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BRIEF COMMUNICATION

Seroprevalence and Risk Factors of Fascioliasis in Yaks, *Bos grunniens*, from Three Counties of Gansu Province, China

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Abstract: The aim of this study was to determine the seroprevalence and risk factors of fascioliasis in yaks, *Bos grunniens*, from 3 counties of Gansu Province in China. A total of 1,584 serum samples, including 974 samples from white yaks from Tianzhu, 464 from black yaks from Maqu, and 146 from black yaks from Luqu County, were collected and analyzed using ELISA to detect IgG antibodies against *Fasciola hepatica*. The overall *F. hepatica* seroprevalence was 28.7% (454/1,584), with 29.2% in white yaks (284/974) and 27.9% in black yaks (170/610). The seroprevalence of *F. hepatica* in yaks from Tianzhu, Luqu, and Maqu was 29.2%, 22.6%, and 29.5%, respectively. Female yaks (30.9%) had higher *F. hepatica* seroprevalence than male yaks (23.4%). Also, *F. hepatica* seroprevalence varied by different age group from 24.1% to 33.8%. Further, the seroprevalence ranged from 21.8% to 39.1% over different seasons. Interestingly, the season and age of yaks were associated with *F. hepatica* infection in yaks in the investigated areas. These findings provided a basis for further studies on this disease in yaks from 3 counties of Gansu Province in northwestern China, which may ultimately support the development of effective control strategies of fascioliasis in these areas.

Key words: *Fasciola hepatica*, *Bos grunniens*, yak, seroprevalence, ELISA, China

The white yak is a unique yak breed and precious semi-wild animal in China [1]. Approximately 50,000 white yaks live only in Tianzhu Tibetan Autonomous County (TTAC), Gansu Province, northwestern China, where low air pressure, low temperature, and low oxygen content exist [1]. Milk and meat of yaks (especially white yaks) is the key source of income for local Tibetans in TTAC (with an altitude of 2,050-4,874 m above the sea level), Maqu (3,500-3,800 m above the sea level), and Luqu (2,900-4,287 m above the sea level), Gansu Province, China [1]. Furthermore, most of Tibetans and their livestock (white yaks and black yaks) live in mountains in Gansu, and due to the lack of infrastructure, Tibetans and yaks drink the water from the river nearby.

Fasciola hepatica and *Fasciola gigantica* are the major causative agents of fascioliasis, an economically important disease in livestock [2,3]. *F. hepatica* infection usually occurs in temperate zones [4], and *F. gigantica* occurs in tropical and subtropical areas. *Fasciola* infection is caused by ingestion of encysted metacercariae through oral route, and the affected host exhibits symptoms of liver damage, loss of productivity, and even death [5].

Recently, fascioliasis is of increasing concern because *Fasciola* not only has a worldwide distribution, but also threatens human health. For example, up to 2.4 million humans are infected and about 180 million are at risk worldwide [6]. Fascioliasis may also cause great economic losses as indicated by the loss of more than US$3,000 million and more than 600 million animals infected per year worldwide [7]. In view of this impact, large numbers of investigations concerning the prevalence of *Fasciola* in bovines have been conducted in the world [5,8].

In China, information about the prevalence of *Fasciola* in yaks (*Bos grunniens*) is limited, a few previous studies demonstrated that *F. hepatica* is the predominant *Fasciola* species in yaks, and these were published in Chinese journals [9,10]. The objective of the present study was to investigate the seropreva-

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Table 1. Seroprevalence of *Fasciola hepatica* infection in yaks (*Bos grunniens*) in Gansu province, northwestern China by enzyme-linked immunosorbent assay

