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Short communication

Prevalence of *Tritrichomonas foetus* in tennessee bulls

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ABSTRACT

The prevalence of bovine trichomonosis (BT) in TN bulls was estimated through both active screening of bulls and review of previous laboratory records. During the active bull screening, preputial smegma specimens were collected from 458 TN beef bulls at 2 cattle slaughterhouses and 2 stockyards, which serve most beef bulls in TN, between March 2014 and June 2015. Each specimen was cultured for *Tritrichomonas foetus* (*T. foetus*) as well as evaluated microscopically every other day for seven days for any protozoa resembling *T. foetus*. An aliquot of the culture media from each specimen was used for DNA extraction and subsequent qPCR testing. Two specimens were considered suspect on microscopic evaluation, but all specimens were negative for *T. foetus* on qPCR. This suggests that the 2 specimens were most likely contaminated by fecal trichomonads. Retrospectively, 1979 *T. foetus* test records from 2 major TN diagnostic laboratories were reviewed between October 2013 and September 2016. True prevalence of BT in TN beef bulls was estimated at < 0.01% from the laboratory records, although the county prevalence differed in 2 TN counties (Marshal: 0.09% and Bedford: 0.5%). Overall, the prevalence of BT in TN is low, and the current screening efforts to help control BT disease in TN are acceptable. Future efforts should focus on educating cattle stakeholders on the importance of optimal specimen collection and handling as well as routine testing for BT before cattle movement. In addition, cattle producers should be reminded of leading risk factors associated with BT in cattle.

1. Introduction

Bovine trichomonosis (BT) is a contagious venereal disease of cattle caused by *Tritrichomonas foetus* (*T. foetus*), an extracellular flagellated protozoan parasite that colonizes the epithelial surface of the bovine reproductive tract (BonDurant, 2005; Michi et al., 2016). Coitus between carrier bulls and susceptible cows or heifers is the main route of transmission (BonDurant, 2005; Michi et al., 2016). *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; Rae et al., 2004; Rodning et al., 2008). Other consequences of BT include a prolonged breeding season, 5% to 12% reduction in weight gain during the suckling/growing period, 4% to 10% reduction in weaning weights, 4% to 10% reduction in monetary returns per calf born, 14% to 50% reduction in annual calf crop, and 5% to 35% reduction in financial return per cow when compared to cows exposed to a fertile uninfected bull (Rae, 1989).

Currently, there is no effective approved therapy for *T. foetus*-infected cattle in the US. While there is a commercial vaccine available that has been shown to help clear *T. foetus* infections in vaccinated cows, it has limited success in mitigating infections and reducing abortion risk (Baltzell et al., 2013; Villarroel et al., 2004). In the absence of effective treatment options and vaccines, BT management strategies include diagnostic testing, reporting, eliminating infected animals, and cattle movement control. These control strategies require knowledge of regional prevalence of BT for implementation to be successful. Estimating the prevalence of BT is therefore a critical first step to implementing successful BT control programs in an area.

There has been growing concern in recent years about the prevalence and economic impact of BT in cattle in several regions of the US, including Tennessee (TN). In California and Florida, investigators found 9 of 57 (15.8%) and 17 of 59 (28.8%) herds with at least one *T. foetus*-positive bull (BonDurant et al., 1990; Rae et al., 2004). Reported individual bull prevalence of *T. foetus* across all bulls tested in various states range from 0.17–7.8% (Rae et al., 2004; Rodning et al., 2008; Szonyi et al., 2012). The status of BT in TN, including prevalence and

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distribution, is unknown. Therefore, the objective of this study was to estimate the prevalence of *T. foetus* infections in TN beef bulls through active screening of bulls as well as the use of previously collected laboratory records.

