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Short communication

Spatio-temporal epidemiology of *Tritrichomonas foetus* infection in Texas bulls based on state-wide diagnostic laboratory data

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ABSTRACT

Texas is the largest cattle producing state and suffers severe economic losses due to abortions caused by the protozoan parasite *Tritrichomonas foetus*. The objective of this study was to use data from the state-wide diagnostic laboratory system of Texas to investigate the occurrence and spatio-temporal distribution of bovine trichomoniasis (BT) in Texas, and to identify spatial disease clusters within the state. The study population consisted of bulls tested for BT in 2010 by the Texas Veterinary Medical Diagnostic Laboratory system that performs at least 95% of all *T. foetus* testing in the state. Preputial samples were cultured and diagnosis was made by real-time polymerase chain reaction (PCR). Data on BT was aggregated at the county level with time aggregation of one month. The scan statistics was used to identify spatial disease clusters. The database included 31,202 test results with a proportion of positives of 3.7%. As expected, BT was present throughout Texas. Testing prevalence was highest in the summer (5.5%). The scan statistics identified a spatial cluster in southeastern Texas, which could only partially be explained by cattle herd density. The findings of this study provide baseline data to monitor the success of BT control activities in Texas and aids in generating hypotheses regarding specific risk factors for the disease. The identification of high-risk areas and periods is also essential to improve intervention efforts.

1. Introduction

Bovine trichomoniasis (BT) is a contagious venereal disease of cattle caused by the flagellate protozoan *Tritrichomonas foetus*. This parasite colonizes the epithelial surfaces of the bovine reproductive tract, causing inflammation, embryonic losses, and infertility in females (Parsonson et al., 1976). Infection with *T. foetus* in bulls is typically asymptomatic and restricted to the epithelial surface of the penis, prepuce and urethra (Parsonson et al., 1974). Once infected, however, males become lifelong carriers of the pathogen. Coitus between carrier bulls and susceptible cows or heifers is the main route of transmission (BonDurant, 2005). Consequently, *T. foetus* causes serious economic losses where natural breeding conditions exist, due to reduced calf crops and culling of infected cattle (Rae et al., 1999; Rodning et al., 2008). No treatment for BT is legally available in the United States. While there is a commercial vaccine available that has been shown to help clear infection in vaccinated cows, it has not been proven to reliably prevent infection (Villarroel et al., 2004).

There has been a growing concern in recent years about the economic impact of BT in several beef cattle rangeland regions of the United States, including Texas.

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Texas is the largest cattle producing state in the nation with an estimated total cattle population of 13.7 million (United States Department of Agriculture, National Agricultural Statistics Service. 2007 Census of Agriculture. Available at: www.nass.usda.gov. Accessed March 21, 2011). It has been reported that BT may cause calf crop losses up to 30% in some herds in Texas (Cima, 2009). The economic impact of an actual *T. foetus* outbreak in a herd of 161 cows in Texas was estimated to exceed $78,000 in the first year, with ongoing losses exceeding $100,000 for a 3-year period (Davidson, J.M. Bovine Trichomoniasis: Understanding this Tricky Profit Taker! Texas A&M University AgriLife Extension Service website. Available at: http://aevm.tamu.edu/files/2010/06/Trich-Article-Feb_2009-Davidson.pdf. Accessed April 15, 2011). Recently BT has become a reportable disease in Texas with control efforts mainly focusing on mandatory testing of breeding bulls and culling of infected animals (Cima, 2009). Testing results from this state-wide control program provide unique and invaluable information to better understand the distribution and epidemiology of BT in Texas, in order to improve prevention measures and reduce economic losses caused by this parasite. Thus, our objective was to use the testing results generated through the control program in the state-wide veterinary diagnostic laboratory network of Texas to investigate the occurrence and spatio-temporal distribution of BT in Texas and to identify spatial clusters of the disease.

