



## Research Article

## A serologic investigation of epizootic hemorrhagic disease virus in China between 2014 and 2019

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## ABSTRACT

Epizootic hemorrhagic disease virus (EHDV) is a member of the genus *Orbivirus*, family *Sedoreoviridae*. It was firstly recognized in 1955 to cause a highly fatal disease of wild white-tailed deer in America. So far, EHDV was detected and isolated in many wild or domestic ruminants, and widely distributed all over the world. Although the domestic cattle and sheep infected by EHDV were usually asymptomatic or subclinical, several outbreaks of epizootic hemorrhagic disease (EHD) in deer and cattle had been reported. Many EHDV strains were isolated and sequenced in last two decades in China, which promoted a general serologic investigation of EHDV in China. In this study, 18,122 sera were collected from asymptomatic or subclinical domestic ruminants (cattle, cow, yaks, sheep, goats, and deer) in 116 regions belonging to 15 provinces in China. All the sera were tested by EHDV C-ELISA, and the results were obtained by big data analysis. EHDV infections were detected in the 14 of 15 provinces, and only Tibet (average altitude  $\geq 4000$  m) which was the highest province in China was free of EHDV. The numbers of seropositive collections in both bovine and goat/sheep were in an inverse proportion to the latitude. However, the seropositive rates in bovine were ranged from 0% to 100%, while the seropositive rates in goat/sheep were no more than 50%. The results suggested that bovine was obviously more susceptible for EHDV infection than goat and sheep, therefore might be a major reservoir of EHDV in China. The prevalence of EHDV was consistent with the distribution of *Culicoides* which were known as the sole insect vectors of EHDV. In particular, the seropositive rates of EHDV were very high in the southern provinces, which required the enhanced surveillance in the future.

## 1. Introduction

A highly fatal disease of wild white-tailed deer appeared in America in 1955 was firstly defined as epizootic hemorrhagic disease (EHD) of deer (Shope et al., 1960). Now, EHD is known as an arthropod transmitted viral disease, which is caused by the epizootic hemorrhagic disease virus (EHDV) belonging to genus *Orbivirus*, family *Sedoreoviridae*, and transmitted by some species of *Culicoides* (Diptera: Ceratopogonidae). Deer and cattle are known as major ruminant hosts of EHDV (OIE, 2021). Positive results of serosurvey or viral nucleic acids detections indicated that yak, sheep, goat, camel, as well as llama are potential hosts of EHDV (Roy, 2007; Cetre-Sossah et al., 2014; Raabis et al., 2014; Maclachlan et al., 2015; Casey et al., 2021), while positive results of bluetongue virus (BTV)-EHDV indirect ELISA (I-ELISA) were found in bears and white rhinoceroses (Dunbar et al., 1998; Miller et al., 2011).

The clinical symptoms of EHD in susceptible mammal hosts, such as fever, serosal hemorrhages, and mucosal edema, are similar to the disease caused by BTV and African horse sickness virus (AHSV) (Roy, 2007; Maclachlan et al., 2015; Yanase et al., 2020). However, most infected mammals, especially domestic livestock, are subclinical or asymptomatic (Savini et al., 2011; Maclachlan et al., 2015).

So far, at least 7 serotypes of EHDV (EHDV-1, -2, -4, -5, -6, -7, -8) were identified (Maan et al., 2017; Shirafuji et al., 2017; Yanase et al., 2020; OIE, 2021; Yamamoto et al., 2021), and at least two new serotypes (EHDV-10 and another unnamed) were introduced recently (Yanase et al., 2020; Yang et al., 2020; Yamamoto et al., 2021). EHDV is reported in Africa, Asia, North America, South America and Oceania, while the center section of Europe is free of EHDV (Savini et al., 2011). EHDV-1, EHDV-2, and EHDV-6 were the common serotypes in the United States and Canada, and EHDV infections in deer and cattle were

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usually reported (Aradaib et al., 2005; Raabis et al., 2014; Allen et al., 2019; Casey et al., 2021; Cottingham et al., 2021). In South America, EHDV-1 was isolated and EHDV-6 was detected in both French Guiana and Ecuador (Verdezoto et al., 2018). In Africa, EHDV RNA or antibodies in cattle, sheep and goat were detected in Tunisia (Mejri et al., 2018), Libya (Mahmoud et al., 2021), Reunion Island (Breard et al., 2004; Sailleau et al., 2012; Cetre-Sossah et al., 2014), Kenya (Toye et al., 2013), Zimbabwe (Gordon et al., 2017) and the island of Mayotte (Dommergues et al., 2019); EHDV-1 and EHDV-6 were identified, but the EHDV-6 was more common in Africa (Cetre-Sossah et al., 2014; Mejri et al., 2018; Mahmoud et al., 2021). One strain of EHDV-4 was reported in Nigeria (Maan et al., 2010). In East Asia, at least 7 serotypes of EHDV (EHDV-1, -2, -5, -6, -7, -8, -10) have been identified (Zhang et al., 2016; Shirafuji et al., 2017; Qi et al., 2019; Yanase et al., 2020). A large epidemic of Ibaraki disease in cattle caused by Ibaraki virus, which was identified as one clade of EHDV-2 later, occurred in Japan between 1959 and 1960 (Omori et al., 1969; Anthony et al., 2009b). A relative small outbreak of this disease was reported in the southern part of Japan in 1987 (Ohashi et al., 1999; Yanase et al., 2020). EHDV-7 strains caused an outbreak of Ibaraki-like disease in cattle in south Japan in 1997 (Kato et al., 2016; Shirafuji et al., 2017). In recent years, the Ibaraki disease was also recorded in Japan in 2013 (Hirashima et al., 2015). In Western Asia, EHDV were reported in Bahrain, Oman and Israel, and 4 serotypes of EHDV (EHDV-1, -2, -6, -7) were identified (Maan et al., 2010; Kedmi et al., 2011; Golender and Bumbarov, 2019). An outbreak in cattle was caused by EHDV-6 during 2007 (Temizel et al., 2009) and the EHDV infections in goitered gazelle were reported in Turkey, which lies on the border between Europe and Asia. In Oceania, at least 5 serotypes of EHDV (EHDV-2, -5, -6, -7, -8) were isolated in Australia (Campbell and St George, 1986; Anthony et al., 2009a; Maan et al., 2010).

