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#### **Short Communication**

# Large-scale serological survey of bovine ephemeral fever in China



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#### ABSTRACT

Bovine ephemeral fever (BEF) is caused by the arthropod-borne bovine ephemeral fever virus (BEFV), which is classified in the family *Rhabdoviridae* and the genus *Ephemerovirus*. A debilitating and sometimes fatal viral disease, BEF affects cattle and water buffalo. The epizootiology of BEF among cattle in China has not been fully determined. We examined the seroprevalence of the BEFV among cattle in China between January 2012 and June 2014. Among the 2822 serum samples collected from various cattle breeds in 26 provinces in China, the seropositive rate for the BEFV ranged from 0% to 81% between regions and species. Our findings show that BEFV was prevalent in the all of the regions tested in our study and provide the first reliable reference regarding BEF surveillance in China.

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#### 1. Introduction

Bovine ephemeral fever (BEF) is caused by the arthropod-borne bovine ephemeral fever virus (BEFV), a member of the family *Rhabdoviridae* and the genus *Ephemerovirus* (Dietzgen and Kuzmin, 2011). The disease is not contagious among vertebrates, and the epizootiology is consistent with insect-borne transmission. Animals with BEF present with high fever, spontaneous abortion, and lameness or paralysis (Yeruham et al., 2003; Hsieh et al., 2005; Walker, 2005). The geographic distribution of BEF in bovines spans Africa, the Middle East, Australia and Asia, including China.

The vectors of the BEFV have not been well defined. The virus has been isolated from both mosquitoes and biting midges (*Culicoides* spp.) in Australia and Africa, but various factors suggest that mosquitoes are the principal vectors

(Walker et al., 2012; Walker, 2013). To date, no insects have been identified as vectors of the BEFV in China. The incidence and prevalence for BEF among cattle in China have not been well characterized, and serological surveillance data for the BEFV in China is scant. We performed a serological survey of BEFV in cattle in 26 provinces in China between January 2012 and June 2014, and present the first report of the seroprevalence of the BEFV in China.

#### 2. Materials and methods

#### 2.1. Serum samples

Serum samples (n = 2822) were obtained from domestic cattle, water buffalo and yak in 26 provinces in China between January 2012 and June 2014, as shown in Table 1. The samples were collected only from the animals that were not inoculated with BEFV a vaccine. Sera that tested positive for rabies virus (RV) was stored at Lanzhou Veterinary Research Institute.

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Table 1 Characteristics of the sera and the prevalence of BEFV in China.

Province	Region	Herds	Date	Total samples	Positive samples	Prevalence (%)
Chongqing		Cattle	6/2013	56	23	41
Gansu	Dingxi	Cattle	10/2012	20	0	0
	Zhangye	Cattle	9/2013	24	3	12.5
Guangdong	Guangzhou	Cattle	7/2013	34	3	8.8
Guangxi	Lingui	Cattle	20/8/2012	35	8	22.8
Ü	Tianyang	Cattle	22/8/2012	31	15	48.3
	Nanning	Cattle	6/2014	330	17	5.2
Guizhou	Qinglong	Water buffaloes	20/8/2012	28	16	57.1
Hainan	Dongfang	Cattle	11/2012	30	9	30
	Wuzhishan	Cattle	11/2012	30	1	3.3
	Qionghai	Cattle	10/2013	30	7	23.3
	Wenchang	Cattle	9/2013	26	1	3.8
	Sanya	Cattle	8/2013	12	0	0
Hebei	Zhengzong	Cattle	10/2013	30	10	33.3
	Luding	Cattle	9/2013	30	11	36.7
Henan	Zhengzhou	Dairy cattle	6/2012	33	12	36.3
Heilongjiang	Qiqihaer	Cattle	9/2013	100	22	22
Hubei	Wuhan	Water buffaloes	7/2013	118	9	7.5
	Wuhan	Water buffaloes	5/2014	40	3	7.6
Hunan	Xinhuang	Cattle	18/8/2012	28	16	57.1
InnerMongolia	Helingeer	Dairy cattle	7/6/2012	60	28	46.7
	Hulunbeier	Dairy cattle	8/2012	117	49	41.9
Jiangsu	Yutai	Water buffaloes	12/3/2013	69	7	10.1
	Nanjing	Dairy cattle	17/2/2013	75	39	52
	Nanjing	Dairy cattle	13/2/2013	100	41	41
Jilin	Changchun	Cattle	21/11/2013	25	14	56
	Yongji	Cattle	21/11/2013	28	7	25
Liaoning	Benxi	Dairy cattle	21/11/2013	29	10	34.5
	Dalian	Cattle	21/11/2013	29	4	13.8
	Anshan	Cattle	21/11/2013	25 25	1	4
Ningxia	Pingjibao	Cattle	7/2012	46	29	63
Qinghai	Xining	Yak	10/2012	90	6	6.7
	Haibei	Yak		90 97	36	37.1
			10/6/2014			
Shandong	Qingdao	Dairy cattle	8/2013	24	10	41.7
Shanxi	Yanan	Cattle	8/2013	20	6	30
	Xi,an	Dairy cattle	6/2012	24	8	33.3
Shaanxi	Taiyuan	Cattle	8/2012	109	88	81
a. 1	Datong	Dairy cattle	9/2012	48	24	50
Sichuan 	Luzhou	Water buffaloes	22/8/2012	30	14	46.7
rianjin 		Dairy cattle	6/2012	48	24	50
Γibet	Wuma	Yak	7/2013	96	36	37.5
Xinjiang	qitai	Cattle	25/6/2013	23	4	17.9
	Jimusa	Cattle	25/6/2013	25	9	36
	Changji	Cattle	26/6/2013	21	5	21.7
	Weili	Cattle	25/6/2013	15	4	26.7
	Manasi	Cattle	25/6/2013	25	5	20
	Fukang	Cattle	26/6/2013	25	5	20
	Mulei	Cattle	25/6/2013	25	4	16
	Hutubi	Cattle	5/6/2013	24	15	64
	Kalamayi	Dairy cattle	6/2012	15	9	60
	Akesu	Cattle	8/2013	25	2	8
Yunnan	Shizong	Cattle	3/9/2013	80	23	28.7
	Puer	Cattle	24/9/2013	80	58	72.5
	Dehong	Cattle	16/9/2013	100	64	64
Zhejiang	Jinhua	Cattle	7/2013	30	8	26.7
	Ruian	Cattle	8/2013	30	17	56.7

### 2.2. Microneutralization assay

Serum samples were first examined for the presence of antibodies against the BEFV using a previously described microneutralization assay (publication no. NY/T543-2002 of the Agricultural Industry Criteria of the People's Republic of China). Duplicate serum samples were diluted

from 1:2 to 1:4 with growth medium in 96-well cell culture plates in a total volume of 25  $\mu$ L/well. A 100- $\mu$ L aliquot of medium containing the BEFV at the 1 TCID<sub>50</sub> (50% tissue culture infective dose) was added to each well, and the plates were incubated for 60 min at 37 °C. Approximately 3  $\times$  10<sup>4</sup> BHK21 cells in 100  $\mu$ L of medium were added to each well. The cells were cultured for 5 days

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