2. Materials and methods

2.1. Active bull screening

2.1.1. Animal selection and specimen collection

Given an estimated prevalence of 3% (and not less than 1.5%; prevalence in the Southeast U.S. has been determined to range from 0 to 6%), a confidence level of 95%, and a population of 44,370 herd bulls (USDA and NASS, 2014), 491 bulls were needed to estimate the prevalence of *T. foetus* in TN beef bulls (Epi Info™ Version 7.0 software, Centers for Disease Control and Prevention, Atlanta, GA, USA). Two slaughterhouses and 2 stockyards were purposively selected as specimen collection sites because they slaughtered or sold a significant portion of beef cattle in TN. The slaughterhouses used are FLP Food and Southeastern Provision and are located in Augusta, GA and Bean Station, TN, respectively. The 2 stockyards are located in Athens, TN and Somerville, TN. Between March 2014 and June 2015, preputial smegma specimens were collected from cull beef bulls at these slaughterhouses and stockyards. For each bull, age, the individual number from a US Department of Agriculture-approved backtag, and the breed of the bull were recorded at the time of specimen collection. Age was estimated by using dentition. Specimens were collected only from bulls with backtag identifications beginning with the prefix "63," indicating TN as the state of last origin; with the first mature incisors erupted, indicating the bull was at least 18 months of age; and a phenotype consistent with beef cattle. On dates the specimen collection sites were visited, preputial smegma specimens were collected from all bulls that met the above criteria. Briefly, a new, clean, dry, bull rasper (Tricamper™) was placed into the preputial fornix of each bull and preputial smegma was obtained. Excessive or overaggressive penile or preputial scraping was avoided as blood in the specimen may negatively affect some BT diagnostics (PCR) (Mukhufhi et al., 2003). The smegma was placed immediately into the self-contained InPouch™TF *T. foetus* culture pouch (BioMed Diagnostics; White City, OR, USA) as per manufacturer's recommendation.

2.1.2. Culture and PCR testing of specimens

The specimens were evaluated microscopically every other day for seven days for any trichomonad activity. Prior to evaluation, the pouch was pulled five times across the edge of a table for mixing. The pouch was first evaluated at 100X magnification; suspect organisms were subsequently evaluated at 400X for positive morphological identification. For DNA extraction, an equal volume from each of three samples was pooled and 100 ul was extracted using a commercial kit according to the manufacturer's protocol (DNeasy Blood and Tissue Kit, Qiagen). qPCR was performed using the VetMAX-Gold Trich Detection *T. foetus* DNA detection Kit (Applied Biosystmes, licensed by the USDA). Each reaction contained specimen DNA, forward and reverse primers, FAM labelled probe, and taq polymerase premix. Negative controls for each batch included nuclease-free water and mock-purified culture. A known positive control was included in every batch. Specimens were amplified using the Step One Real-time PCR system (Life Technologies). A Ct value less than 38 was considered PCR-positive, values between 38 and 40 were considered PCR-suspect, and specimens that did not yield a Ct value in 40 cycles of amplification were PCR-negative. Some trichomonad isolates may not propagate *in vitro*, or the trichomonads may die prior to microscopic and/or molecular detection (PCR) (Clavijo et al., 2011). This is especially important given that DNAases may destroy residual DNA of dead trichomonads during the incubation and microscopic evaluation phase and lead to false-negative PCR results (Rubino et al., 1991). For this active BT bull screening survey, on Day 3 of incubation, a 500 µl aliquot of the culture media from each specimen was placed in separate, sterile, labelled 1.5 ml microcentrifuge tube and frozen at −20C for use in DNA extraction and subsequent qPCR testing. Obtaining an aliquot on Day 3 allowed time for replication of propagating trichomonads but minimized DNA degradation in non-propagating or dead trichomonads, thus maximizing the potential to obtain PCR positive specimens. Because only a single specimen result was assessed for each bull sample, the subsequent use of the term 'sample' denotes a specimen from a beef bull sample. The justification for performing both culture and PCR on each sample was the improved sensitivity (approximately 78%) compared to either test performed alone (approximately 65%) (Cobo et al., 2007).

2.2. Laboratory records evaluation

The Tennessee Department of Agriculture C. E. Kord Animal Health Diagnostic Laboratory and the University of Tennessee College of Veterinary Medicine (UTCVM) Parasitology Laboratory, located in Nashville and Knoxville, TN, respectively, generated computer records for all *T. foetus* diagnostics (culture and/or PCR) performed between October 2013 and September 2016. Records included some, if not all, of the following information: date of sample collection, date specimen was received by the laboratory, name of and address associated with the veterinarian that collected the sample, owner or farm affiliated with the sample, sample and/or bull identification, breed and age of bull associated with the sample, laboratory that processed and performed the diagnostic test(s) on the sample, test(s) performed, and test results.