In China, quite a few EHDV reports were documented. Eight cattle with Ibaraki disease-like symptoms were received by a veterinary station in September 1985 (Xue et al., 1987), which might be the first case of EHD in Chinese mainland. Subsequently, EHDV antibodies were detected from the sera of cattle, sheep and goat for the first time in Chinese mainland in 1990 (Li, 1990). So far, although there was no outbreak of EHD reported in Chinese mainland, some strains belonging to six serotypes (EHDV-1, -5, -6, -7, -8, -10) were isolated (Zhang et al., 2016; Qi et al., 2019; Yang et al., 2019), and a novel strain not belonging to any known serotype was isolated from a cattle in Yunnan recently (Yang et al., 2020). The aim of the study is to investigate the prevalence of EHDV in half of Chinese provinces in recent years.

## 2. Materials and methods

### 2.1. Sample collection

Totally, 18,122 serum samples were collected from asymptomatic or subclinical domestic animals (cattle, cow, yaks, sheep, goats, and deer) aged no less than 6 months in 116 regions belonging to 15 provinces (Chongqing, Guangdong, Guangxi, Guizhou, Hebei, Hubei, Inner Mongolia, Jiangsu, Jilin, Liaoning, Shanxi, Sichuan, Tibet, Xinjiang, and Yunnan) in China between 2014 and 2019. So far, no EHDV vaccine was used in China, and these ruminants were not immunized by EHDV vaccines previously.

### 2.2. C-ELISA

The C-ELISA method was used to test the antibodies against EHDV proteins as described by the World Organization for Animal Health (OIE, 2021). Briefly, the EHDV-6 virions purified by a continuous sucrose gradient centrifugation and deactivated by diethylene imide were used to coat 96-well microplates (Costar, Corning, Kennebunk, USA). Serogroup-reactive anti-EHDV polyclonal antibody produced by EHDV-2 (identified as EHDV-10 later) virions immunized guinea pig (Zhu et al., 2018) was used as a competing antibody. The two EHDV strains used in this methods was isolated from cattle in Shizong of Yunnan, China in 2012 (Zhu et al., 2018). For all the tests, 40  $\mu$ L of diluents was added into the antigen coated well, and then 10  $\mu$ L of samples (positive control: a cattle serum positive for EHDV antibodies, negative control: a cattle serum negative for EHDV antibodies, and tested sera) was added in duplicate. Color was developed by TMB substrate (SurModics, Eden Prairie, USA) and stopped by 1 mol/L of sulphuric acid. Optical densities under 450 nm ( $OD_{450}$ ) values were read by a microplate reader (Multiskan Ascent, Thermo Scientific, Waltham, USA). Percent inhibition (PI) was calculated as  $PI = 1 - OD_{\text{sample}}/OD_{\text{negative}}$ , and a mean PI value of the duplicate tests was used as the final result.  $PI \geq 50\%$  was considered positive, and PI between 40% and 50% was considered to be suspicious. The specificity of this C-ELISA method had been tested using the antisera of 6 serotypes (EHDV-1, -2, -5, -6, -7, -8), Akabane virus (AKAV), bovine ephemeral fever virus (BEFV), 24 serotypes (1–24) of BTV, Chuzan virus (CHUV), foot and mouth disease virus (FMDV), goatpox virus (GPV), and peste des petits ruminants virus (PPRV), respectively, by Zhu et al. (2018). The PI values of the EHDV antisera were more than 85%. Some BTV antisera did cross-react in the C-ELISA, but the PI values of these samples were lower than 25%, which were judged as negative results by the cutoff value of 50% (Zhu et al., 2018).

**Table 1**

Seroprevalence of EHDV in tested domestic ruminants from 15 provinces in China between 2014 and 2019.