2. Methods

The study population consisted of bulls routinely tested for infection with *T. foetus* between January 1st and December 31st of 2010 at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL). The study period of 2010 was selected because this was the first full calendar year when mandatory BT testing was implemented in Texas. Texas regulation requires testing of all non-virgin bulls for *T. foetus* prior to interstate or intrastate commerce. Therefore, the majority of the tested bulls were breeding bulls requiring a disease-free certificate, with a few test results available on other bulls (non-breeding bulls, breeding bulls not participating in commerce). The TVMDL is one of the largest veterinary diagnostic laboratory systems in the nation, and it performs at least 95% of *T. foetus* testing in the state of Texas. All available data from both full-service TVMDL laboratory locations, Amarillo and College Station, were included in the study. Preputial wash samples were transported and cultured in a commercial growth packet (InPouch TF Test, BioMed Diagnostics, Inc., White City, OR). Samples were inoculated in the self-contained InPouch TF *T. foetus* culture pouch (BioMed Diagnostics; White City, OR). The medium is selective for the transport and growth of *T. foetus* while inhibiting the growth of contaminating microorganisms. The inoculated culture pouches were placed vertically into a 37 °C portable incubator. The pouches were microscopically observed daily for six days for the presence of motile trichomonads. The samples remained in the incubator at all times except for daily microscopic evaluations. An aliquot of the culture media was used for DNA extraction. Subsequent real-time PCR was performed as previously described (McMillen and Lew, 2006). Master mix contained primers (T.foeForward: 5′-GCGGCTGGATTAGCTTTCTTT-3′; T.foeReverse: 5′-GGCGCGCAATGTGCAT-3′; and probe (T.foeProbe: 5′-ACAAGTTCGATCTTTG-3′; 5′FAM/3′MGBNFQ) at a concentration of 500 nM and 300 nM each, respectively, and 12.5 μl of TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA) in a 25-μl total volume reaction. Temperature and time of thermocycling parameters used in the present study were as recommended by the master mix manufacturer.

Data was aggregated at the county level, using the submitting veterinary clinic's county as a proxy for each tested animal's location. This approach was necessary to preserve confidentiality of animal owners and was based on the assumption that local veterinarians were involved in sample collection, and their offices were in proximity to the study subjects' residence. Testing prevalence was calculated as the proportion of positive tests out of the total number of tests performed. This approach was valid because RT-PCR testing requires only one negative test result to declare the bull free of infection and obtain a disease free certificate (as compared to three consecutive negative test results in the case of culture alone). Thus, although we cannot rule out that a minor fraction of bulls were tested more than once during the study period, such occurrence was considered rare. To visualize the testing prevalence in each county in Texas, a choropleth map was created using ArcGIS 9.3 (ESRI, Redlands, CA).

The scan statistics method (SaTScan version 9.0, Martin Kulldorff and Information Management Services Inc., Boston, MA) was used to identify spatial clusters of BT in Texas (Kulldorff, 1997). The number of *T. foetus* positive tests in each county was assumed to follow the Poisson distribution, while the number of animals tested per county constituted the population at risk in the respective county. Both circular and elliptical spatial cluster shapes were investigated. The maximum spatial cluster size was set at 50% of the population at risk (default setting). *P*-Values were obtained based on Monte Carlo simulations after 999 random replications of the dataset, under the null hypothesis of spatial randomness. Clusters with statistical significance of $p < 0.05$ were plotted on the map. For the significant clusters, the relative risk (RR) was also reported (risk of being BT positive within the cluster, compared to the population's risk). To investigate the sensitivity of the results to the Poisson model assumptions, the Bernoulli model was also fitted to the data. The results were almost identical and thus results were only presented for the Poisson model.

It was assumed that cattle herd density would be a strong predictor of the spatial BT risk distribution. From the disease control perspective, our primary interest was to identify high-risk areas that are amenable to intervention (i.e. high-risk areas where the elevated risk could not be explained by herd density) and to display these areas in a visually appealing format. The best approach to achieve this goal was the use of mapping techniques, specifically choropleth mapping with overlay. Beef cattle herd density estimates for each county in Texas were obtained from the 2007 census of the National Agricultural