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# Epidemiological investigation and risk factors of *Echinococcus granulosus* in yaks (*Bos grunniens*), Tibetan pigs and Tibetans on Qinghai Tibetan plateau

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ABSTRACT

*Echinococcus granulosus* (*E. granulosus*) is a diverse zoonotic parasite and causes Cystic echinococcosis (CE) disease in humans and livestock. However, scare information is available about the epidemic situation of *E. granulosus* infection in yaks, Tibetan pigs and native Tibetans on the Qinghai Tibetan plateau. Therefore, a study was carried out to find prevalence and risk factors of *E. granulosus* in yaks, Tibetan pigs and Tibetans. Serum samples from yaks (1371), Tibetan pigs (454) and Tibetans (600) were collected and assessed by commercial ELISA kits. Multivariable logistic regression model was performed to find the variables possibly associated with exposure of *E. granulosus* infection in yaks, Tibetan pigs and Tibetan.

The overall prevalence of *E. granulosus* in yaks was 6.49%. In different regions, the prevalence were ranged from 3.43% to 11.79%. In male and female yaks, the prevalence was 5.67% and 7.04%, respectively. In different ages, the prevalence were ranged from 2.20% to 10.9%. While, in different years, the prevalence was 3.61% in 2014, 9.66% in 2015, and 6.33% in 2016. According to the conditional stepwise logistic regression, three factors (region, age and year) were demonstrated to be risk factors influencing the prevalence of *E. granulosus* in yaks significantly ($P < 0.05$). A total 33/454 of Tibetan pigs were positive for *E. granulosus* with the distribution of 5.47, 5.70 and 13.27% prevalence in Gongbo'gvamda, Mainling, and Nyingchi region, respectively. In male and female Tibetan pigs, the prevalence was 7.12% and 7.49% respectively, while region was considered as a significant ($P < 0.05$) risk factor influencing the *E. granulosus* infection in Tibetan pigs. The total prevalence of *E. granulosus* infection in Tibetans was 1.83%, while in male and female Tibetans, the prevalence was 1.41% and 2.21%, respectively. In different ages, the prevalence were ranged from 0 to 3.21%. In Tibetans contacting animals or not was 2.41% and 0.54% respectively, and breeding dogs or not was 3.0% and 1.09%, respectively. Risk factors (gender age, contact animal and breed dog) were not significant ($P > 0.05$). The present results reported the prevalence and associated risk factors of *E. granulosus* in yaks, Tibetan pigs and native Tibetans. These findings could have important epidemiological significance and a direct influence on the remote plateau.

## 1. Introduction

The larval stage of the tapeworm *Echinococcus granulosus (E. granulosus*) causes a zoonotic life-threatening disease, Cystic echinococcosis (CE) (Chaligiannisa et al., 2015; Scioscia et al., 2016). This parasite has a worldwide geographical distribution and is considered as an important problem for animal and human health. It causes significant economic losses due to the formidable adaptability of the larval stage (hydatid cyst) to domestic and wild mammalian intermediate hosts, including humans (Eckert et al., 2000; Craig et al., 2007). The eradication of this disease remains a great challenge, especially in least developed countries (Larrieu and Zanini, 2012). The transmission of *E. granulosusis* primarily maintained with many wild canids as definitive hosts and livestock animal as intermediate hosts (Scioscia et al., 2016). Humans become infected through the ingestion of parasite eggs which are passed in the feces of infected carnivores (Pleydell et al., 2008). In humans CE infection clinical manifested from asymptomatic infection to severe disease and possibly death (Li et al., 2011).

The yak (*Bos grunniens*), is a long haired bovine species found throughout the Himalayan region of the South Central Asia (China, Russia, Bhutan, Nepal, Mongolia, India, and other countries) at high cold altitude (3000 m) (Li et al., 2014; Li et al., 2016a). Approximately

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14 million (90%) of the world yak population live on the Qinghai-Tibetan Plateau in China (Li et al., 2015). Due to the usefulness of its milk, meat, dung, and wool, make this animal important for native herdsmen (Li et al., 2014; Li et al., 2015). Infectious diseases have been a serious threat for livestock (Elhaig et al., 2016; Qayyum et al., 2016; Yilmaz et al., 2016). So, in such remote plateaus, any zoonotic parasitic disease in yak may cause economical losses and potential threat to public health (Li et al., 2014; Li et al., 2016a).

Tibetan pig is an economically important local pig breed in South-East of Tibetan Plateau and adjacent area of P.R. China (Qinghai, Tibet, Sichuan, Gansu and Yunnan) at 3000 m above the sea level and cold region (Li et al., 2016b; Li et al., 2017a). Pigs feed on free range system combined with dry-lot husbandry, which makes Tibetan pig's disease resistance along with lean carcass and high-quality meat production (Li et al., 2017b). Owing to its higher tenderness and texture, the Tibetan pig meat is highly proteinaceous and delicious in nature, which makes the pigs an important source of income to herdsmen (Li et al., 2016b). So, any zoonotic disease in the pigs may cause serious economic losses to pig production, and potential threat to human health (Li et al., 2017c).

Previously, *E. granulosus* has been reported in people in 21 provinces in China, especially in western provinces of Qinghai, Gansu, Sichuan, Ningxia, Xinjiang, Inner Mongolia, Tibet, and Yunnan (Li et al., 2010; Han et al., 2016). *E. granulosus* has been confirmed of infection in sheep and yaks on the Qinghai Tibetan plateau by mitochondrial cytochrome C oxidase subunit 2 (cox2) gene (Hu et al., 2015). Since 2010, CE was listed in the subjects of major national infectious disease control programs in China (Han et al., 2016). However, limited information is available bout the epidemic condition of *E. granulosus* in yaks, Tibetan pigs and Tibetans on the Qinghai Tibetan plateau. Therefore, a survey was conducted to investigate the prevalence of *E. granulosus* in yaks, Tibetan pigs and Tibetans on the high altitude areas in China.