Province	Positive samples/total samples (positive rate %)							Total
	2014	2015	2016	2017	2018	2019		
Chongqing	NA	NA	0/20 (0.0)	19/140 (13.6)	113/600 (18.8)	51/135 (37.8)	183/895 (20.4)	
Guangdong	91/233 (39.1)	188/299 (62.9)	106/169 (62.7)	155/195 (79.5)	64/110 (58.2)	NA	604/1006 (60.0)	
Guangxi	84/230 (36.5)	68/248 (27.4)	NA	NA	85/145 (58.6)	152/328 (46.3)	389/951 (40.9)	
Guizhou	NA	NA	NA	71/115 (61.7)	64/95 (67.4)	43/377 (11.4)	178/587 (30.3)	
Hebei	NA	NA	NA	7/150 (4.7)	1/277 (0.4)	2/350 (0.6)	10/777 (1.3)	
Hubei	NA	NA	2/221 (0.9)	1/152 (0.7)	144/183 (78.7)	126/502 (25.1)	273/1058 (25.8)	
Inner Mongolia	0/266 (0.0)	2/140 (1.4)	2/460 (0.4)	0/450 (0.0)	5/540 (0.9)	19/320 (5.9)	28/2176 (1.3)	
Jiangsu	NA	19/60 (31.7)	NA	NA	NA	NA	19/60 (31.7)	
Jilin	NA	NA	NA	3/100 (3.0)	0/160 (0.0)	0/508 (0.0)	3/768 (0.4)	
Liaoning	NA	NA	NA	6/335 (1.8)	NA	NA	6/335 (1.8)	
Shanxi	NA	1/292 (0.3)	4/373 (1.1)	36/537 (6.7)	NA	NA	41/1202 (3.4)	
Sichuan	NA	NA	NA	55/231 (23.8)	NA	NA	55/231 (23.8)	
Tibet	NA	NA	NA	0/107 (0.0)	0/240 (0.0)	NA	0/347 (0.0)	
Xinjiang	NA	8/400 (2.0)	6/982 (0.6)	0/121 (0.0)	4/198 (2.0)	19/772 (2.5)	37/2473 (1.5)	
Yunnan	150/550 (27.3)	NA	7/154 (4.5) <sup>a</sup>	2491/2784 (89.5)	332/867 (38.3)	651/901 (72.3)	3631/5256 (69.1)	
Total	325/1279 (25.4)	286/1439 (19.9)	127/2379 (5.3)	2844/5417 (52.5)	812/3415 (23.8)	1063/4193 (25.4)	5457/18,122 (30.1)	

NA, not available.

<sup>a</sup> These animals included 98 yaks from Shangri-la.

### 2.3. Data preparation

The source places of samples are classified into three grades, namely province, region, and county. Since the data were large and much information of the exact collection sites was absent, county was set as the basic collection site in this study. According to the information provided by the collectors, animal objects were classified to seven categories, namely unclassified bovine (usually cattle but might contain a few Asia buffaloes, cows or yaks), unclassified goat/sheep, cow, yak, goat, sheep, and deer. For convenience, “group” was set as a basic unit in this study. A group was defined as a cluster of sera samples collected from one of the categories in one county at one time (year). The number of samples per group was variable. A group was considered as a positive group if it contained a positive sample. As the exact place or coordinates of the farms were absent, all the coordinates of the collection sites were acquired from web map (<https://api.map.baidu.com/lbsapi/getpoint/index.html?qq-pf-to=pcqq-group> and <https://www.d1xz.net/xp/jingwei/>) and accurate to the main area of every county especially the county towns.

### 2.4. Data analysis

For big data analysis, the geographical position, coordinate, collection year, animal species, animal amount, EHDV seropositive rate of every group were listed in a spreadsheet. Scatter diagrams and box

diagrams were constructed by the R programming language (Greene et al., 2014; Lortie et al., 2020) using these data. For frequency statistics, median values and 95% confidence intervals (CI) were calculated by descriptive statistics (bootstrap = 1000) using software PASW Statistics (version 18) (<http://www.spss.com.hk/statistics/>). Two sketch maps were drawn based on the standard map [map number: GS (2019) 1676] downloaded from <http://bzdt.ch.mnr.gov.cn/>.