2.3. Analysis

In estimating the true prevalence of BT, previously described Se and Sp results of single PCR alone, single culture alone, and a combination of single PCR and culture were used (Cobo et al., 2007). These sensitivity and specificity results for PCR, culture, and both are 65.9% and 98.3%, 67.8% and 98.9%, and 78.3% and 98.5%, respectively. True prevalence estimates were calculated as described previously (Reiczigel et al., 2010), and estimates less than zero were not consistent with assumed Se and Sp values and were indicated by " < 0". Confidence limits were calculated as described previously (Reiczigel et al., 2010). Aerial maps of the active bull screening and the laboratory records review were created using a commercial software (ArcMap in ArcGIS 10.4.1, ESRI 2016).

3. Results

In the active BT bull screening, a total of 458 beef bulls were sampled from 2 slaughterhouses (FPL Food and Southeastern Provision) and 2 stockyards (Athens and Somerville, TN) (Fig. 1). Nine individuals collected samples from these 458 beef bulls between March 2014 and June 2015. The distributions of these sampling were: FLP Food (207 samples; 14 sample collection dates; median of 14.5 samples per date; 27 stockyards specific backtag identifications), Southeastern Provision (98 samples; 28 sample collection dates; median of 3.5 samples per date; 18 stockyard specific backtag identifications), Athens stockyard (138 samples; 19 sample collection dates; median of 7 samples per date; a single stockyard), and Somerville (15 samples; 5 sample collection dates; median of 2 samples per date; a single stockyard). Angus bulls represented 71% of the sample population, and the median age for all bulls tested was 5 years [mean = 5.76 years; 346 bulls (75%) were 5 years of age or older; Table 1]. These bulls originated from 24 of 95 counties in TN. This county information corresponds to the stockyard where the animal received its backtag identification and may not necessarily correspond to the county of residence before sale and subsequent slaughter. There were approximately 39 stockyards in 36 counties approved to sell cattle in TN during the survey, and bulls originated from 24 of those 36 counties. The top five counties were

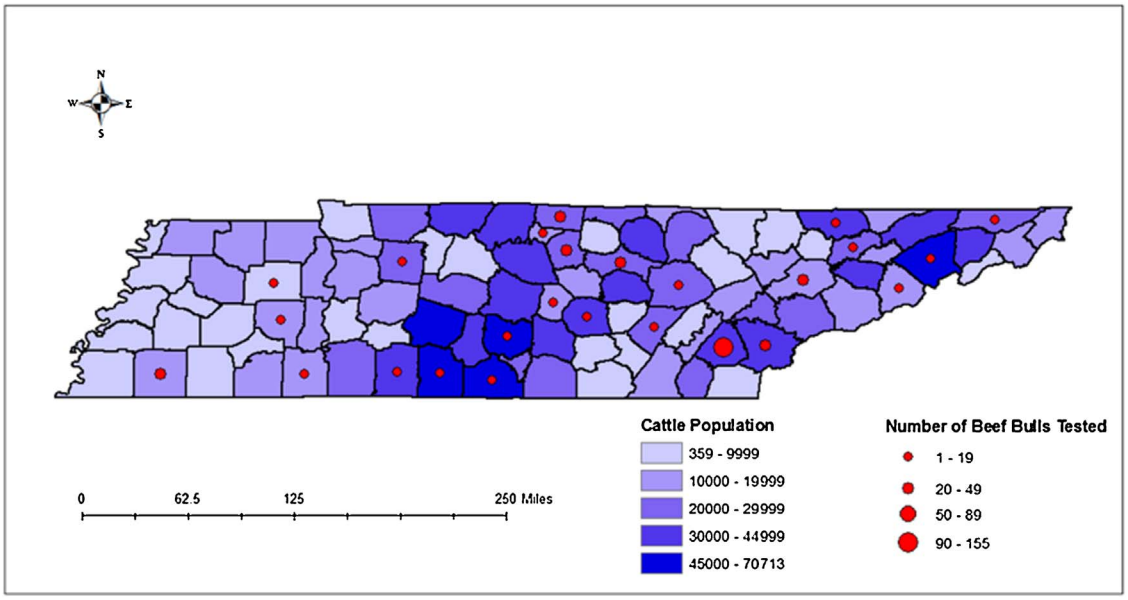

Fig. 1. Choropleth map of beef cattle population density per county in TN and the number of beef bulls tested and their distribution based on active surveillance data for bovine trichomonosis from 2014 to 2015.

