Case Review

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Tritrichomonas foetus infections in female beef cattle with abortion in Wyoming, USAChaoqun Yao^{1,2,3,4}¹Department of Biomedical Sciences, Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, St Kitts, West Indies²One Health Center for Zoonoses and Tropical Veterinary Medicine, Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, St Kitts, West Indies³Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82070, USA⁴Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY 82070, USA

Introduction: Bovine trichomoniasis has been endemic in the USA since its discovery in the 1930s. Testing of bulls used for reproduction is currently mandated in 26 states to control spread of the disease. Although individual head prevalence in Wyoming has decreased since 2000 when the state's regulation started, the herd prevalence remains steady and the disease continues to have a wide geographic distribution. One factor neglected in current regulations is the role of infected cows/heifers in transmission. The latter may harbour *Tritrichomonas foetus*, the causative organism, up to a few weeks post-abortion/parturition. This capacity enables them to spread the disease in spite of extensive bull testing.

Case presentation: The purpose of the present study was to determine the prevalence among Wyoming beef cattle of detectable *T. foetus* infection in cows/heifers with a history of abortion for which samples had been tested. This retrospective study included all submissions to the Wyoming State Veterinary Laboratory between 2000 and 2010. Cows/heifers with a history of abortion among Wyoming producers were tested for trichomoniasis. Overall prevalence was 9.7 %. Furthermore, 4.5 % of aborted fetuses were positive.

Conclusion: Our data collectively demonstrates that a percentage of cows/heifers that recently experienced abortion are positive for *T. foetus* and may play an important role in maintaining endemicity of bovine trichomoniasis.

Keywords: abortion; beef cattle; bovine trichomoniasis; cow; *Tritrichomonas foetus*.

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Introduction

Bovine trichomoniasis, caused by *Tritrichomonas foetus*, is a venereal disease of cattle. Infected cows/heifers exhibit vaginitis, endometritis, early abortion and infertility (Bondurant, 2005; Felleisen, 1999). The disease has a significant economic impact on the US producers of both dairy and beef cattle, mainly due to reduced calf crops, decreased revenue returns, culling and replacement of infected bulls, and cost of veterinary services. It is estimated that beef herds with 20–40 % *T. foetus*-positive bulls had a reduction of 14–50 % in annual calf crops, a reduction of 4–10 % in monetary return per calf born, and a reduction of 5–35 % in the return per cow (Rae, 1989). Each infected cow in a dairy herd leads to an estimated economic loss of \$665 (Goodger & Skirrow, 1986). This economic loss could well be a determining factor for financial failure of a cattle producer.

Bovine trichomoniasis is distributed worldwide, with cases reported in America, Asia, Australia, Europe and South Africa where natural bull service is a means of cattle breeding (Yao, 2013). *T. foetus* undergoes only the trophozoite stage and is mainly transmitted between bulls and cows/heifers by coitus. It was shown that a single service by an infected bull resulted in 95 % infection of previously uninfected susceptible nulliparous cows (Parsonson *et al.*, 1976). Interestingly, transmission from infected cows/heifers to bulls appears less efficient. Clark *et al.* (1974a) showed that it took three to six services for *T. foetus*-positive heifers to infect Hereford bulls more than 4 years old, and nine matings to infect one of two 3-year-old bulls. On the other hand, bulls directly transmit *T. foetus* protozoa from infected to non-infected cows during mating if the mating interval is less than 20 min, without becoming infected themselves (Clark *et al.*, 1977).

Bovine trichomoniasis has been found in many US states from all regions of the country (Abbitt & Meyerholz, 1979; BonDurant *et al.*, 1990; Gay *et al.*, 1996; Hoevers *et al.*,

Abbreviations: LIMS, Laboratory Information Management System; WSVL, Wyoming State Veterinary Laboratory.

