



**Full Length Article**

## Sero-Prevalence of Hydatidosis in Camel Population in Different Ecological Zones of Balochistan Province, Pakistan

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### Abstract

The present investigation describes the sero-prevalence of cystic echinococcosis (hydatidosis) in camel population located at four different ecological zones of Balochistan province, Pakistan. To this end, a total of 334 camel serum samples were tested for hydatidosis through iELISA using antigen B (EgAgB) isolated from hydrated cysts. The association of different risk factors with prevalence of hydatidosis was determined by collecting information on pre-designed questionnaire. The test results at a cut-off value of 0.5 revealed that 35.92% camels were positive for cystic echinococcosis. The highest prevalence of this disease was recorded in District Musakhel (40.00%), followed in decreasing order by Zhob (37.04%), Loralai (32.08%) and Barkhan (30.00%). Different risk factors, including breed (Pahwal, Raigi, Kohi), age (>10years, 5-10 years, <5 years), gender (male, female) and season (Winter, Spring, Summer, Autumn) were found statistically associated ( $P < 0.05$ ) with the sero-prevalence of hydatidosis in study areas. The multivariate analysis indicated that prevalence of hydatidosis was significantly higher in females, Pahwalbreed, Winter and animals having >10 years of age ( $P < 0.05$ ). The present study may provide base line data to plan hydatidosis control program in camel in the province. © 2020 Friends Science Publishers

**Key words:** Sero-prevalence; Cystic echinococcosis; iElisa; Camel; Balochistan

### Introduction

Camel (*Caemelus dromedarius*) is a multiuse animal in semi-arid and arid zones of the world. Camel provides meat, milk and byproducts which is the good useful addition to the food chain (Hussain *et al.* 2016; Qureshi *et al.* 2019). In Pakistan, there are 21 breeds of camels reared for different purposes. However, the major types of camels are mountainous and riverine, which are mostly reared by pastoral nomads (Ali *et al.* 2009; Marghazani 2018). Hydatidosis (also known as cystic echinococcosis) in ruminants is caused by the larval stage of dog tapeworm "*Echinococcus granulosus*". It is one of the major parasitic problems in camel that effects the production potential of camel because of the development of many cysts in different organs, known as "Hydatid Cyst". The cyst is slow growing, and severity of infection depends on the size of cyst. When cyst gains full size, it creates pressure on tissues or organs, followed by necrosis and organ disruption. Most of the meat animals are slaughtered before these cysts getting full size.

However, the life span of camel is 40 to 50 years which is four times more than other domestic animals so the chances of developing full sized hydatid cyst are more in camel as compared to other domestic animals (Ahmadi and Dalimi 2006; Ijaz *et al.* 2018).

It is also considered as Top Neglected Tropical Zoonotic Disease, which has been reported in many hosts, including humans, round the globe (Jenkins 2005; Hüttner *et al.* 2008; Liao *et al.* 2016; Khan *et al.* 2018). Dogs are definitive host for this parasite while food animals such as sheep, goats, cattle and camels are likely to act as intermediate hosts (Torgerson and Budke 2003). In humans, cystic echinococcosis is listed as most severe parasitic disease by the World Health Organization. The hydatid cysts develop in liver and lungs of humans, leading to organ dysfunction (Brunetti and Junghanss 2009). G1 and G3 strains of *E. granulosus* are most commonly prevalent species in Humans. However, recent studies have shown that the *E. granulosus* strain G6 commonly occurring in camels are also prevalent in humans, indicating that camel may play a significant role in transmission of this parasite to

humans (Kinkar *et al.* 2018; Laurimäe *et al.* 2018).

Infection can be diagnosed through conventional, serological and molecular techniques. However, serological diagnosis has been preferred over conventional approaches due to their high specificity and sensitivity (Abdybekova and Torgerson 2012; Elmajdoub and Rahman 2015). Molecular diagnosis may be an option but requires more expertise and instrumentation. Serological methods standardized for detection of specific antibodies in sera of infected animals are indirect haemagglutination (IHA) and enzyme linked immunosorbent assay (ELISA) (Latif *et al.* 2010; Sazmand *et al.* 2013; Liao *et al.* 2016).