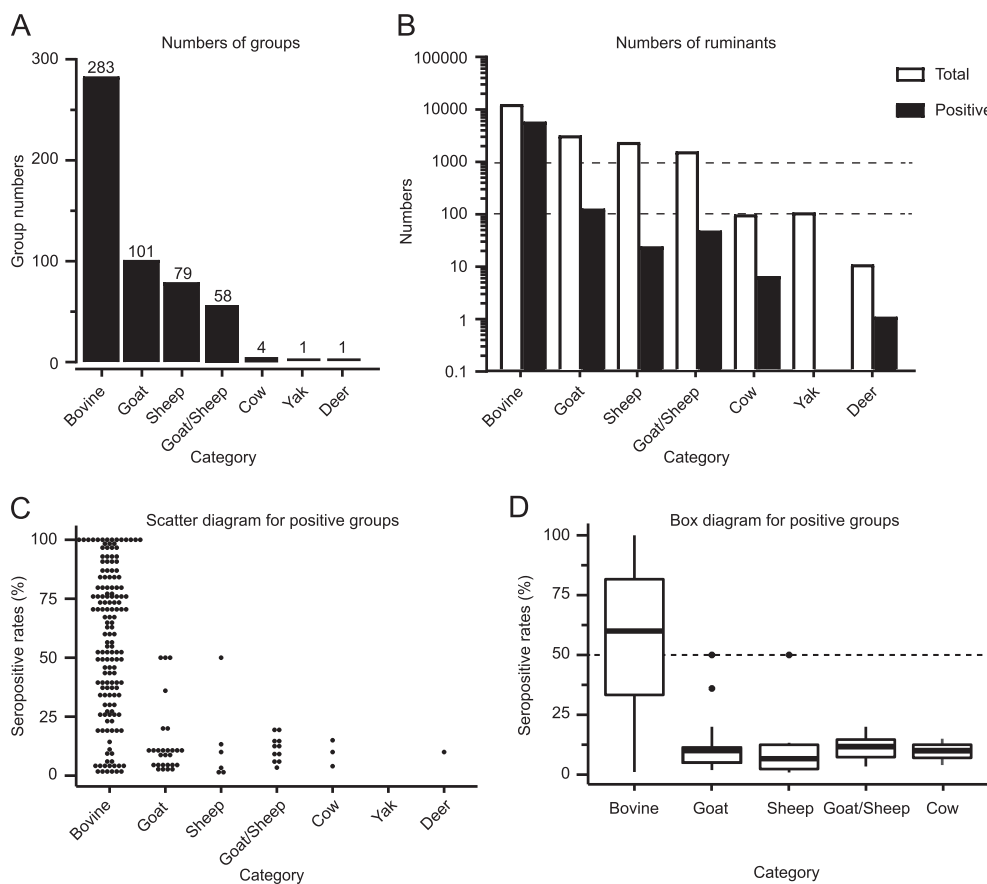
## 3. Results

### 3.1. EHDV seroprevalence

In this study, totally, 5457 of 18,122 (30.1%) domestic ruminants were positive for EHDV antibodies tested by C-ELISA (Table 1). The results (Table 1, Supplementary Table S1; Supplementary Table S2) indicated that EHDV were distributed widely in China in recent years, and the positive rates were more than 30% in at least five provinces (Guangdong, Guangxi, Guizhou, Jiangsu, and Yunnan). Only Tibet was free of EHDV in this study. A group of seronegative yaks in Shangri-la heavily reduced the seropositive rate in Yunnan in 2016 (Table 1).

### 3.2. Ruminant hosts of EHDV

Totally, 288 groups of bovine sera including cattle, cows and yaks, and 238 groups of goats and sheep sera were tested (Fig. 1A). Concretely,



**Fig. 1.** Tested ruminants and the seropositive rates of EHDV. The definition of group was described in materials and methods. **A** The numbers of groups. **B** The numbers of total samples (ruminants) and coupled seropositive samples. The intervals of axis y were in the pattern of logarithm ( $\log_{10}$ ) and the numbers represented the actual ruminant numbers. **C** The scatter diagram showing the distribution of seropositive groups. **D** The box diagram showing the distribution of seropositive groups. The bold transverse line, box, and vertical line indicate the median, a range of 25% (Q1) quartile to 75% (Q3) quartile, and the range of lower extreme to upper extreme, respectively; the dots represent the outliers (McGill et al., 1978).

**Table 2**  
General seropositive rates of EHDV in different categories.

Animal category	General seropositive rate (%) calculated by	
	Animal numbers <sup>a</sup>	Groups <sup>b</sup>
Unclassified bovine <sup>c</sup>	46.0 (5268/11,451)	53.4 (151/283)
Cows	6.7 (6/90)	75.0 (3/4)
Yaks	0 (0/98)	0.0 (0/1)
Unclassified goats/sheep <sup>d</sup>	3.1 (70/1436)	19.0 (11/58)
Goats	4.0 (116/2876)	26.7 (27/101)
Sheep	1.0 (22/2161)	7.6 (6/79)
Deer	10.0 (1/10)	100.0 (1/1)