2003; Rae *et al.*, 2004; Rhyan *et al.*, 1988, 1999; Rodning *et al.*, 2008; Szonyi *et al.*, 2012; Yao *et al.*, 2011). To curtail the spread of this disease, 26 states had trichomoniasis control/management programme regulations in place as of 1 April 2014 (NIAA/USAHA, 2014). A core component of these state regulations is that all bulls used for reproduction test negative for *T. foetus* prior to importation, trade or to grazing public properties. Documentation on the effectiveness of these regulations in curtailing disease spread is rare. Individual bull prevalence dropped from 1.7 % to 0.2 % in Wyoming between 2000 and 2010 as a result of state regulation (Yao *et al.*, 2011). However, the regulation appears to have had very marginal effects on two important parameters gauging disease control, i.e. diminished geographical distribution and decreased herd prevalence. Between 2007 and 2010, 15 of 23 counties still had at least one positive herd and the average herd prevalence was 2.2 % (Yao *et al.*, 2011). Lately, it was found through a questionnaire of all Wyoming cattle producers that *T. foetus* infections were significantly associated with neighboring positive herd(s), grazing public allotments and co-mingling with other herds (Jin *et al.*, 2014).

Generally, infections of cows/heifers are transient, often lasting only a few months. Further, the recovered females develop short-lived immunity to re-infection. After an early abortion peaking at about 7–10 weeks of pregnancy and an additional 2–4 weeks for a new oestrus cycle, these cows/heifers become pregnant again if bulls are still available, resulting in an extended breeding season being observed in many *T. foetus*-positive herds. However, some infected females carry the pathogen for a much longer period of time. Skirrow (1987) found two of seven infected cows maintained infections for 6 and 9 weeks, respectively, after parturition. Furthermore, infected cows/heifers pass the pathogen to virgin or uninfected bulls by mating and the infections are successfully established in these males (Clark *et al.*, 1974a, b).

Bovine trichomoniasis is transmitted between cattle during coitus. There are many reports on infections in bulls. In contrast, only a few studies on trichomoniasis in cows/heifers are available. For example, a survey of uterine lavages collected from 21 open cows with a history of reproductive problems in two herds in Brazil resulted in a 61.8 % infection rate with *T. foetus* (Gonzalez-Carmona *et al.*, 2012). In Argentina, *T. foetus* was isolated from three and two of 76 pregnant and 64 non-pregnant cows, respectively (Mancebo *et al.*, 1995).

The Wyoming State Veterinary Laboratory (WSVL) has been diagnosing bovine trichomoniasis for veterinarians and producers throughout the state and several adjacent states since the 1980s, two decades before the state actually started to regulate the disease. Accession numbers, unique numbers assigned for each batch of specimens received, and sample numbers increased significantly upon mandatory bull testing in 2000. The laboratory has been testing 6000–8000 bovine samples for *T. foetus* annually since

then. The majority of these samples are collected from the preputial sheath of bulls while a much smaller fraction are from aborted fetuses and cows/heifers with a history of abortion.

The purpose of the current study was to determine the prevalence of *T. foetus* infection in cows/heifers with a history of recent abortion from which samples were submitted to the WSVL for *T. foetus* testing. A retrospective approach was taken to include all accessions of cows/heifers with abortion between 2000 and 2010 in the Laboratory Information Management System (LIMS) data system. A significant proportion of this population (9.7 %) were *T. foetus*-positive. These data support the notion that infected cows/heifers play a pivotal role in the endemicity of bovine trichomoniasis.

Methods

Data

The data bank used was WSVL's electronic data in LIMS. All accessions and cases in the data bank relating to bovine trichomoniasis between 2000 and 2010 were retrieved and exported to Excel. The criteria for data retrieval included: (1) bovine species; (2) trichomoniasis testing, no matter whether it was by culture or by PCR; and (3) owner's residence in Wyoming when testing was requested, no matter where the sample-submitting veterinarian was located. Information sought for each entry included accession number, history, sample type and number, type of test requested and test result. Annual data were exported to the Excel spreadsheet separately. To validate the accuracy of the retrieved data, all accessions and cases for the year 2010 for which hard copies were available were manually keyed into Excel by two individuals who checked each other's work to minimize human error. These individuals and the person downloading the data were blinded to each other's tasks. The manual data set was used as a standard against which the retrieved data was checked. Complete agreement was found among the two data sets, which confirmed accuracy of the retrieved data. Afterwards, annual data for the remaining years between 2000 and 2010 were separately retrieved using the same protocol without validation.