In Pakistan, hydatidosis with high prevalence in domestic animals has been reported by some epidemiologists in some regions (Mirani *et al.* 2000; Latif *et al.* 2010; Ehsan *et al.* 2017). However, no study has been carried out regarding occurrence of this disease in camels which are main source of income for nomads in Balochistan. Importantly, in pastoralist farming, the camel herd is accompanied by dogs for security purposes which are main host for this parasite, indicating that the camel in Balochistan may be suffering from hydatidosis with a possibility of reduced production. Therefore, the current study was designed to investigate the sero-prevalence of hydatidosis in camel population of Balochistan, Pakistan.

## Material and Methods

### Study area

This study was conducted in geographically and ecologically four different camel raising districts of Balochistan, Pakistan *i.e.*, Barkhan, Musakhel, Loralai and Zhob. The Latitude and Longitude of Balochistan is 28.49 and 65.09, respectively. Balochistan contains 44% land mass of Pakistan. The four districts are located at northern side of Balochistan and are hilly areas with high monsoon rain fall. The following prevailing conditions in the study districts may favour the growth and development of this parasite: pastoralist pattern grazing, mix herds, keeping dogs with herds, high population of Wolf, Fox, Jekale and Hyena in mountains.

### Estimation of sample size

For sample size calculation, statistical formula was used by keeping expected prevalence as 50% with CI as 95% and desired absolute precision as 6% (Thrusfield 2007). Multistage cluster sampling was done for selection of camels in the study area. According to cluster sampling, primary units along with secondary and tertiary units were selected as union councils, number of farms and camels, respectively. The secondary units were selected based on number of camels per herd (>20) with no history of anti-parasitic medication for last one year. Based on above

statistical methods and criteria, 334 camels from 11 herds in 16 union councils were screened for cystic echinococcosis.

### Sample collection and processing

From each animal, approximately 5 mL blood sample was aseptically collected from jugular vein using 21.5-gauge sterile needle in a gel-clot activator vacutainer (Improvacuter, Germany). All samples were transported to laboratory and centrifuged at 4000 rpm for 5 min. Then, the serum was separated in cryovial tubes and stored at -20°C until further experiment. From herds' owners, complete information regarding possible risk factors, including previous medication, age, sex and breed were collected on pre-designed questionnaire.

### Serological analysis

For detection of hydatidosis in camel, an indirect enzyme linked immunosorbent assay (iELISA) was performed using antigen B (EgAgB) of hydatid cyst by following the steps and conditions described by Al-Kitani *et al.* (2017). The EgAgB was kindly provided by Animal Health Research Centre, Ministry of Agriculture & Fisheries, Oman (Al-Kitani *et al.* 2017).

### Statistical analysis

The relationship between each factor (age, sex, breed, study districts, season) and seroprevalence of hydatidosis was computed using both the univariate analysis through chi-square test and multivariate analysis through binary logistic regression model with backward step approach (Thrusfield 2007). The risk factors were further divided into two categories, including individual level model (age, breed, gender) and herd level model (districts, season). The evaluation of model fitness and classification of outliers was performed at 0.5 cut off point. The 95% CI for all these tests were also computed. All the analyses were performed at 0.5% level of significance using S.P.S.S. software (IBM S.P.S.S. Statistics for Windows, Version 20.0) (Urdaz-Rodriguez *et al.* 2009).