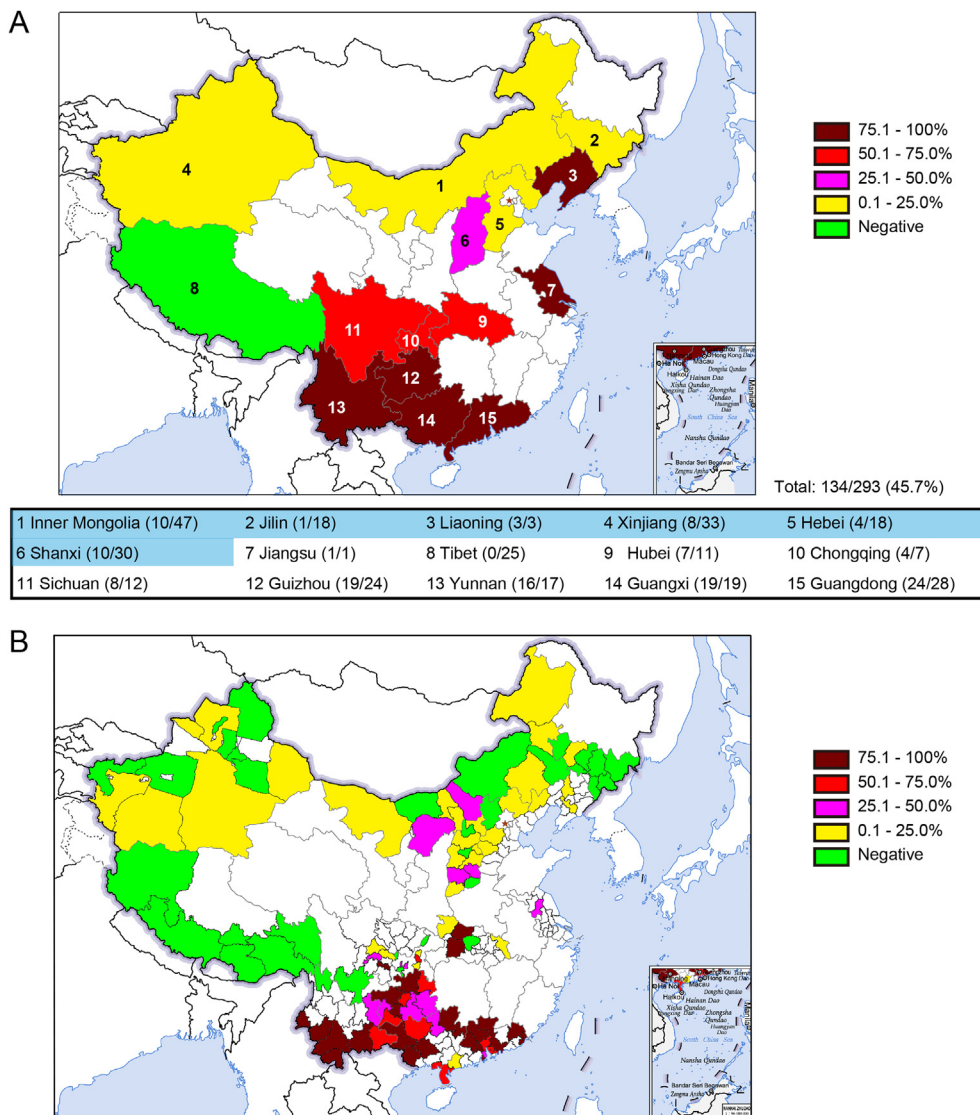
<sup>a</sup> The numbers of positive samples and the total samples, and their ratios were shown.

<sup>b</sup> The numbers of positive groups and the total groups, as well as their ratios were shown. The definition of group was described in materials and methods.

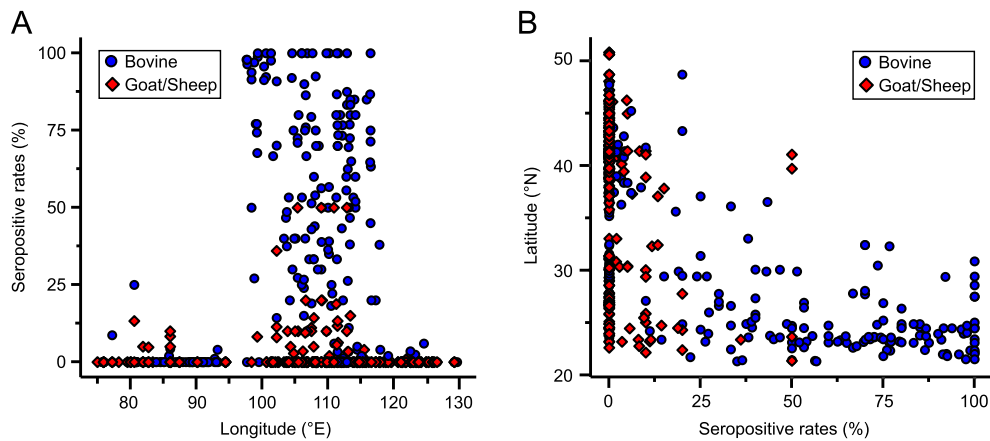
<sup>c</sup> Samples mainly comprise of cattle, but may contained buffaloes, cows, and yaks.

<sup>d</sup> Samples of goats or sheep without classification, but goats are common in south China and sheep are common in north China usually.

the samples were composed of 11,451 unclassified bovine (usually cattle but might contain a few Asia buffaloes, cows or yaks), 90 cows, 98 yaks, 1436 unclassified goats and sheep, 2876 goats, 2161 sheep, and 10 deer (Table 2, Fig. 1B). Generally, the bovine category had the highest positive rate (46.0%), and the positive rates of other categories were 10.0% (deer), 6.7% (cows), 4.0% (goats), 3.1% (goats/sheep), 1.0% (sheep), and 0.0% (yaks), respectively (Table 2, Fig. 1B). A scatter diagram showed that the number of seropositive bovine groups was obviously larger than the number of seropositive goats/sheep groups, and nearly half of positive bovine groups had a positive rate more than 50%, while the positive rates of goat/sheep groups never exceeded 50% (Fig. 1C). The medians of seropositive rates calculated by all the positive groups among bovine, goat, sheep, goat/sheep, cow were 60.0% [95% CI (50.0%–70.0%)], 10.0% [95% CI (5.0%–10.0%)], 6.7% [95% CI (1.5%–31.7%)], 11.7% [95% CI (6.3%–15.0%)], and 10.0% [95% CI (4.0%–15.0%)], respectively (Fig. 1D). As negative groups were joined, the medians of seropositive rates among bovine category and cow category lowered to 4.0% [95% CI (0%–22.2%), n = 283] and 7.0% [95% CI (0%–