Cases

Accessions were identified by searching annual data sets using key words comprising: uterine discharge, foetus, vagina, dead (death), abortion, cervical and calf (calves). Each identified entry was manually checked to make sure its samples were from an aborted foetus or cow/heifer. Samples from the latter included uterine discharges, uterine samples, cervical mucus and vaginal discharges. All entries were tabulated by year in a single Excel file, and redundant items were removed. Test results were recorded by year, host (female versus foetus), female reproductive organs (uterus, cervix and vagina) and testing method (culture versus PCR). In addition, the time when the abortion occurred was recorded for all applicable cases.

Testing

Veterinarians specified testing methods when submitting samples to WSVL using a standard submission form. Samples were tested for *T. foetus* by culturing or PCR as previously described (Yao *et al.*, 2011). Briefly, samples for culturing were inoculated into Diamond's medium (Diamond, 1957), which was prepared in-house, and incubated at 37 °C for 48–72 h. Cultures were checked microscopically in dark field for live trichomonads with characteristic rolling motion. DNA was extracted from directly submitted frozen samples or from 24 h cultures as previously described (Chen & Li, 2001). Primers TFR3 and TFR4 were used in PCR to amplify a 347 bp DNA fragment located in the genomic region spanning the 5.8S ribosomal RNA and the internal transcribed spacer as previously described (Felleisen *et al.*, 1998). Each PCR consisted of 94 °C for 5 min, and 40 cycles of 94 °C for 30 s, 67 °C for 30 s and 72 °C for 90 s, followed by 72 °C for 15 min in the presence of 2.5 µM each primer. For each batch of samples a negative control of water and a positive control of cultured *T. foetus* were included to gauge whether both the DNA extraction and PCR were working properly. PCR products were photographically documented after electrophoresis on 1.5 % agarose gels. Positive results were determined by the presence of a 347 bp band.

Results

Between 2000 and 2010 a total of 93 cows/heifers with abortion were tested for *T. foetus* by culturing and/or PCR. Samples from the cervix ($n=29$), vagina ($n=11$) and uterus ($n=53$) resulted in zero (0 %), one (9.1 %) and eight (15.1 %) positives for *T. foetus*, respectively. The overall prevalence was 9.7 %. One of 22 aborted fetuses (4.5 %) also tested positive. Four aborted fetuses with accompanying placentas were submitted; one placenta tested positive (25.0 %). Positive samples were only detected in 2002, 2003 and 2008. Interestingly, all positive results were from cultured samples, whereas among 36 samples tested by PCR, none was positive (Table 1).

Records from 17 of the 115 abortion cases stated the stage of pregnancy when the abortion occurred. Eight animals aborted at less than 90 days, four at 5–6 months, and five in late term of pregnancy. Four cows/heifers tested positive for *T. foetus* among the eight animals aborting in the first 90 days of pregnancy, whereas all nine animals aborting at 5 months or later were negative.

Discussion

Here we report that in Wyoming between 2000 and 2010 the overall prevalence of *T. foetus* was 9.7 % among 97 cows/heifers with history of abortion from which specimens had been submitted to WSVL for trichomoniasis testing. The highest rate of positives (15.1 %) was found in the uterus, followed by the vagina (9.1 %), whereas cervical samples were negative. Although not a controlled set of samples, these data suggest that samples collected

from the uterus and the cervix represent the best and the worst, respectively, for *T. foetus* testing in cows/heifers with abortions. BonDurant *et al.* (2003) found that cervico-vaginal mucus aspirates of four experimentally infected heifers all tested positive by PCR. In an Argentinian study, *T. foetus* was recovered from the vaginas of all five positive animals, whereas only two of the five were positive based on uterine samples (Mancebo *et al.*, 1995). In another experimental study involving eight cows killed from 15 to 50 days after infection, *T. foetus* was found by culture in the vagina and cervix of all cows, in the uterus in six and in the oviducts of four (Parsonson *et al.*, 1976). As a result of this discrepancy, a well-designed experiment is warranted to test the sensitivity of samples collected from the vagina, cervix and uterus of *T. foetus*-positive cows/heifers. Current protocols advise that samples should be collected from all three locations to maximize the likelihood for *T. foetus* detection.