## Results

Antibodies against cyst form of *E. granulosus* were detected in camels belonging to all study areas of Balochistan with varied prevalence. The overall prevalence of hydatidosis in camel population in study districts was 35.92% (120/334), as determined through iELISA. The univariate analysis indicated that various factors influenced the prevalence of hydatidosis in camels in the study area. The higher prevalence of the disease was observed in district Musakhel (40.00%,  $\chi^2=7.12$ ,  $P=0.010$ ) as compared to those in Zhob (37.04%), Loralai (32.08) and Barkhan (30.00%). Gender-wise prevalence of hydatidosis was higher in female camels

**Table 1:** Univariate and multivariate analyses for assessment of association of risk factors with the occurrence of hydatidosis in camels in Balochistan Province, Pakistan

| Category    | Variable   | Prevalence (%) (Positive/ total samples) | 95%CI     | Univariate analysis |           | Multivariate analysis |         |
|-------------|------------|--|-----------|---------------------|-----------|-----------------------|---------|
|             |            |  |           | Chi-square          | P Value   | OR                    | P value |
| Gender      | Female     | 41.43 (104/251)                          | 33.9-50.8 | $\chi^2=14.27$      | $P=0.013$ | 2.96                  | 0.0004  |
|             | Male       | 19.27 (16/83)                            | 15.3-20.5 |                     |           | -                     | -       |
| Age         | >10 years  | 61.25 (49/80)                            | 55.5-68.9 | $\chi^2=34.36$      | $P=0.014$ | 8.34                  | 0.0001  |
|             | 5-10 years | 37.58 (53/141)                           | 31.8-44.2 |                     |           | 3.18                  | 0.0002  |
|             | <5 years   | 15.93 (18/113)                           | 13.4-16.5 |                     |           | -                     | -       |
| Breed       | Raigi      | 32.04 (33/103)                           | 25.4-41.1 | $\chi^2=5.66$       | $P=0.010$ | 2.33                  | 0.0079  |
|             | Pahwal     | 62.26 (66/106)                           | 57.6-68.9 |                     |           | 8.17                  | 0.0001  |
|             | Kohi       | 16.80 (21/125)                           | 12.3-20.8 |                     |           | -                     | -       |
| Herd Region | Musakheil  | 40.00 (52/130)                           | 36.4-44.7 | $\chi^2=0.06$       | $P=0.014$ | 1.56                  | 0.0016  |
|             | Loralai    | 32.08 (17/53)                            | 28.3-34.7 |                     |           | 1.10                  | 0.0085  |
|             | Zhob       | 37.04 (30/81)                            | 35.2-41.2 |                     |           | 1.37                  | 0.0036  |
|             | Barkhan    | 30.00 (21/70)                            | 24.4-32.5 |                     |           | -                     | -       |
| Season      | Spring     | 44.00 (44/100)                           | 39.8-48.9 | $\chi^2=1.44$       | $P=0.016$ | 3.45                  | 0.0008  |
|             | Summer     | 24.44 (22/90)                            | 22.1-27.4 |                     |           | 1.41                  | 0.0037  |
|             | Winter     | 55.41 (41/74)                            | 51.2-60.8 |                     |           | 5.45                  | 0.0001  |
|             | Autumn     | 18.57 (13/70)                            | 14.9-21.1 |                     |           | -                     | -       |

(41.43%,  $\chi^2=14.27$ ,  $P=0.003$ ) as compared to those of males (19.27%). Among different breeds, the higher prevalence of hydatidosis was observed in Pahwal breed (62.26%,  $\chi^2=5.66$ ,  $P=0.001$ ), followed in decreasing order by Raigi (32.04%) and Kohi breeds of camels (16.80%). Age-wise prevalence was higher in >10 years age group (61.25%,  $\chi^2=34.36$ ,  $P=0.014$ ), as compared to those of 5–10 years (37.58%) and <5 years age groups (15.93%) in decreasing order. According to seasons, the prevalence of hydatidosis was significantly higher ( $\chi^2=1.44$ ,  $P=0.016$ ) in Winter (55.41%) and lower in Autumn (18.57%). However, no significant difference in the prevalence of hydatidosis was observed during Summer (24.44%) and Autumn (18.57%). The details regarding number of samples and confidence intervals have been mentioned in Table 1.

