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Short Communication

# Epidemiological and Clinical Features of *Fasciola hepatica* Infection in Yaks (*Bos grunniens*) on Tibetan Plateau, China

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**Authors' Contribution**
**QXW and HLD conceived and designed the experiments. FBG, JYZ, ZYC and HLD performed the experiments. MK, MAG, RZA and HLD contributed reagents, materials, and analysis tools. HLD wrote the manuscript.**

**Key words**
**Yaks, *Fasciola hepatica*, Seroprevalence, Infection, Tibetan plateau**

## ABSTRACT

Fascioliasis is a common foodborne and waterborne zoonotic trematode parasitic disease infection worldwide. However, yak's fascioliasis is rarely reported on Tibetan Plateau. In this study, serological survey and associated risk factors of *Fasciola hepatica* (*F. hepatica*) infection in Tibetan yaks was investigated using an ELISA assay. The results showed that the 305/849 (35.92%) studied animals were sero-positive for *F. hepatica* with the further distribution of 24.20% (38/157), 38.02% (92/242) and 38.89% (175/450) from Bayi, Mainling, and Gongbo'gyamda areas, respectively. The seroprevalence of *F. hepatica* increased from 20.75% to 48.8% with age of yaks, with the further distribution of 20.75%, 30.23%, 39.55%, 37.70% and 48.8% for age groups: <0.5 year, 0.5-1 year, >1-2 year, >2-4 years and >4 years, respectively. The prevalence in the male and female yaks was 32.51% and 39.05%, respectively. The physical examination showed a poor body condition with emaciation, weakness, loss of appetite, anemia, jaundice, and abdominal edema in infected yaks, while on dissection, many prominent nodules of parasitic migration were present on the liver surface. Our results provided a baseline data for the exposure of yaks to *F. hepatica* infection in the study area. Therefore, some important strategies and surveillance should be adopted to control *F. hepatica* on Tibetan Plateau, China.

Infectious diseases including Fascioliasis is one of the important zoonotic parasitic diseases caused by the foodborne trematode species, widely distributed among animals and humans as public health concern (Ijaz *et al*., 2018; Mehmood *et al*., 2017; Wang *et al*., 2018). The adult *F. hepatica* parasitizes in liver and bile ducts of ruminants such as cattle, sheep, horses and other mammals (including rodents and humans), which are considered as the normal definitive hosts, while wild mammals can serve as important reservoirs (Shimalov and Shimalov, 2000). In humans, the *F. gigantica* can lead to the hepatitis and cholangitis, and cause unrecoverable mechanical liver injury. However, fascioliasis is usually misdiagnosed and neglected in human in China, and less attention has been paid to the prevention and control clinically (Ai *et al*., 2017). Though, about 2.4 million people in over 60 countries have suffered fascioliasis, and more than 180 million people are at risk of fascioliasis (Nithiuthai *et al*., 2004). In livestock, the outbreaks of fascioliasis can lead to the unrecoverable mechanical liver injury, inflammation of liver and bile ducts with significant economic losses (including reduction in milk, meat, wool) to livestock industry (Li *et al*., 2019b; Mehmood *et al*., 2017). In China, the control of fascioliasis has become more important due

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to its changing in epidemiologic pattern and aquaculture model, especially in rural and pastoral areas. *F. hepatica* is maintained in an enzootic cycle between ruminant and other amplifying vertebrate hosts, mainly pigs, dogs, mouse, humans, etc. (Liu *et al*., 2009; Mehmood *et al*., 2017; Shimalov and Shimalov, 2000). In previous study, the prevalence of *F. hepatica* was 87.35% based on examination of 767 faecal samples isolated from 58 farms in Guangxi (Zhang *et al*., 2019). Moreover, about 28.5% of sheep were reported to have *F. hepatica* infection in Heilongjiang from 1999-2003 (Wang *et al*., 2006). In China, a total of 306 human cases of fascioliasis have been recorded, and the situation of fascioliasis infection is probably underestimated due to poor diagnostic tests, and the neglect of this disease (Ai *et al*., 2019). Meanwhile, 33 human fascioliasis cases have been reported from 2003 to 2018 (Ai *et al*., 2019).

Yak (*Bos grunniens*) is an ancient original indigenous bovine species, which were mostly found in high altitude pasture (Dong *et al*., 2019). It is mainly distributed in the Himalayan region with the high cold altitude plateaus in southwest of China, Nepal, India, Bhutan, etc. The yak's meat, milk and wool is considered as the most important and irreplaceable source of income in pastoral areas of Tibet for Tibetan herdsmen (Dong *et al*., 2019; Wu *et al*., 2018a). However, free-ranging yaks graze on mountainous area, and no artificial desensitization of parasite has been employed, which increased the risk of parasites infection among the yak population, and have caused a significant economic losses (Dong *et al*., 2019; Wu *et al*., 2018b). Very little information is available on the prevalence and risk factors of *F. hepatica* infection in yaks at such high altitude in Tibet. Therefore, this species is not a good host for *F. hepatica*, but once infected, it can act as reservoir and transmit the infection to other animals, and even humans. The current study was conducted to determine the prevalence of *F. hepatica* infection in Tibetan yaks, which aims to provide a reference basis for comprehensive prevention and control of *F. hepatica* in the future.