**Fig. 2.** EHDV prevalence in 15 provinces in China between 2014 and 2019. The levels of positive rates were shown by five colors. The six north provinces were highlighted by blue background. **A** The ratio of the positive counties to all the tested counties in each province between 2014 and 2019, and a general ratio of total 134 positive counties to all the 293 counties investigated were shown. **B** The positive rate of each region was represented by the highest seropositive rate from all groups in this region between 2014 and 2019. For Chongqing, regions were replaced by counties, since this province did not have regions.



**Fig. 3.** Distribution of the collections of sera and EHDV prevalence in China between 2014 and 2019. Every dot represented a group of samples explained in the “Data preparation”. The distributions of EHDV along the longitude (A) and the latitude (B) in bovine groups (blue) and goat/sheep groups (red) were shown.

15.0%),  $n = 4$ ] respectively, while the values of other three categories (goat, sheep, goat/sheep) lowered to 0% [95% CI (0%–0%)].

### 3.3. Geographical distribution of EHDV

General statistical results showed that more than 75% counties were EHDV positive in six provinces (Guangdong, Guangxi, Guizhou, Jiangsu, Jilin, and Yunnan), four of which were the southernmost provinces in this study (Fig. 2A). Furthermore, the prevalence of all the tested regions showed by the highest seropositive rate of EHDV among all the groups in every region enucleated that the seropositive rates of EHDV in domestic ruminants were obviously lower in north China and Tibet (Fig. 2B).

The seropositive rates of EHDV along the longitude showed that EHDV infection occurred between the longitude of 77°E and 125°E, and mainly appeared between 96°E and 118°E for all the ruminants (Fig. 3A). As for latitude distribution, the seropositive rates in bovine (ranged from 0% to 100%) were in an inverse proportion to the latitude between 30°N and 50°N, and usually exceeded 50% between 21°N and 30°N (Fig. 3B). Usually the seropositive rates in goats or sheep were no more than 25% and showed little difference in the area between 21°N and 47°N (Fig. 3B). The smooth fitting curves formed by the locally estimated scatterplot smoothing (LOESS) model also suggested that the seropositive rate of EHDV in bovine was in an inverse proportion to the latitude (Supplementary Fig. S1).

Furthermore, the geographical distribution, amount of animal and seropositive rate of EHDV in each group, as well as the densities of groups along the longitude and latitude, were shown in Fig. 4. The numbers of bovine groups were approximately equal in north (higher than 35°N) and south (lower than 35°N), while the goats/sheep groups in north were a little more than that in south. However, the amount of bovine in south was obviously larger than that in north, and the amount of goats and sheep in south was a little larger than that in north. Seropositive rates in south were higher than that in north both in bovine and goats/sheep (Fig. 4).

## 4. Discussion

In this study, more than 18 thousands of sera were collected from the domestic ruminants (cattle, cows, yaks, goats, sheep, and deer) without symptoms from 15 provinces in China between 2014 and 2019, and used for the serologic tests by EHDV C-ELISA.