The multivariate model using binary logistic regression was applied for the determination of association of different risk factors with prevalence of hydatidosis at both individual (age, breed, gender) and herd level (Districts, seasons) (Table 1). The model fitness tests, including Lemeshow and Hosmer Test ( $\chi^2=14.08$ ,  $df=2$ ,  $P=0.001$ ) and Nagelkerke R Square (0.038), indicated that binary regression model is a weak model to investigate the association of individual level factors with the prevalence of hydatidosis. However, similar to the statistical findings of univariate analysis, the prevalence of hydatidosis was higher ( $P < 0.05$ ) in females (OR=2.96), Pahwal breed (OR=8.17) and above than 10 years age group (OR=8.34) as compared to those of respective variables in each of these categories. At herd level, Lemeshow and Hosmer Test ( $\chi^2=0.732$ ,  $df=2$ ,  $P = 0.583$ ) and Nagelkerke R Square (0.038) indicated that binary logistic regression model is best fitted to investigate the association of risk factors with the prevalence of hydatidosis at herd level. In contrast to the results of univariable analysis, the prevalence of hydatidosis was found significantly different among different seasons ( $P < 0.05$ ), being higher prevalence in Winter (OR=5.45), followed in decreasing order by Spring (OR=3.45), Summer

(OR=1.41) and Autumn. However, among study districts, the prevalence of hydatidosis was higher in Musakhel district (OR=1.56,  $P<0.05$ ), followed in decreasing order by Zhob (OR=1.37), Loralai (OR=1.10) and Barkhan, as indicated by univariate analysis. The detailed information regarding association of risk factors with prevalence of hydatidosis along with confidence intervals, Odds' ratios and P values through univariate and multivariate analyses has been mentioned in Table 1.

## Discussion

The prevalence of hydatidosis in Pakistan has been reported in wide range of host in many areas such as 8.85% in Sheep, 6.21% in Goat (Iqbal et al. 2012), 7.19% in cattle and 7.52% in buffalo (Latif et al. 2010) in various areas on Punjab Province. Although, a few studies have been conducted to ascertain the occurrence of this disease in camels (Latif et al. 2010). However, the epidemiological data of hydatidosis in camels in Balochistan is not collected so far, which is having largest population of this animal in Pakistan. Interestingly, a higher prevalence of hydatidosis has been reported in sheep (46.74%) in Quetta district of Balochistan (Ahmed et al. 2006), highlighting the occurrence of this diseases in camel in the province. Therefore, the present study was designed to investigate the sero-prevalence of cyst form of *E. granulosus* in four districts of Balochistan province, including Barkhan, Musakhel, Loralai and Zhob, which have high camel population in the province.

In the present study, 35.92% camels were sero-positive for hydatidosis, including 30.00% in Barkhan, 40% in Musakhel, 32.08% in Loralai and 37.04% in Zhob district. Though, in another province of Pakistan, namely Punjab, a report published in 2012 indicated the prevalence of this disease in camels as 17.29% (Latif et al. 2010), which is lower than that of present study. This higher

prevalence of this disease in camels in Balochistan may be due to following reasons: (1) the camels in the province are generally kept in pastoralist conditions, including utilization of large grazing areas receiving wide range of animal species which may favor the disease spread, (2) Likewise, poor diet and draught stress weaken their resistance to disease, (3) Deployment of large population of dogs (definitive host) which are main reservoir of this parasite. Another factor is the lack of implementation of quarantine measures in the province for camels coming from neighboring countries. As for example, Balochistan share its border with Afghanistan and Iran, where a high prevalence of this disease in camel (60%) has been reported (Ahmadi 2005).

The hydatidosis has been reported in camels in many countries, although lower than that of present study, such as 12.54% prevalence in Libya (Elmajdoub and Rahman 2015), 32.85% in Saudi Arabia (Ibrahim 2010), 1.28% in Oman (Idris *et al.* 1999). In contrast, a higher prevalence of hydatidosis in camel (60%) has been documented in some areas of Iran (Ahmadi 2005). This varied prevalence of hydatidosis in camel among different countries may be due to different climatic conditions, farming system, on-farm management and housing practices of that area and sample size in each study.