*Materials and methods*

The study was permitted by the ethics committee of Tibet Agriculture and Animal Husbandry University (Permit number: 2018040501). A total of 849 blood samples were collected from the jugular vein by local veterinary practitioners from three regions of Tibet (Bayi, Mainling, and Gongbo'gyamda) from 2018 to 2019 (Supplementary Fig. 1). The present study was carried out in Nyingchi, in southeastern Tibet, China. This area is geographically isolated from Tibet and Sichuan Provinces by Himalayas, and shares borders with Yunnan and Qinghai Provinces (Wu *et al*., 2018a; Zhang *et al*., 2018). The average height of the altitude is about 3100 meters in the study site (Li *et al*., 2019a, b). All collected samples were centrifuged at 3500 × g for 10-20 min, and serum was used for further analysis. A total of 10 yaks with typical symptoms of *F. hepatica* infection were slaughtered at local slaughterhouses. After autopsy, the prominent nodules of parasitic migration in the surface of livers were examined, and the severity of parasitic infection were recorded.

The serum samples were tested by using a commercial ELISA kits according to the manufacturer's instructions (Shanghai Yuduo, Shanghai, China). Briefly, the critical value (cut off) was calculated as: cut off = Negative control + 0.15; if the samples $OD_{450}$ < cut off were interpreted as negative, while the samples $OD_{450}$ > cut off were considered as positive.

The data were analyzed with Chi-square test (SPSS, version 21.0). Furthermore, the odds ratios (OR) were also calculated based on likelihood ratio.

*Results and discussion*

*F. hepatica* infection results in significant losses because of mechanical injury of liver, hepatitis, cholangitis, and even death of animals. A total of 305 out of 849 serum samples (35.92%) were found positive to *F. hepatica* in yaks trough ELISA test, with the further distribution of 24.20% (38/157), 38.02% (92/242) and 38.89% (175/450) from Bayi, Mainling, and Gongbo'gyamda areas, respectively. Our results showed that the seroprevalence of *F. hepatica* in yaks was high in Gongbo'gyamda and Mainling. It may be related to climatic conditions of this area with a high temperature, humidity, rainfall, and other weather conditions which are favorable for the existence and spread of metacercariae of *F. hepatica*. Secondly, the yaks in Gongbo'gyamda and Mainling regions are on free grazing, which increases the spread of *F. hepatica* infection.

The seroprevalence of *F. hepatica* increased from 20.75%-48.8% with age of yaks, with the further distribution of 20.75%, 30.23%, 39.55%, 37.70% and 48.8% for age groups: <0.5 year, 0.5-1 year, >1-2 year, >2-4 years and >4 years, respectively. The seroprevalence in male and female yaks was 32.51% and 39.05%, respectively (Table I). Yaks are the production partner and source of wealth for Tibetans. Female yaks (especially for old female yaks) were used for breeding, that will not be killed. So, female yaks have many chances to have greater exposure risks than male yaks. In present study, the overall seroprevalence of *F. hepatica* was 35.92%, which was higher than that reported in yaks in Gansu (28.7%) (Zhang *et al*., 2017), cattle (28.6%) and goats (26.0%) in Yunnan, China (Chen *et al*., 2013), and buffaloes in Brazil (3%) (Pritsch *et al*., 2019); while, lower than the seroprevalence

in buffaloes (44.7%) in Hunan, China (Liu *et al*., 2009). Meanwhile, three mainly pre-identified probable risk factors including regions, age and gender were evaluated. The results showed that the seroprevalence of *F. hepatica* in females (39.05%; 95% CI 34.5%-43.8%) was 1.33 times (OR=1.33) more than male (32.51%; 95% CI 28.0%-37.3%). The gender based results have no statistically significant to influence the seroprevalence of *F. hepatica* among yaks, it has also suggested that the incidence of *F. hepatica* case have no relationship with exact gender. Odds ratio showed that yaks lived in Gongbo'gyamda (OR=1.99) and Mainling (OR=1.87) has 1.99 and 1.87-fold higher risk of *F. hepatica* infection as compared with yaks lived in Bayi (24.20%), respectively. Moreover, the results found the seroprevalence of *F. hepatica* in yaks of >4 years, >2-4 years, >1-2 year, and 0.5-1 year was 3.64, 2.31, 2.50, and 1.65 fold (OR=3.23, 95% CI=5.8-14.9) higher risk than yaks of <0.5 year, respectively. The seroprevalence and risk of *F. hepatica* in yaks increase with age as well, which was in accordance with some previous reports of cumulative effect with the age in the seroprevalence of *F. hepatica* in ruminants (Robinson *et al*., 2013; Sanchez-Andrade *et al*., 2002; Zhang *et al*., 2017). The present study suggests that adult yaks have more chances to expose with *F. hepatica* through free-range outside the pasturing area, while calves' yaks (<0.5 years of age) have less chances to encounter with metacercariae of *F. hepatica*. In our study, the physical examination showed a poor body condition with emaciation, weakness, loss of appetite, anemia, jaundice, and abdominal edema in infected yaks. After dissection, many prominent nodules of parasitic migration can be seen on the liver surface, and many yaks were found infested with *F. hepatica* in liver revealing the high infection rate in yaks. Our physical examination and pathological findings concomitant with the previous finding of Mehmood *et al*. (2017) and Wang *et al*. (2006).

