). Xinjiang is an important trading route for cattle from central Asia to China, and has an extensive livestock breeding industry. However, there are no data on the presence of *E. bieneusi* on cattle in Xinjiang. To better understand the epidemiology of *E. bieneusi*, the present study was conducted to determine the prevalence and genotype distribution of *E. bieneusi* in dairy calves in Xinjiang.

2. Materials and methods

2.1. Ethics statement

This study was performed according to the recommendations of the Chinese Laboratory Animal Administration Act of 1988. The research

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protocol was reviewed and approved by the Research Ethics Committee at Henan Agricultural University. Prior to fecal specimen collection, permission was obtained from the animal owners whenever possible.

2.2. Study area and sample collection

From August to September 2013, a total of 514 fecal samples were collected from randomly selected Holstein calves at 15 intensively reared dairy farms near the cities of Wujiaqu, Changji, Urumqi, Korla, Tacheng, Zhaosu, and Aksu in the Xinjiang Uyghur Autonomous region, in the northwest of China (Table 1), with 200–5000 animals per farm. Two age groups were considered, pre-weaned calves (0–60 days) ($n = 237$) and post-weaned calves (61–150 days ($n = 277$). The animals were all apparently healthy at the time of sampling. Fecal samples were collected directly from the rectum using disposable gloves and plastic containers. Samples were stored at 4 °C until DNA extraction.

2.3. DNA extraction and PCR amplification

Genomic DNA was extracted from approximately 200 mg of each fecal sample using the E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) using the manufacturer's directions. Extracted DNA sample was stored at −20 °C until analysis.

E. bieneusi was detected by nested PCR amplification of the ITS of the ribosomal RNA, as previously described [16]. Each sample was analyzed twice using 2 μL of extracted DNA as a template. KOD-Plus amplification enzyme (Toyobo Co. Ltd., Osaka, Japan) was used for PCR amplification.

2.4. Sequence analysis

All secondary PCR amplicons were bi-directionally sequenced on an ABI PRISM™ 3730 XL DNA Analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences were identified by alignment with reference sequences downloaded from GenBank using MEGA 5 software (http://www.megasoftware.net/). Nucleotide sequences obtained in the present study have been deposited in GenBank under accession numbers KU531571–KU53157676.

2.5. Statistical analysis

A chi-square test was used to compare the prevalence of *E. bieneusi* infection between different ages of animals. Statistical significance was set at a value of $p < 0.05$.

3. Results

A total of 514 fecal samples were examined for *E. bieneusi* by PCR and 85 (16.5%) were positive. Of the 15 farms tested, *E. bieneusi* was identified on 12 (80.0%). The prevalence of *E. bieneusi* in dairy calves on the positive farms in this study ranged from 6.1% to 36.4%, with the highest prevalence on a farm in Korla (A) (Table 1). A slightly higher prevalence of *E. bieneusi* (17.7%) was observed in pre-weaned calves compared with post-weaned calves (15.5%) (Table 1); however, this different between age groups was not significant ($p > 0.05$).

Eighty five *E. bieneusi* nucleotide sequences of the ITS were compared with those in the GenBank database using BLAST analysis. Six

Table 1
Prevalence and genotypes of *E. bieneusi* in dairy calves on 15 farms in Xinjiang, China.

Farm	Age group (days)	No.of positive/No. of examined (%)	*E. bieneusi* genotype identified (n)
Wujiaqu (A)	Pre-weaned calves (<60 d)	3/18 (16.7)	J (3)
Wujiaqu (B)	Pre-weaned calves (<60 d)	4/16 (25.0)	J (4)
	Post-weaned calves (61–150 d)	3/15 (20.0)	J (3)
	Sub total	7/31 (22.6)	J (7)
Wujiaqu (C)	Pre-weaned calves (<60 d)	5/20 (25.0)	D (1), I (2), J (2)
Changji	Pre-weaned calves (<60 d)	2/14 (14.3)	D (1), J (1)
	Post-weaned calves (61–150 d)	1/19 (5.3)	J (1)
	Sub total	3/33 (9.1)	D (1), J (2)
Urumqi	Pre-weaned calves (<60 d)	2/4 (50.0)	J (2)
	Post-weaned calves (61–150 d)	4/28 (14.3)	J (4)
	Sub total	6/32 (18.8)	J (6)
Korla (A)	Pre-weaned calves (<60 d)	4/11 (36.4)	BEB4 (4)
Korla (B)	Post-weaned calves (61–150 d)	0/13 (0)	
Korla (C)	Pre-weaned calves (<60 d)	3/4 (75.0)	I (3)
	Post-weaned calves (61–150 d)	8/28 (28.6)	I (7), J (1)
	Sub total	11/32 (34.4)	I (10), J (1)
Korla (D)	Pre-weaned calves (<60 d)	0/13 (0)	
	Post-weaned calves (61–150 d)	2/20 (10.0)	EbpC (1), J (1)
	Sub total	2/33 (6.1)	EbpC (1), J (1)
Tacheng	Pre-weaned calves (<60 d)	0/3 (0)	
	Post-weaned calves (61–150 d)	0/5 (0)	
	Sub total	0/8 (0)	
Zhaosu	Pre-weaned calves (<60 d)	0/8 (0)	
Aksu (A)	Pre-weaned calves (<60 d)	4/22 (18.2)	CC4 (1), J (3)
	Post-weaned calves (61–150 d)	1/26 (3.8)	J (1)
	Sub total	5/48 (10.4)	CC4 (1), J (4)
Aksu (B)	Pre-weaned calves (<60 d)	7/26 (26.9)	I (2), J (5)
	Post-weaned calves (61–150 d)	3/32 (9.4)	J (3)
	Sub total	10/58 (17.2)	I (2), J (8)
Aksu (C)	Pre-weaned calves (<60 d)	6/49 (12.2)	I (1), J (5)
	Post-weaned calves (61–150 d)	1/27 (3.7)	J (1)
	Sub total	7/70 (10.0)	I (1), J (6)
Aksu (D)	Pre-weaned calves (<60 d)	6/46 (13.0)	EbpC (1), I (1), J (4)
	Post-weaned calves (61–150 d)	16/53 (30.2)	I (3), J (13)
	Sub total	22/99 (22.2)	EbpC (1), I (4), J (17)
Sub total	Pre-weaned calves (<60 d)	42/237 (17.7)	BEB4 (4), CC4 (1), D (2), EbpC (1), I (8), J (26)
	Post-weaned calves (61–150 d)	43/277 (15.5)	EbpC (1), I (11), J (31)
Total		85/514 (16.5)	BEB4 (4), CC4 (1), D (2), EbpC (2), I (19), J (57)