With respect to animal gender, it has largely been reported that the occurrence of parasitic infections is higher in females as compared to those of males, as the growth and development of parasite larval stages increased due to immunosuppression in pregnancy and parturition stress. Moreover, the prolonged keeping of females for several years for breeding and milking purpose make them more exposed to parasites as compared to males which are slaughtered at young ages for meat purpose (Hussen *et al.* 2019). Accordingly, various studies have shown a higher prevalence of hydatidosis in females as compared to those of males (Salih *et al.* 2011; Pour *et al.* 2012; Khan *et al.* 2013). Similarly, in the present study, females had higher prevalence of this disease (41.43%) than those of males (19.27%). Apart from the above reasons, male animals in the study area are kept at farm due to aggressive behavior, while, female animals mostly graze in a pasture which may increase the chances of ingestion of infective stage of this parasite through contaminated vegetation (Personal observation).

Age of the animals is another reported factor influencing the prevalence of many parasitic infections (Brunetti and Junghanss 2009). In the present study, highest prevalence of hydatidosis was recorded in older animals (>10 years old age group), followed in decreasing order by 5–10 years old and <5 years old age groups. However, there is dearth of knowledge regarding association of age with transmission dynamics of this parasite in camels. The possible reasons of this higher seroprevalence in older animal may include, (1) weaken immune system against pathogens (Varcasia *et al.* 2007), (2) persistence of

antibodies due to prolonged survival of hydatid cyst on organs of host for many years (Liao *et al.* 2016), (3) higher population of female animals in this age group, which were more infected in the present study (Personal observation). Nevertheless, various scientists in other regions documented higher prevalence of this disease in old age groups of other animal species (Lahmar *et al.* 1999; Capuano *et al.* 2006; Ibrahim *et al.* 2008; Rinaldi *et al.* 2008).

Among various breeds of camel, the highest prevalence of hydatidosis was recorded in Pahwal breed as compared to those of Raigi and Kohi. This varied prevalence of the disease among various breeds may be linked with feeding habits of these breeds, as Pahwal breed graze in pastures and Kohi breed browse leaves of trees, herbs and shrubs (Raziq *et al.* 2010). Moreover, Kohi and Raigi are local breeds of the study area, which are well adapted to harsh and drought conditions of Balochistan and presumed to be resistant to parasitic infections (Raziq *et al.* 2010).

The highest prevalence of hydatidosis was documented in camels in District Musakhel than those of other districts. The higher prevalence of hydatidosis in camels in this district may be due to following reasons: (1) highest population of camels, including large number of females, (2) presence of large number of wild animals, including Wolf, Fox, Jackal and Hyena which are definitive host of this parasite, further contaminating the pasture through feces, (3) existence of pastoral system.

According to seasons, a higher prevalence of hydatidosis was observed during Winter and Spring as compared to Autumn and Summer. In Winter, due to cold and dry climatic conditions, less fodder and fewer grazing areas are available for camels in Balochistan, where pastoralist farming system is prevailing. Consequently, the animals may become malnourished with weakened immunity; and they start licking bones and faeces on the ground which may increase the likelihood of transmission of this parasite through feco-oral route (Personal observation). On the other hand, the higher prevalence of hydatidosis in camels of study area in Spring may be due to rainy period increasing the spread of infective stage of this parasite from one area to surroundings.

## Conclusion

The overall prevalence of hydatidosis was 35.92% in four districts of Balochistan. The animals having age more than 10 years had more prevalence as compared to younger animals. In female camels, the prevalence of hydatidosis was higher as compared to those of male animals. Among different breeds of camel, the prevalence of hydatidosis was higher in Pahwal breed as compared to those of Kohi and Raigi. Nevertheless, this high prevalence of hydatidosis in camel is also highlighting that the human population linked with camel farming in the study area may be at risk of getting this infection, as evident

by previous studies showing high prevalence of camel *Echinococcus* strains in humans.

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