Table 1
Breed and median age of bulls from TN in the active bovine trichomonosis bull screening survey.

Bull breed	No. bulls	Percent	Median age (years)
Angus	325	71.0	5
Brahma	5	1.1	5
Brangus	3	0.7	12
Charolais	45	9.8	5
Gelbveih	2	0.4	8.5
Hereford	26	5.7	7
Highlander	1	0.2	9
Limousin	13	2.8	6
Longhorn	6	1.3	5
Mixed	14	3.1	5
Romagnola	1	0.2	12
Shorthorn	1	0.2	4
Simmental	16	3.5	5
Total	458	100	5

McMinn (155 samples), Putnam (39 samples), Monroe (26 samples), Macon (23 samples) and Smith (22 samples).

Following Day 1 and Day 3 of culturing, all 458 samples were negative for BT. However, on Day 5, there were 2 suspect samples, 13 unread samples, and 443 negative results for BT. Nevertheless, after Day 7, a total of 457 samples yielded negative results for BT, while one sample was not read. Subsequent PCR screenings of these 458 samples for BT yielded all negative results.

In the laboratory record review, 1979 TN bulls were screened for BT in the 2 laboratories between October 2013 and September 2016. The average number of tests performed per month ranged from 7 to 314 with the greatest number of tests performed during the spring and fall months in comparison to the rest of the year (Fig. 2). Sample submissions were associated with approximately 205 different owners or farms with a mean of 9.7 (median of 1; range 1–669) samples per owner or farm.

Samples were submitted by 73 different veterinarians with a mean of 27 (median of 4; range of 1–686) samples per veterinarian. No date of collection was provided for 185 (9%) of the sample in the laboratory record review. For the 1794 samples with a date of collection, the median duration of time between date of sample collection and the laboratory receiving the sample was 1 day (mean of 1.2 days; range of 0–6 days) with 76 (4%) of samples with a date of collection taking more than 2 days to arrive at the laboratory.

Of the bulls tested, 1674 (85%) had no age reported, while the remaining 305 (15%) were assigned an age. The median age of bulls assigned an age was 2 years [mean of 2.6 years; 44 (14%) of bulls with an assigned age were 5 years of age or older; minimum and maximum ages of 1 and 14, respectively]. Of the bulls tested, 989 (50%) were Angus, 374 (19%) had no breed information reported, 270 (13%) were mixed breeds, 239 (12%) were Hereford, and the remaining 117 (6%) were of less prevalent breeds.

These bulls originated from 42 of 95 counties in TN (Fig. 3). The county information corresponds to the county of the veterinarian that collected the sample for screening and not necessarily the county of residence for the bull. There was a significant difference ($p < 0.001$) between counties associated with bulls tested for BT in TN and those that were not with respect to the following variables: the total cattle population, number of beef farms, and number of dairy farms (Table 2).

Ninety-three percent (1845 samples) of the screening was performed at the C.E. Kord laboratory, while the balance of 7% (135 samples) was screened at the UTCVM laboratory. Of the 1979 collected samples, 1972 were screened using PCR. Of these, 3 were positive, 1968 were negative, and 3 were rejected due to the compromised integrity of the specimens. However, only 559 samples were screened using culture. Of these, 2 were positive, 554 were negative, and 3 were rejected due to the compromised integrity of the specimens. All 559 samples screened using culture were also screened using PCR.

Of the 42 counties with submissions, the estimated true prevalence of BT in 20 counties was presented, where positive samples were identified (Bedford and Marshall; Fig. 3) and the top most contributors in bull screening for BT were revealed (Table 3). In Bedford County, one bull was positive by PCR on two separate specimen tests, and the estimated true prevalence of BT in Bedford County was 0.5%. Two bulls from Marshal County were positive by both PCR and culture, and the estimated true prevalence of BT in Marshal County was 0.077%. The overall estimated true prevalence of BT in TN was < 0.01% for either PCR alone, culture alone, or a combination of PCR and culture.

