

Serological evidence of antibodies against *Chlamydophila abortus* in free-ranging yak (*Poephagus grunniens*) in Arunachal Pradesh, India

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Summary

Serum samples were randomly collected from 172 free-ranging yak (*Poephagus grunniens*, presently *Bos grunniens*) from six different yak tracts of Arunachal Pradesh, India, and subjected to enzyme-linked immunosorbent assay to detect the presence of specific antibodies against *Chlamydophila abortus*. The overall prevalence of this disease in yak was 35%. The prevalence of *Cp. abortus*-specific antibodies was significantly higher in yak cows (41%) than among bulls (25%). The highest prevalence (39%; 95% confidence interval [CI] = 27, 55) was found in yak between one and three years of age, while the lowest prevalence (20%; 95% CI = 10, 41) was reported in yak below one year of age.

Keywords

Chlamydophila abortus – Enzyme-linked immunosorbent assay – India – Seroprevalence – Yak.

Introduction

Chlamydophila, an obligate, intracellular, Gram-negative coccoid pathogen, is responsible for a wide range of clinical syndromes in animals and human beings (5). The family *Chlamydiaceae* previously contained only the single genus, *Chlamydia*. However, a recent reclassification has established two genera: *Chlamydia* (*C.*) and *Chlamydophila* (*Cp.*). Chlamydial infections are associated with a variety of reproductive disorders in cattle, including:

- failure to conceive
- abortion
- endometritis
- vaginitis (4, 13).

In addition, pneumonia, enteritis, encephalitis, arthritis and keratoconjunctivitis have also been reported (7, 12). In sheep and goats, *Cp. abortus* is known to cause ovine enzootic abortion.

Yak (*Poephagus grunniens*) are a unique bovine species of great economic importance in high hill- and snow-bound regions from 3,000 m to 5,000 m above mean sea level (msl), in the People's Democratic Republic of China, Mongolia, Bhutan, Nepal, Russia and India (14). The yak population in India has decreased over the years, and the yak is now considered a threatened species (2). Various reasons have been reported for this decline in population, including reproductive disorders (1, 2). The present study describes the seroprevalence of antibodies against *Cp. abortus* among free-ranging yak.

Materials and methods

This study was conducted in different yak tracts of the West Kameng (2,217 msl, from 91° 30' to 92° 40' East in longitude, and 26° 54' to 28° 01' North in latitude) and Tawang (3,500 msl, at a latitude of 27° 48' and longitude of 97° 30') districts of Arunachal Pradesh, India. Blood samples were randomly collected from 172 free-ranging yak from six different locations. The separated sera were de-complemented and subjected to serological analysis using the CHEKIT *Cp. abortus* enzyme-linked immunosorbent assay (ELISA) kit, to detect the presence of *Cp. abortus*-specific antibodies.

Chlamydomphila abortus-coated microtitre plates were supplied with the kit. One hundred microlitres of test and control (positive and negative) sera (1:400 dilutions in CHEKIT wash solution) in duplicate were added to the respective wells. The plates were then incubated for 1 h at 37°C and subsequently washed. Then, 100 µl of CHEKIT anti-ruminant immunoglobulin G (IgG) horseradish peroxidase conjugate was added to each well and the plates were incubated again for 1 h at 37°C. After washing, 100 µl of CHEKIT tetramethylbenzidine substrate was added and further incubation took place for 15 min at room temperature. The reaction was stopped and absorbance was taken at 450 nm. The percentage positivity was calculated using the following formula, where OD stands for optical density and PP for percentage positivity:

$$PP (\%) = \frac{OD_{\text{test}} - OD_{\text{neg}} \times 100\%}{OD_{\text{pos}} - OD_{\text{neg}}}$$

(OD_{test} = average OD of the test sample, OD_{pos} = OD of the positive control and OD_{neg} = OD of the negative control)

Parallelism was assessed between the serial dilutions of positive control sera and yak sera. A higher degree of parallelism was observed in the percentage positivity curve between the serially diluted positive control sera and yak

sera ($p < 0.01$), using the standard regression test. In a previous study, the authors observed that yak IgG is highly similar to bovine IgG, if not identical (1). Therefore, a cut-off value similar to that recommended for bovine ruminants by the manufacturer was used to determine the *Chlamydomphila*-specific antibodies in yak serum.

A value greater than or equal to 40% was considered positive. The group with a PP > 30% but < 40% was interpreted as suspect and serum samples with a PP ≤ -30% were considered negative.

The chi-squared (χ^2) test was used to compare the seroprevalence in relation to the age and sex of the animals tested. The confidence interval was determined by Fisher's exact test. All analysis was performed using Graph Pad Prism software, version 4.0.

Results and discussion

Of 172 animals, 60 were found to have tested positive (Table I) for the presence of antibodies against *Cp. abortus* (prevalence 35%: 95% CI = 28, 42). However, based on the OD value, the results from 21 yak (12.2%: 95% CI = 10, 41) were interpreted as suspect.