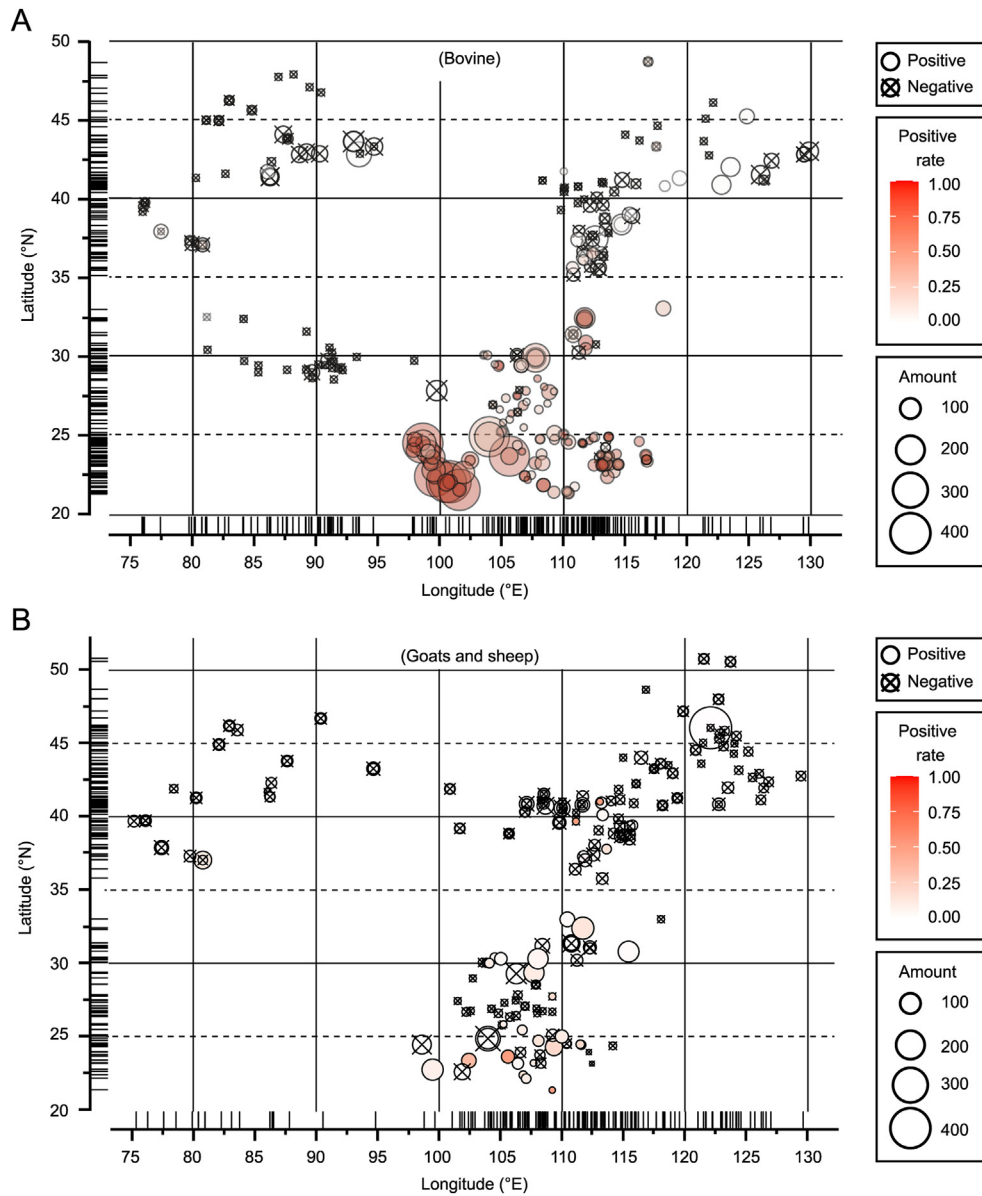
For serological tests, I-ELISA and gel immunodiffusion were found to have major drawback of being unable to distinguish between antibodies against EHDV and BTV (OIE, 2021). A serogroup specific C-ELISA kit detecting antibodies against EHDV VP7 was recommended by OIE as the

optimal choice for EHDV infection surveillance (OIE, 2021). In this study, a C-ELISA method previously developed by us was applied. This method could distinguish between antibodies against EHDV and BTV (serotypes 1 to 24) well, although the antibodies against several serotypes of BTV exerted weak cross-reaction in the C-ELISA (Zhu et al., 2018).

It is reported that EHDV has various vertebrate hosts especially ruminants (Roy, 2007; Cetre-Sossah et al., 2014; Raabis et al., 2014; Maclachlan et al., 2015; Casey et al., 2021). In this study, cattle, cows, goats, sheep, and deer were found to be infected by EHDV. Only one group of yaks (Shangri-la, Yunnan Province) was submitted for serological assay, and these yaks were free from EHDV infection. The results of this investigation suggest that bovines are more susceptible than goats and sheep, which is in accord with the investigation in Israel (Kedmi et al., 2011), Reunion Island (Cetre-Sossah et al., 2014), and Zimbabwe (Gordon et al., 2017). Also, most of the EHDV reports worldwide were associated with cattle rather than goats and sheep (Aradaib et al., 2005; Yamizel et al., 2009; Verdezoto et al., 2018; Dommergues et al., 2019; Yanase et al., 2020). Therefore bovine probably is a major EHDV reservoir in China, especially in south provinces.

EHDV infections were detected in the 14 of 15 provinces, and only Tibet which was the highest province in China was free of EHDV. In this study, EHDV infections were detected between the longitude of 77.42°E and 124.81°E, and between the latitude of 21.28°N and 48.67°N (Table 3, Figs. 3 and 4). This scope obviously exceeded the northernmost record (42.9849°N) of EHDV infection in Canada (Allen et al., 2019). The EHDV infections mainly occurred between the longitude of 97°E and 118°E (Figs. 3A and 4), since the Qinghai-Tibet Plateau locate in the western China, and some eastern provinces were out of investigation. Generally, the seropositive rates in south China were higher than that in north China, and the tested ruminants in high altitude localities (Tibet with an average altitude no less than 4000 m, and Shangri-la with an average altitude more than 3000 m) were free from EHDV infection. EHDV was believed to be one of *Culicoides*-dependent infectious diseases for livestock (Mellor et al., 2000; Roy, 2007). Consistent with this, the incidence of EHDV infection was in a direct proportion to the vitality of *Culicoides* which are active under warm and wet weather and are inhibited by low temperature (Mellor et al., 2000; Wilson and Mellor, 2008). Although no EHDV infection was detected in Tibet and Shangri-la in this investigation, seroprevalence of BTV and detection of *Tibet Orbivirus* in the Qinghai-Tibet Plateau were previously reported (Li et al., 2014; Ma et al., 2017). Because BTV is *Culicoides*-borne virus, and *Tibet Orbivirus* was *Culicoides*-borne and mosquito-borne virus, it is possible that EHDV also spreads through bovine on the plateau.