Our data also showed that 1 (4.5 %) of 22 aborted fetuses was positive for *T. foetus*. A limitation of the current study is that the samples were not concomitantly tested for other abortion-causing pathogens such as viruses, bacteria, fungi and parasites (Eaglesome & Garcia, 1997; Thibier & Guerin, 2000). In a survey of pathogens by PCR from 80 aborted bovine fetuses in Beijing, China, infectious agents were detected in 45 (56.3 %), 22 (48.9 %) of which represented co-infections with two or three infectious agents. The pathogens identified and their rates were: bovine rhinotracheitis virus (36.3 %), *Neospora caninum* (31.3 %), bovine viral diarrhoea virus (7.5 %), *Brucella abortus* (6.3 %), *T. foetus* (5 %) and *Toxoplasma gondii* (1.3 %) (Yang *et al.*, 2012).

Bovine trichomoniasis causes abortion usually during early gestation (Bondurant, 2005). In heifers experimentally infected with *T. foetus*, conceptus deaths peaked at 50 to 70 days gestation (Parsonson *et al.*, 1976). *T. foetus* was not found among 126 fetuses older than first trimester of gestational age (Campero *et al.*, 2003). Similarly, we report that among eight abortions in the first trimester four were *T. foetus*-positive, whereas none of nine with later abortions was. In a study of 13 cases of bovine abortion attributed to *T. foetus* during 1982–1987 (Rhyan *et al.*, 1988), fetuses were found to vary in gestational age from 2 months to term. Eleven of 12 fetuses were of gestational age 5 months or older, three of those being in the last gestational month. The authors attributed these results to possible bias as herd managers may not have recognized early abortions.

In summary, data presented here show an overall prevalence of 9.7 and 4.5 % *T. foetus* among cows/heifers with abortion and aborted fetuses, respectively, in Wyoming between 2000 and 2010. They support the need for testing open cows for *T. foetus* as a part of an integrated approach to control and eventual elimination of bovine trichomoniasis. Besides testing bulls as required by state rules, other suggested measures include the use of artificial insemination, using

Table 1. Bovine trichomoniasis testing of vaginal, cervical and uterine samples of cows/heifers with abortion and of the abomasal fluid of aborted fetuses in Wyoming between 2000 and 2010

Year	PCR N_{tot} cows/heifers (N_{pos})	Culture N_{tot} cows/heifers (N_{pos})	PCR N_{tot} fetuses (N_{pos})	Culture N_{tot} fetuses (N_{pos})
2000	0	0	0	0
2001	0	8 (0)	0	0
2002	0	12 (2)	0	0
2003	0	14 (7)	0	1 (0)
2004	0	9 (0)	0	1 (0)
2005	0	9 (0)	0	1 (0)
2006	0	2 (0)	0	0
2007	4 (0)	7 (0)	2 (0)*	2 (0)
2008	1 (0)	2 (0)	3 (0)	1 (1)†
2009	4 (0)‡	2 (0)	2 (0)	2 (0)§
2010	17 (0)	14 (0)	3 (0)	4 (0)
Total	26 (0)	79 (9)	10 (0)	12 (1)

*Two placentas were also tested with fetuses.

†Both abomasal and placental fluid were culture-positive.

‡One sample was also cultured.

§One placenta was also tested with a fetus.

||Eleven were also cultured.

bulls younger than 3 years old, maintaining closed herds, and grazing private/well-fenced allotments (Jin *et al.*, 2014). The more widely an integrated approach is adapted, the sooner bovine trichomoniasis will be controlled and eventually eliminated from the US bovine herds.

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