In sheep and cattle, the main source of infection in the environment is the placenta and aborted foetus materials. These tissues are shed in the environment and remain infective for a long time, during the calving or lambing season, and animals which come into contact with them can be infected either by ingestion or inhalation. Similar studies and observations are not available for yak, since yak are generally reared under the transhumance system of migration. The herdspeople have their permanent settlements at lower altitudes. During summer, they move to alpine pastures with their entire stock. With the onset of winter, they move back to middle altitudes with their

Table I
The seroprevalence of antibodies against *Chlamydomphila abortus* in yak in India, by age and sex

Profile	Total number of animals tested	Percentage	Seropositive		χ^2	Degree of freedom*	p-value
			Percentage	95% CI			
Sex							
Male	64	25%		16, 38	4.38	1	0.03
Female	108	41%		32, 51			
Age							
6 m to 1 year	30	20%		10, 41	3.56	2	0.16
1-3 years	86	39%		30, 51			
> 3 years	56	38%		27, 53			

CI: confidence interval

χ^2 : chi-squared value

*Degree of freedom: number of values in the final calculation that are free to vary

animals (2). Thus, yak share common grazing land and water resources with other domestic and wild ruminants. Moreover, yak remain in herds of between 50 and 200 animals. It seems probable that such close proximity and shared pastures are possible factors in the high prevalence of *Cp. abortus* antibodies in yak. However, it is very difficult to draw any definite conclusion, as the pathogenicity and pathogenesis of chlamydiosis in yak are not yet known.

Interestingly, the prevalence of *Cp. abortus*-specific antibodies was significantly higher among yak cows (41%) than bulls (25%) (Table 1). Sexual transmission has been suggested as a less common method of infection in sheep. However, there are also indications of sexual transmission in cattle and boar (6, 11). The presence of the pathogen in fresh and cryo-preserved semen, preputial washing and in the male genital tracts of bulls, rams and bucks has been reported (10). No such study has been conducted on yak. However, the possibility of sexual transmission cannot be ignored and servicing with an infected bull could be responsible for the increased seropositivity among yak cows. However, reports of sexual transmission are rare in both cattle and sheep.

There was no significant difference between the prevalence of *Cp. abortus*-specific antibodies in yak of one to three years of age (39%: 95% CI = 30, 51) and in yak older than three years (38%: 95% CI = 27, 53) (Table 1). The prevalence was much lower in yak less than one year old (20%: 95% CI = 10, 41). This is probably because advancing age brings greater exposure to infected or carrier animals.

Conclusion

This study indicates that antibodies against *Chlamydophila* spp. are widespread among the yak of Arunachal Pradesh. Given the substantial evidence of reproductive anomalies (2) in yak, such as repeat breeding, infertility and abortion, the possible role of *Cp. abortus* (4, 13) cannot be underestimated. Moreover, the fact that it is a zoonosis is also of serious concern (3, 8). Tribal farmers, and women in particular, come into close contact with their yak. *Chlamydophila abortus* has been recorded as causing abortion in pregnant women who handle infected sheep and goats (3).

However, the possibility of some antigenically similar pathogen, such as *Cp. pecorum*, rather than *Cp. abortus* itself, cannot be excluded. The National Research Centre on Yak is situated in a very remote corner of an inaccessible, snow-bound Himalayan tract. There is no established facility for the isolation and confirmation of *Cp. abortus*, nor are other tests, such as the complement fixation test (CFT), easily available. Thus, the CHEKIT *Cp. abortus* ELISA was the only reference test available for this study. Nonetheless, the CHEKIT chlamydia test kit has demonstrated 100% specificity and 89% to 95% sensitivity in goats. For bovine herds, the chlamydia ELISA was found to be more sensitive than the CFT (9). This preliminary study will help researchers to understand more of the previously unknown epidemiology of *Cp. abortus* in yak.



Détection sérologique d'anticorps dirigés contre *Chlamydophila abortus* chez des yaks (*Poephagus grunniens*) vivant en liberté dans l'Arunachal Pradesh, Inde

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Résumé

Des échantillons de sérum ont été prélevés sur 172 yaks (*Poephagus grunniens*, actuellement *Bos grunniens*) vivant en liberté, sélectionnés de manière aléatoire dans six sites différents de l'Arunachal Pradesh, Inde ; la recherche d'anticorps spécifiques dirigés contre *Chlamydophila abortus* a été réalisée au moyen d'une épreuve immuno-enzymatique. La prévalence globale chez le yak était de 35 %.

La prevalencia d'anticorpos específicos était significativement plus élevée chez les femelles (41 %) que chez les mâles (25 %). La prévalence la plus élevée (39 % avec un intervalle de confiance [IC] de 95 % compris entre 27 et 55) a été enregistrée chez les yaks âgés de un à trois ans ; la prévalence la plus faible a été enregistrée chez les yaks âgés de moins d'un an (20 % ; IC de 95 % compris entre 10 et 41).

Mots-clés

Chlamydophila abortus – Épreuve immuno-enzymatique – Inde – Prévalence sérologique – Yak.



DetECCIÓN serológica de anticuerpos contra *Chlamydophila abortus* en yaks (*Poephagus grunniens*) criados en libertad en Arunachal Pradesh, India

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Resumen

Para realizar este estudio se tomaron muestras aleatorias de suero de 172 yaks (*Poephagus grunniens*, actualmente *Bos grunniens*) criados en libertad en seis zonas de Arunachal Pradesh, India, y se las sometió a la prueba de inmunoabsorción enzimática a efectos de detectar la presencia de anticuerpos específicos contra *Chlamydophila abortus*. La prevalencia total de la enfermedad observada en los yaks fue del 35%. En las hembras, la prevalencia de los anticuerpos específicos contra *Cp. abortus* resultó mucho más elevada (41%) que en los machos (25%). La mayor prevalencia (39%; IC 95% = 27, 55) se encontró en los yaks de uno a tres años de edad y la menor (20%; IC 95% = 10, 41) en los animales menores de un año.

Palabras clave

Chlamydophila abortus – India – Prueba de inmunoabsorción enzimática – Seroprevalencia – Yak.



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