The virulence of EHDV varies among different strains. So far, some strains of EHDV-2 including the Ibaraki virus, which caused large scale



**Fig. 4.** The coordinate distributions of the ruminants tested in this study. **A** The groups of unclassified bovine, cattle, cows, and yaks. **B** The groups of unclassified goats/sheep, goats, and sheep. Every point represented a single group of samples, and their collection sites were located by the geographical coordinate. The EHDV seropositive and negative groups were shown by simple circle and circle with cross, respectively. The seropositive rate and the ruminant amount of every group were shown by the gradient colors and the dot area, respectively. The rugs adjoining to the axes exhibited the densities of groups along the longitude or latitude.

**Table 3**

The extreme geographical sites where EHDV seropositive sera were collected in China in this study.

Province	Region	County	Longitude (°E)	Latitude (°N)	Positive rate (%) <sup>a</sup>	Total <sup>b</sup>	Extreme
Inner Mongolia	Hulun-buir	Xinbarhu-left <sup>c</sup>	116.82	48.67	20 (4/20)	30	North
Jilin	Songyuan	Ningjiang	124.81	45.20	6.0 (3/50)	50	East
Xinjiang	Kashi	Yecheng <sup>d</sup>	77.42	37.89	8.7 (4/46)	54	West
Guangdong	Zhanjiang	Zhanjiang	110.37	21.28	56.7 (17/30)	50	South

Note: all these records come from unclassified bovine.

<sup>a</sup> The positive rate from the group with the highest positive rate in the same county.

<sup>b</sup> Total number of tested unclassified bovine in the same county.

<sup>c</sup> In this county, a group of goats (0/10) and a group of sheep (0/10) were tested and were negative.

<sup>d</sup> In this county, a group of goats (0/46) and a group of sheep (0/30) were tested and were negative.

lethal cases in white-tailed deer and cattle (Allen et al., 2019; Yanase et al., 2020; Casey et al., 2021; Cottingham et al., 2021), were considered to be the most violent strains. EHDV-6 was frequently reported worldwide and

caused one outbreak (46 cases) in Japan in 2015 (Kamomae et al., 2018), but usually nonlethal (Sailleau et al., 2012; Zhang et al., 2016; Domergues et al., 2019; Cottingham et al., 2021; Mahmoud et al., 2021). Six

known serotypes of EHDV (EHDV-1, -5, -6, -7, -8, -10) and one novel serotype were isolated in China up to now (Zhang et al., 2016; Qi et al., 2019; Yang et al., 2019, 2020). But these works for serotype identification were mainly originated from Yunnan and Guangxi, and the data on the distribution of EHDV serotypes in China were missing.

Although the bovine were infected by EHDV frequently in southern provinces such as Yunnan, Guangdong, Guangxi and Guizhou (Figs. 2 and 4), there was only one EHD case (mild symptom) reported in Mangshi County, Yunnan (Yang et al., 2020). It is probably because no highly virulent strain, like Ibaraki virus, spread in recent years in these provinces. However, the bovine in south China is under serious threat of EHD. It is necessary to monitor the EHDV strains in bovine especially the bovine with EHD symptoms in south provinces. Besides, cleaning up the dung of livestock in time during summer will reduce the amount of *Culicoides*, and therefore reduce the transmitting risk of EHDV in south China.

Some domestic ruminants died of uncertain diseases recently in Yunnan Province. It is necessary to develop molecular diagnostic techniques for the infectious diseases including EHD and to monitor the highly virulent EHDV strains in China in the future. It is also important to surveil EHDV in Hainan island, which is the southernmost province in China but outside the investigation of EHDV by far.

## 5. Conclusions

In this study, antibodies against EHDV were detected in cattle, cows, goats, sheep and deer in China. Bovine with seropositive rates ranged from 0% to 100% was obviously more susceptible for EHDV infection than the goat and sheep with seropositive rates ranged from 0% to 50%. The seropositive rates of EHDV were high in the southern provinces, which required the enhanced surveillance in the future.

## Data availability

All the data generated during the current study are included in the manuscript.

## Ethics statement

The processes of blood collections from animals (cattle, cows, yaks, deer, goats and sheep) were approved by their hosts, and the animal health was protected. The study was approved by the Ethics Committee of Yunnan Animal Science and Veterinary Institute.

## Author contributions

Yingliang Duan: data curation, formal analysis, visualization, writing-original draft, writing-review & editing. Zhenxing Yang: data curation, investigation, methodology. Pei Zhu: investigation, resources. Lei Xiao: resources. Zhanhong Li: resources. Zhuoran Li: resources. Le Li: resources. Jianbo Zhu: data curation, funding acquisition, project administration, resources.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virs.2022.06.005>.

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