Variable	Category	No. tested	No. positive	% (95% CI)	*P*-value	OR (95% CI)
Region	Luqu	146	33	22.6 (15.82-29.39)	0.23	Reference
	Tianzhu	974	284	29.2 (26.30-32.01)		1.41 (0.93-2.13)
	Maqu	464	137	29.6 (25.38-33.68)		1.44 (0.93-2.22)
Gender	Male	471	110	23.4 (19.53-27.18)	0.2	Reference
	Female	1,113	344	30.9 (28.19-33.62)		1.47 (1.15-1.88)
Species	Black yaks	610	170	27.9 (24.31-31.43)	0.58	Reference
	White yaks	974	284	29.2 (26.30-32.01)		1.07 (0.85-1.33)
Age (year)	0 < year ≤ 1	286	69	24.1 (19.17-29.09)	0.02	Reference
	1 < years ≤ 2	292	81	27.7 (22.60-32.88)		1.21 (0.83-1.75)
	2 < years ≤ 4	521	140	26.9 (23.07-30.68)		1.16 (0.83-1.61)
	4 < years	485	164	33.8 (29.60-38.03)		1.61 (1.16-2.24)
Season	Summer (Apr. to Jun.)	354	77	21.8 (17.45-26.05)	< 0.0001	Reference
	Spring (Jan. to Mar.)	428	94	22.0 (18.04-25.89)		1.01 (0.72-1.42)
	Autumn (Jul. to Sep.)	467	152	32.6 (28.30-36.80)		1.74 (1.26-2.39)
	Winter (Oct. to Dec.)	335	131	39.1 (33.88-44.33)		2.31 (1.65-3.23)
Total		1,584	454	28.7 (26.44-30.89)		

lence of *F. hepatica* infection in white yaks and black yaks in Gansu Province, northwestern China, with the goal to provide "baseline" information for estimating the effectiveness of future control strategies against *F. hepatica* infection in yaks in China.

The study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (approval no. LVRIAEC2013-010). A total of 1,584 serum samples were randomly collected from 3 counties (974 from Tianzhu, 36°31′-37°55′N, 102°07′-103°46′E; 464 from Maqu, 33°06′-34°30′N, 100°45′-102°29′E; 146 from Luqu County, 33°58′-34°48′N, 101°35′-102°58′E) between April 2013 and March 2014. The 4 seasons from spring to winter were defined as January-March, April-June, July-September, and October-December, respectively. All the investigation sites have a short period of warm season (June to October). The blood samples were transported quickly on ice to the laboratory, and centrifuged at 3,000 g for 10 min, and then serum was obtained, and stored at -20˚C until used. All information of the yaks examined, i.e., body color, age and sex, geographical region, and surveyed season, were recorded.

The level of antibodies against *F. hepatica* were examined using ELISA by the SVANOVIR® *F. hepatica*-Ab ELISA test (Svanova, Boeringer, Uppsala, Sweden) according to the manufacturer's recommendations. The excretory-secretory (ES)-antigen did not cross-react with *Ostertagia ostertagi* infections. The ELISA kit used in this study has a high specificity (88%) and sensitivity (92%), and has been widely used for detection of *F. hepatica* infection in bovine previously [11,12]. The cut-off was set at 0.3, meaning that ratios below this level were considered as negative. The positive and negative controls were supplied in the kit and included in each test.

The variation in *F. hepatica* prevalence (y) of yaks from different geographical locations ($\chi1$), species ($\chi2$), genders ($\chi3$), season groups ($\chi4$), and ages ($\chi5$) was analyzed by the chi-square test using SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA) [13]. Each of these variables was included in the binary Logit model as an independent variable based on the multivariable regression analysis. The best model was judged by Fisher's scoring algorithm. All tests were 2-sided, and values of $P < 0.05$ were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated to explore the strength of the association between *F. hepatica*-seropositivity and the conditions investigated.

Of the 1,584 tested serum samples, 454 (28.7%) were positive for *F. hepatica* antibody, with 29.2% (284/974) in white yaks and 27.9% (170/610) in black yaks. The positive rate in female yaks (30.9%, 344/1,113) was higher than in males (23.4%, 110/471) (Table 1). The seroprevalence was 29.2% (284/974), 22.6% (33/146), and 29.5% (137/464) in yaks from Tianzhu, Luqu, and Maqu County, in Gansu Province, China, respectively. They were different by age groups and ranged from 24.1% (69/286) to 33.8% (164/485). The seasonal prevalences were 22.0% (94/428), 21.8% (77/354), 32.6% (152/467), and 39.1% (131/335) in spring, summer, autumn, and winter, respectively (Table 1).