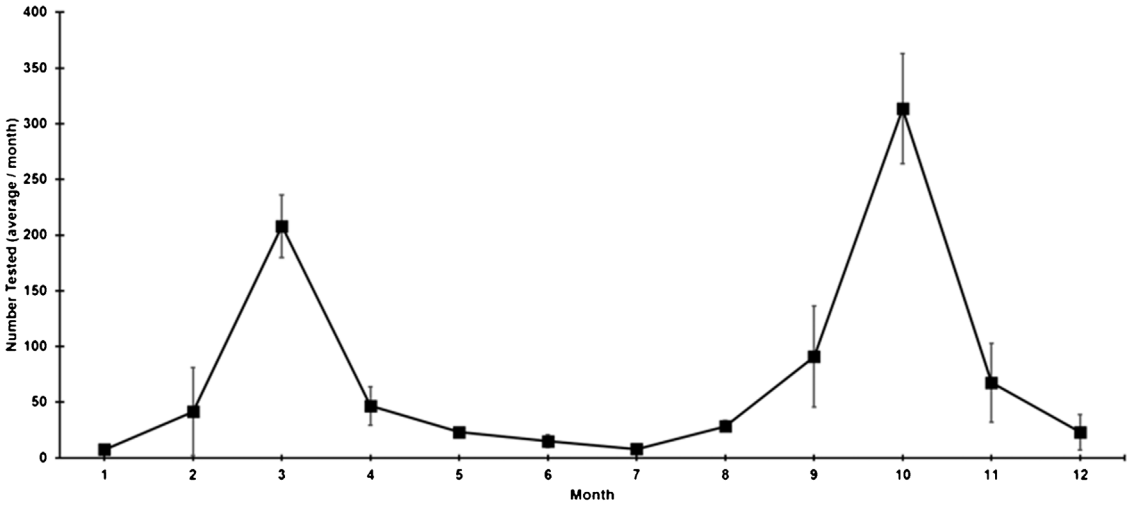

Fig. 2. Monthly time series of the average number of *T. foetus* tests performed (+ SEM) from 2014 to 2016 in TN as part of the laboratory record review.

4. Discussion

The duration of record assessment and the total number of sample results evaluated support the claim that the true prevalence of BT in TN bulls is < 0.01%. Non-randomized surveys conducted in extensive grazing systems in the U.S. reported individual bull prevalence of *T. foetus* infection in the range of 1.25–6.0% (Rae et al., 2004; Szonyi et al., 2012). While estimating the true BT prevalence in TN would not be possible, the methods used to test prevalence in the present study were comparable to the prevalence estimates obtained in previous surveys. The prevalence of BT among TN bulls reported in these surveys seemed to be less than the prevalence reported in many western states (4–7%) (Bondurant et al., 1990; Szonyi et al., 2012) and FL (6%) (Rae et al., 2004). However, the surveys reported here did indicate that BT was present in TN but at a prevalence more similar to that reported in AL (0.27%) (Rodning et al., 2008).

Several factors may have contributed to a lower than expected prevalence of BT in TN bulls. During the active bull screening survey, no BT positive samples were identified. Possibly, this observation reflects the true estimate of BT in TN. However, a number of factors could be responsible for not identifying a positive sample. First, the total number of animals sampled (458) was less than the calculated number of samples (491) needed to estimate the prevalence of BT in TN. It is possible that BT positive samples would have been identified if additional animals were screened to match the calculated sample size. The contract funding the active bull screening survey was terminated before the target sample size could be met. Given the outcome of the laboratory record review in this study, the assumed prevalence (3%) used for the sample size calculation in the active bull screening survey likely overestimated the true prevalence of BT in TN. Based upon the active surveillance reported here, the true prevalence of BT in TN bulls is likely < 0.01%. Assuming an estimated prevalence of 0.01% was used in estimating the sample size, samples from 20,588 beef bulls would be needed to estimate the prevalence of *T. foetus* in TN beef bulls. Also, samples were collected only once from bulls included in these surveys without regard to sexual rest, which could have also led to false-negative results. Regardless of the sampling and diagnostic technique used, it is generally recommended that bulls be sexually rested for 1–2 weeks before testing for *T. foetus*; otherwise, false-negative results are more likely because coitus removes the organisms from the penis and prepuce (Peter, 1997). Given the sensitivity of a single *T. foetus* culture or PCR, false-negative results are also possible even if a bull has been

