

Genetic diversity of Hydatid cysts obtained from bovines in Uruguay

Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by the metacestode of *Echinococcus granulosus* sensu lato (s.l.). This cestode requires two hosts to complete its life cycle. On one hand, an intermediate host, which includes a wide range of domestic and wild mammals, including humans, where the metacestode develops. On the other hand, a definitive host, usually a canid, that harbors the adult form in its small intestine. Although the taxonomy of *E. granulosus* s.l. remains highly controversial, the following species have been recognized to date: *E. granulosus* sensu stricto (G1, G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. intermedius* (G6 and G7), *E. canadensis* (G8 and G10), and *E. felidis* (lion strain). This disease is widely distributed globally, and in South America, it is considered endemic and hyperendemic in some regions. Based on sequencing data, *E. granulosus* s.s. has the widest distribution, followed by *E. ortleppi*. In Uruguay, information on the species causing CE is very limited, highlighting the importance of determining them. This study aims to molecularly characterize hydatid cysts obtained from cattle processed in slaughterhouses in Uruguay. For this, 341 hydatid cysts from the lungs of cattle sent for slaughter were collected from July to November 2022. Prior to extraction, the fertility (presence/absence of protoscolices) of each cyst was assessed, classifying them as fertile (128) or infertile (213). DNA extraction was performed using a piece of the germinal layer, and a fragment of the cytochrome c oxidase subunit 1 was amplified by PCR. The amplicons of expected size were sent for sequencing, and identity was assessed by BLAST. Out of the total samples obtained, 19 have been analyzed so far, with 11 confirmed as *E. ortleppi* and the remaining 8 as *E. granulosus* s.s. (G1-G3). Regarding the fertility of the cysts, 11 were fertile (9 *E. ortleppi* and 2 *E. granulosus* s.s.), while 8 were infertile (2 *E. ortleppi* and 6 *E. granulosus* s.s.). These preliminary results on the preferential presence of *E. ortleppi* in fertile bovine hydatid cysts would confirm the existence of an autochthonous cycle primarily involving cattle in Uruguay. Currently, molecular characterization of 40 of the cysts (20 fertile and 20 infertile) is ongoing to establish a more detailed pattern regarding the presence of *E. ortleppi* in cattle in Uruguay.

Keywords: *echinococcus ortleppi*, uruguay, cattle.

Coxiella burnetii y *Neospora caninum* como factores de riesgo para pérdidas gestacionales en un rodeo de cría

Coxiella burnetii and *Neospora caninum* as risk factors for pregnancy losses in a beef cattle herd

Rabaza A¹, Eisler MC, García-Pintos C, Cuadro F, Morales R, Menchaca A, Giannitti F*, Zarantonelli L*. ¹arabaza@pasteur.edu.uy Laboratorio de Bacterias Zoonóticas, Unidad Mixta Pasteur + INIA (UMPI), Institut Pasteur de Montevideo, 11400 Uruguay.

Coxiella burnetii es una bacteria intracelular de gran transmisibilidad, baja dosis infectiva y distribución mundial que produce la zoonosis Fiebre Q (coxielosis). En rumiantes produce trastornos reproductivos, incluidos abortos, retención de placenta, metritis e infertilidad. Se han realizado numerosos estudios sobre abortos en pequeños rumiantes y su prevalencia en el ganado, sin embargo, pocos estudios han explorado la asociación entre *C. burnetii* y las pérdidas reproductivas bovinas. El protozoo *Neospora caninum* infecta a bovinos como hospederos intermediarios produciendo abortos y fallas reproductivas y es endémico en el rodeo bovino de Uruguay (Banales et al., 2006; Dubey, 2011). Los fetos y placentas son fundamentales en las investigaciones diagnósticas de abortos; sin