In the present study, the overall *F. hepatica* seroprevalence was 28.7% (454/1,584), which was lower than that reported in yaks in Xinghai tested by autopsy method [9,10], cattle in Switzerland [14], Poland [14], Australia [15], Ireland [16], and Vietnam [17] examined by ELISA, but higher than that of cattle in Spain [5] and Denmark [18], cow in Germany [19] tested by ELISA. It has been demonstrated that *F. hepatica* infection mainly occurs in cattle [20] and goats [20], otherwise, *F. gigantica* usually infect water buffaloes. So, these differences could be related to the animal susceptibility to fascioliasis. Moreover, local climatic conditions, farming regimen, animal welfare, as well as sampling time and sample sizes may also affect the results.

Table 1 presents the relationship between *F. hepatica*-positivity in yaks based on the univariate analysis. The impacts of multiple variables on *F. hepatica* were evaluated by forward stepwise logistic regression analysis using Fisher's scoring technique. In the final model, 2 variables had effects on the disease, described by the equation "y=-0.3731x4-0.2329x5+2.5191". Season had a strong effect on the risk of *F. hepatica*, which was in accordance with previous studies [10,21-24].

Almost all life in the investigated areas (Maqu, Luqu, and Tianzhu counties) drink water from the river nearby. The infected yaks can shed eggs into the environment (including rivers), which is a potential source for snail (can survive during July to October) infection, and the infected snails can shed cercariae into the environment. Cercariae then develop into metacercariae (in rivers and/or grass) which infect the definitive hosts (including yaks). This forms the vicious circle. Statistical analysis suggested that the *F. hepatica* seroprevalence was significantly higher in yaks in winter (39.1%, 95% CI 33.9-44.3) and autumn (32.6%, 95% CI 28.3-36.8) than yaks in spring (22.0%, 95% CI 18.0-25.9) and summer (21.8%, 95% CI 17.5-26.1, $P<0.0001$) (Table 1). This may be due to the fact that the climatic conditions in the surveyed regions during July to October were favorable for the development of *F. hepatica*. These results also suggest that temperature should be considered when carrying out control programs in the investigation areas.

Mammalian hosts acquire *F. hepatica* infection through ingestion of contaminated vegetation or water containing viable metacercariae of *F. hepatica* throughout their lifetimes [25,26], so older hosts have more opportunities to ingest encysted dormant larvae. In the present study, age was considered as a risk factor associated with *F. hepatica* infection in yaks.

Multivariable regression analysis showed that yaks of more than 4 years (OR=1.61, 95% CI 1.16-2.24), of 2-4 years (OR=1.16, 95% CI 0.83-1.61) and of 1-2 years (OR=1.21, 95% CI 0.83-1.75) has a 1.61 times, 1.16 times, and 1.21 fold higher risk of infection compared to yaks of 0-1 year (24.1%), respectively (Table 1). The trend of *F. hepatica* seroprevalence increased with age of yaks, which was in accordance with previous reports of an age-cumulative effect in the seroprevalence of *F. hepatica* in cattle [25,27].

In the present study, a higher seroprevalence was found in Maqu County of higher altitude than that in Luqu and TTAC with lower altitudes, although the difference was not statistically significant ($P=0.23$). This could be related to many factors, such as farming regimen, animal welfare, as well as sampling time and sample sizes, so whether there is a direct relationship between altitude and fascioliasis prevalence should be determined in further studies. A previous study indicated that *F. gigantica* also infects yaks in India [28]. In China, *F. gigantica* infection has also been reported in humans [29] and water buffaloes [30], but no information concerning *F. gigantica* infection in yaks was recorded. Therefore, whether *F. gigantica* can infect yaks in China should be investigated in future studies.

Taken together, the present survey indicated high *F. hepatica* seroprevalence (28.7%, 454/1,584) in yaks from 3 counties of Gansu Province in northwestern China, with 29.2% (284/974) and 27.9% (170/610) in white yaks and black yaks, respectively, which can cause economic losses to the local yak industry, and also has a potential threat to the health of Tibetans in these areas. Moreover, season and age of yak were found to be correlated with *F. hepatica* infection in yaks in the investigated areas. This information provides new local disease surveillance data, which can be very valuable for any future control interventions.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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