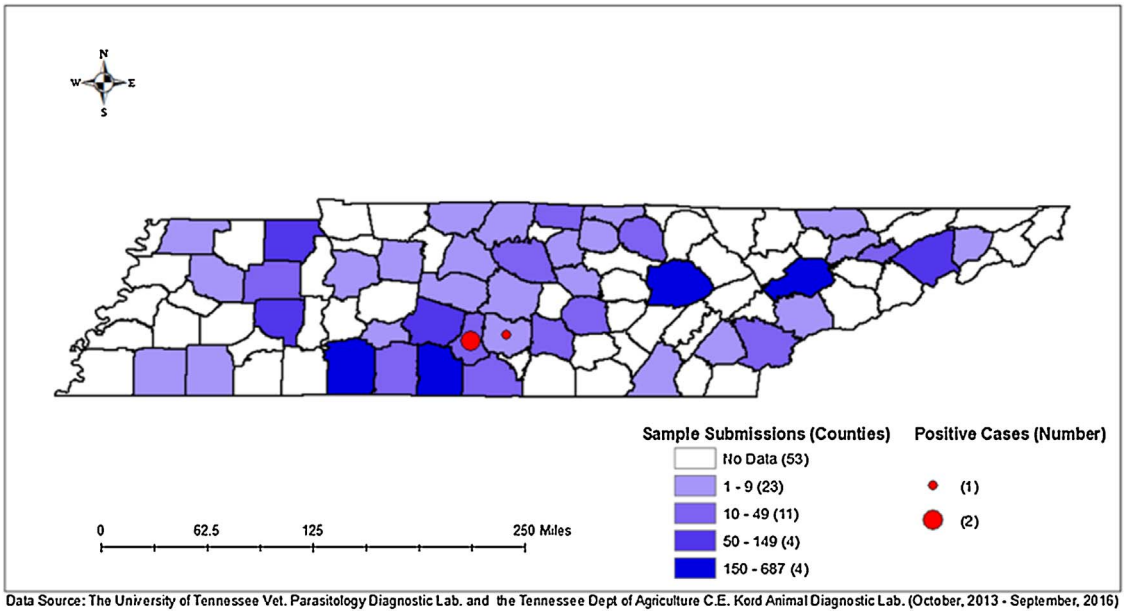

Fig. 3. Choropleth map of bull bovine trichomonosis sample submissions and positive cases per county in TN based on state-wide diagnostic laboratory data from 2013 to 2016.

Table 2
Distribution of cattle and farm demographics between counties associated with bulls tested for bovine trichomonosis in TN and those that did were not in the laboratory record review (2013–2016).

Counties associated with bulls tested for bovine trichomonosis (n = 42)							
Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	3652	70,713	27,745	22,820	32,670	15,804	24,654
Number of farms	116	1703	570	475	665	304	574
Beef farms	96	1460	494	412	576	263	502
Dairy farms	2	97	16	10	21	17	12
Counties not associated with bulls tested for bovine trichomonosis (n = 53)							
Variable	Minimum	Maximum	Mean	Lower 95% CL for mean	Upper 95% CL for mean	Standard deviation	Median
Cattle population	359	37,437	13,038	10,701	15,375	8478	10,368
Number of farms	1	932	281	232	330	177	261
Beef farms	4	829	241	200	284	153	220
Dairy farms	0	25	6	5	8	5	5

sexually rested because the amount of *T. foetus* organisms present on the penis and/or prepuce can fluctuate. Hence, because the utilized tests are not highly sensitive, it is possible that a BT positive could have been missed.

Lastly, a BT positive bull may have been identified if the present study evaluated bulls from more than 24 of the 95 counties in the state.