embargo, la obtención de estos tejidos a campo es desafiante especialmente en sistemas de cría extensivos de base pastoril como los predominantes en Sudamérica. Dada esta limitante, exploramos la seropositividad a ambos patógenos como factor de riesgo de pérdidas gestacionales en un rodeo bovino. Se realizó un estudio de casos y controles emparejado para investigar la relación entre la presencia de inmunoglobulinas anti-*C. burnetii* y anti-*Neospora* y la ocurrencia de pérdidas reproductivas en un rodeo de cría de Uruguay con una tasa de preñez del 85% (640/753). Este rodeo se seleccionó después de la detección de *C. burnetii* en el útero de una vaca abortada mediante qPCR. Tras la confirmación de la preñez mediante ecografía, las gestaciones se monitorearon mediante palpación transrectal y ultrasonografía. Todas las vacas con pérdida de preñez (n=35, casos) durante seis meses fueron evaluadas y fueron emparejadas por edad, paridad y edad fetal con vacas compañeras gestantes (n=100, controles). La tasa de pérdida de gestación fue del 5,4% (35/640), registradas principalmente entre el día 30 y 90 (71,4%, 25/35). El suero obtenido en el momento de detección de la pérdida gestacional se analizó mediante ELISA para detectar IgG anti-*C. burnetii* (PrioCHECK™-Ruminant-Q-Fever-Ab-PlateKit) y anti-*N. caninum* (IDScreen™-*Neospora-caninum*-IndirectIDVet). Se ajustó un modelo de regresión logística condicional para estimar los odds ratios (OR) y el correspondiente intervalo de confianza (IC) del 95%. La tasa general de seropositividad para *C. burnetii* fue del 4,4% (6/135), siendo significativamente mayor en los casos (11,4%, 4/35) que en los controles (2,0%, 2/100), indicando que las vacas seropositivas tuvieron un mayor riesgo de aborto (OR=6, IC95% 1,5-22,5; p=0,005). La seropositividad general a *N. caninum* fue 29,6% (40/135), siendo esta tasa también significativamente mayor en los casos (45,7%, 16/35) que en los controles (24,0%, 24/100, OR=2,6; IC95% 1,2-5,4; p=0,006). La significancia de la asociación entre la exposición a *C. burnetii* y el aborto disminuyó cuando *N. caninum* se consideró como un factor de confusión en el modelo, lo que significa que esta variable contribuye a la asociación observada. Estos resultados destacan la complejidad de esta coexposición. Se ha propuesto que la coinfección entre *C. burnetii* y *N. caninum* determina una inmunomodulación que aumenta la probabilidad de ocurrencia de aborto (García-Ispuerto et-al., 2010). En sistemas extensivos de producción donde es difícil acceder a placentas/fetos, un enfoque serológico de casos y controles emparejado sería ventajoso para investigar *C. burnetii* y *N. caninum*.

Palabras clave: aborto; zoonosis; fiebre Q.

Coxiella burnetii is a highly transmissible intracellular bacterium that causes Q fever, a notifiable zoonotic disease distributed worldwide. This bacterium can lead to reproductive disorders in ruminants, including abortion, retained placenta, metritis and infertility. Although numerous studies have been conducted on abortions in small ruminants and the prevalence in cattle, fewer studies have formally explored the association between *C. burnetii* and reproductive losses in cattle. The apicomplexan protozoa *Neospora caninum* infects cattle as intermediate hosts and has a complex life cycle and transmission routes. It leads to abortion and reproductive failure worldwide and is endemic in the Uruguayan bovine population (Banales et-al., 2006; Dubey, 2011). Studying fetuses and placenta is crucial when investigating pregnancy losses; however, obtaining them under field conditions in extensive pasture-based beef production systems common in South America, is challenging. Given this limitation, we aim to explore the role of *C. burnetii* as a risk factor for gestational losses within a beef cattle herd by measuring exposure through the detection of anti-*Coxiella* IgG. A matched case-control study was conducted to explore the relationship between the presence of anti-*C. burnetii* IgG and reproductive losses in a mixed breed beef herd in Uruguay bred using artificial insemination with pregnancy rate of 85% (640/753). This herd was selected after *C.*

burnetii was detected in the uterus of an aborted cow using qPCR. Following pregnancy confirmation by ultrasound, the continuation or loss of the pregnancy was assessed through transrectal palpation. All cows with pregnancy losses (n=35, cases) over six months were selected and matched by age, parity and fetal age to pregnant herd mates (n=100, controls). The pregnancy loss rate was 5.4% (35/640), occurring primarily between 30-90 days of pregnancy (71.4%, 25/35). Serum obtained at the time of the diagnosis of pregnancy loss was analysed by two ELISA assays for anti-*C. burnetii* IgG (PrioCHECK™- Ruminant-Q-Fever-Ab-PlateKit) and anti-*Neospora caninum* IgG (IDScreen™-*Neospora- caninum*-IndirectIDVet). A conditional logistic regression model was fitted to estimate the odds ratios (OR) and corresponding 95% confidence interval (CI). The overall *C. burnetii* seropositivity rate was 4.4% (6/135), this rate being significantly higher in cases (11.4%, 4/35) than control animals (2.0%, 2/100), indicating that seropositive dams had a higher risk of abortion (OR=6, IC95% 1.5-22.5; p=0.005). The overall seropositivity to *N. caninum* was 29.6% (40/135), this rate being also significantly higher in cases (45.7%, 16/35) than controls (24.0%, 24/100, OR=2.6; IC95% 1.2-5.4; p=0.006). The significance of the association between *C. burnetii* exposure and abortion diminished when exposure to *N. caninum* was considered as a confounder in the model, meaning that this confounding factor drives part of the observed association. The reduced but still meaningful significance underscores the complexity of the relationship and emphasises the importance of accounting for other variables that could influence the results. *C. burnetii* and *N. caninum* coinfection is believed to increase the likelihood of abortion in cows through pregnancy immunomodulation (García-Ispuerto *et-al.*, 2010). In extensive beef production systems where accessing placentas/fetuses is difficult, a matched case-control serological approach would be advantageous for investigating *C. burnetii* and *N. caninum*.

Keywords: abortion; zoonosis; Q fever.

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