## 2. Materials and methods

### 2.1. Ethics statement

Blood Samples were collected under the permission of the relevant institutions. All procedures were performed under the instructions and approval of Laboratory Animals Research Centre of Hubei province, Qinghai, Sichuan, Gansu and Tibet in P. R. China and the ethics committee of Huazhong Agricultural University, China (Permit number: 4200695757).

### 2.2. Serum samples

A total of 1371 blood samples were collected during 2014–2016 from yaks on the Qinghai Tibetan plateau (Qinghai 195, Tibet 255. Sichuan 321 and Gansu 600) (Fig1, Table 1), and the information related to region, gender and age was captured on prescribed Performa. The blood samples were centrifuged at 1000 × *g* for 10 min, and serum was separated and stored at −20 °C till further analysis.

A total of 454 serum samples from Tibetan pigs (1.5–2 years age) were collected from a slaughter house in Nyingchi prefecture between December and February 2014-15, and from different counties of the Nyingchi prefecture (Table 2). After collection, each of the sample was centrifuged at 4000 × g for 10 min, and serum was separated and stored at −20 °C till further analysis.

A total of 600 blood samples were collected during July and September in 2015 from Tibetans people who came to the hospital in Nyingchi, Tibet (Table 3). While the information on gender and age was recorded on a prescribe Performa. The blood samples were centrifuged at 1000 × *g* for 10 min, and serum was separated and stored at −20 °C till further analysis.

### 2.3. Determination of antibodies against E. granulosus in yaks

All serum samples were tested for antibodies to Echinococcosis by a commercial enzyme linked immunosorbent assay (ELISA) kit (*Bovine Echinococcosis* Ag ELISA Kit, Duoyu Biological Pharmaceuticals Co., Ltd, Shanghai, China) according to the manufacturer's instructions. The test value was based on the optical density (OD) values of OD 450. To ensure validity, the average OD 450 of positive controls was ≥ 1.00; the average OD 450 of negative controls was ≤ 0.15. The critical (cut off) value = the average OD 450 of negative controls + 0.15. The results were interpreted as positive when the OD 450 ≥ cut off value; negative when the OD 450 < cut off value.

### 2.4. Determination of antibodies against E. granulosus in Tibetan pigs

All serum samples were tested for antibodies to *Echinococcosis* by piloting a commercial enzyme linked immunosorbent assay (ELISA) kit (Pig *Echinococcosis* Ag ELISA Kit, Jianlun Biological Pharmaceuticals Co., Ltd, Guangzhou, China) according to the manufacturer's instructions. The test value was based on the optical density (OD) values of OD 450. The critical (cut off) value = the average OD 450 of negative controls * 2.1, when the average OD 450 of negative controls < 0.05, cut off value = 0.08* 2.1 = 0.168. The results were interpreted as: positive when the OD 450 ≥ cut off value; negative when the OD 450 < cut off value.

### 2.5. Determination of antibodies against E. granulosus in Tibetans

All serum samples were tested for antibodies to *Echinococcosis* by piloting a commercial enzyme linked immunosorbent assay (ELISA) kit (Human *Echinococcosis* Ag ELISA Kit, Yikang Biological Pharmaceuticals Co., Ltd, Guangzhou, China) according to the manufacturer's instructions. The test value was based on the optical density (OD) values of OD 450. The critical (cut off) value = the average OD 450 of negative controls * 2.1, when the average OD 450 of negative controls < 0.05, cut off value = 0.05* 2.1 = 0.105. The results were interpreted as: positive when the OD 450 ≥ cut off value; negative when the OD 450 < cut off value.

### 2.6. Statistical analysis

Multivariable logistic regression model was employed to find variables, potentially associated with exposure to *E. granulosus* infection in yaks, Tibetan pigs and Tibetan. Statistically significant levels within factors and interactions was recognized, when probability (P) value found < 0.05. Odds-ratios (OR) was kept with 95% confidence intervals (CI) and statistical analyses was performed through the IBM SPSS Statistics 20.0 (SPSS Somers, NY).

## 3. Results

For yaks, the overall prevalence of *E. granulosus* was 6.49% (95% CI: 5.2–7.9). In different regions, the prevalence were ranged from 3.43% (95% CI: 1.7–6.0) to 11.79% (95% CI: 7.6–17.2). In male and female yaks, the prevalence was 5.67% (95% CI: 3.9–7.9) and 7.04% (95% CI: 5.4–9.0), respectively. In different ages, the prevalence were ranged from 2.20% (95% CI: 0.3–7.7) to 10.9% (95% CI: 8.2–14.0). In different years, the prevalence was 3.61% (95% CI: 2.0–6.0) in 2014, 9.66% (95% CI: 6.9–13.1) in 2015, and 6.33% (95% CI: 4.5–8.6) in 2016 (Table 1). According to conditional stepwise logistic regression, region, age and year demonstrated the more influencing risk factors in present study. Furthermore, yaks in Tibet (8.63%) had two times (OR = 2.661, 95% CI = 1.265–5.596, ρ = 0.008 < 0.01) higher risk of infection compared to yaks in Sichuan (3.43%); whereas yaks in Qinghai (11.79%) had a three times (OR = 3.768, 95% CI = 1.794–7.917, ρ < 0.001 < 0.01) higher risk of infection compared to yaks in