Our study on the prevalence of *F. hepatica* provides a guideline in clinical medication, and it will also help to build important strategies and maintain a strong surveillance program to control and monitor the emerging *F. hepatica* infection in yaks and other food animals in Tibet.

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**Table I. Seroprevalence of *F. hepatica* in Yaks in different areas, ages, and genders on Tibetan Plateau, China.**

| Categories | Variables | Positive/ examined | Prevalence (%) | OR value | 95% CI |
|---|---|---|---|---|---|
| Regions | Bayi | 38/157 | 24.20% | Ref | 17.7%-31.7% |
| | Mainling | 92/242 | 38.02% | 1.87 | 31.9%-44.5% |
| | Gongbo'gyamda | 175/450 | 38.89% | 1.99 | 34.4%-43.6% |
| Ages | <0.5 year | 22/106 | 20.75% | Ref | 13.5%-29.7% |
| | 0.5-1 year | 65/215 | 30.23% | 1.65 | 24.2%-36.8% |
| | >1-2 year | 87/220 | 39.55% | 2.50 | 33.0%-46.3% |
| | >2-4 years | 69/183 | 37.70% | 2.31 | 30.7%-45.2% |
| | >4 years | 61/125 | 48.80% | 3.64 | 39.8%-57.9% |
| Genders | Male | 132/406 | 32.51% | Ref | 28.0%-37.3% |
| | Female | 173/443 | 39.05% | 1.33 | 34.5%-43.8% |
| | Total | 305/849 | 35.92% | | 32.7%-39.3% |

*Supplementary material*

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20190823220816

*Statement of conflict of interest*

The authors declare no conflict of interest.

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## Supplementary Material

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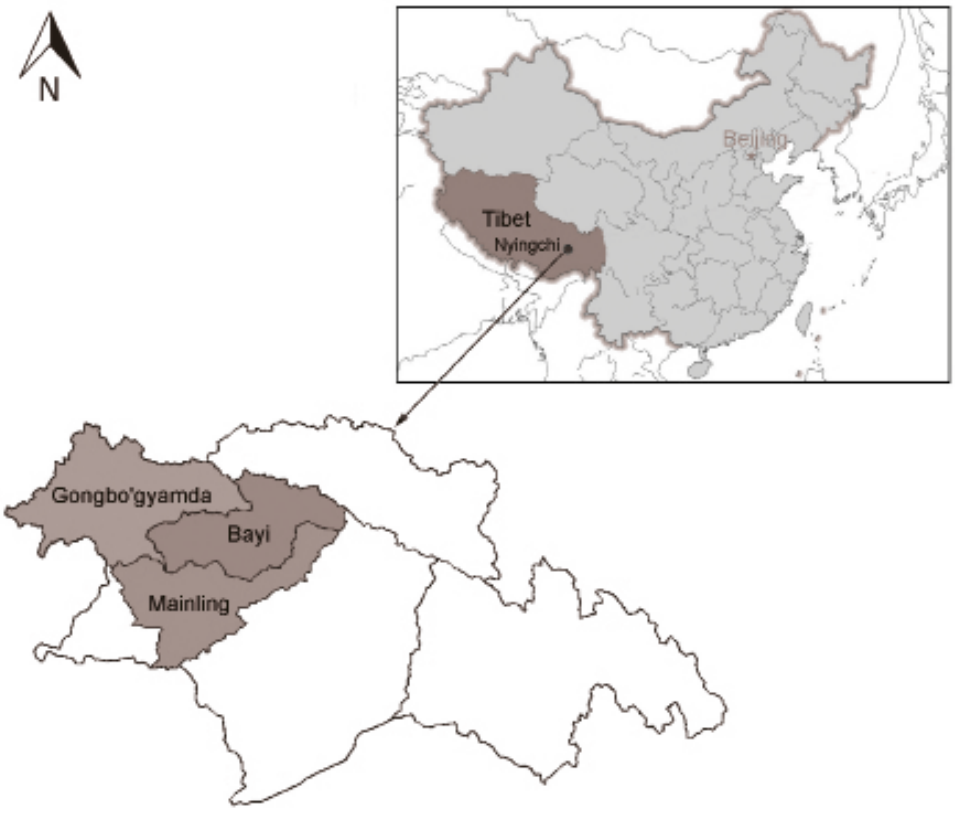


Supplementary Fig. 1: The map of geographical distribution of *F. hepatica* investigation in Tibet, China.

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