Age and herd size are risk factors for bulls acquiring a chronic *T. foetus* infection. The median age of bulls associated with preputial smegma samples in the present active bull screening survey was 5 years (mean age of 5.8 years) with 346 (75%) of the bulls being 5 years of age or older. In a survey of CA beef herds, 2% of bulls 3 years of age and younger were infected with *T. foetus* compared to 6.7% of bulls 4 years of age and older (BonDurant et al., 1990). An epidemiological study in FL found the mean age of BT infected bulls was 5.5 years, and the mean age of uninfected bulls was 3.9 years (Rae et al., 1999). Another study in FL found bulls greater than 5 years of age were 2.2 times more likely to be infected with *T. foetus* than bulls 5 years of age or younger (Rae et al., 2004). Therefore, the population of bulls sampled in the present bull BT screening should have been at greater risk of harboring a *T. foetus* infection than the entire population of TN beef bulls. However, only 305 (15%) of bulls associated with the laboratory record review were assigned an age, and the median age of those bulls was 2 years. Moreover, 261 (86%) of bulls assigned an age were less than 5 years old. Ironically, it was in the laboratory record evaluation, where the risk ought to be smaller, that positive samples were found. Our finding from the lab data suggests that the conventional wisdom that BT being mostly identified in old bulls may not actually be true.

There was no data available regarding the reason for testing the 1979 sample submissions included in the laboratory record review; therefore, the risk of *T. foetus* infection of the bulls associated with those samples was unknown. Perhaps many of the preputial samples were submitted for routine surveillance or regulatory purposes prior to sale, purchase, breeding, or interstate transport, with no indication of reproductive problems in the herd of origin. In the present laboratory record review, there was a spike in sample submissions for BT testing in spring and fall months. With the spike in spring and fall sample submissions and the age of bulls associated with samples assigned an age being relatively young, it is most likely that the samples for the laboratory record review were submitted prior to sale or purchase of young bulls.

A lower prevalence of BT in TN may be because there are mostly small-sized herds in the state. In the epidemiological survey conducted by Rae et al. (Rae et al., 2004), medium-sized herds were much less likely to be infected with *T. foetus* (100–499 cows, 10%) than large

Table 3
Cattle population and prevalence of bovine trichomonosis in selected counties in TN (laboratory record review 2013–2016).

County[a]	Total cattle population	Number of beef farms	Number of dairy farms	Number of bulls screened for Trichomonosis by PCR (no. Positive)	Number of bulls screened for Trichomonosis by culture (no. Positive)	Estimated true prevalence for Trichomonosis by PCR (95% CI)	Estimated true prevalence for Trichomonosis by culture (95% CI)	Estimated true prevalence for Trichomonosis by PCR and culture (95% CI)
Bedford	52,710	681	14	3 (1)	0 (0)	0.493 (0–1)	N/A	0.414 (0.002–1)
Marshall	37,063	521	38	27 (2)	27 (2)	0.089 (0–0.336)	0.095 (0.003–0.332)	0.077 (0–0.283)
Giles	60,336	992	23	687 (0)	9 (0)	< 0 (0–0)	< 0 (0–0.458)	< 0 (0–0.392)
Cumberland	22,251	362	15	177 (0)	77 (0)	< 0 (0–0.005)	< 0 (0–0.053)	< 0 (0–0.041)
Wayne	27,676	425	7	168 (0)	167 (0)	< 0 (0–0.007)	< 0 (0–0.016)	< 0 (0–0.008)
Knox	15,312	453	5	163 (0)	22 (0)	< 0 (0–0.008)	< 0 (0–0.201)	< 0 (0–0.170)
Henderson	17,837	324	10	129 (0)	28 (0)	< 0 (0–0.016)	< 0 (0–0.154)	< 0 (0–0.129)
Henry	19,844	320	17	107 (0)	68 (0)	< 0 (0–0.025)	< 0 (0–0.062)	< 0 (0–0.049)
Greene	70,713	1460	63	83 (0)	65 (0)	< 0 (0–0.040)	< 0 (0–0.066)	< 0 (0–0.052)
Maury	52,108	791	13	72 (0)	7 (0)	< 0 (0–0.051)	< 0 (0–0.549)	< 0 (0–0.472)
Lincoln	61,592	910	17	43 (0)	15 (0)	< 0 (0–0.089)	< 0 (0–0.306)	< 0 (0–0.260)
Macon	22,595	519	5	43 (0)	13 (0)	< 0 (0–0.089)	< 0 (0–0.321)	< 0 (0–0.274)
Coffee	31,389	392	14	40 (0)	4 (0)	< 0 (0–0.097)	< 0 (0–0.774)	< 0 (0–0.667)
Lawrence	44,333	801	97	28 (0)	1 (0)	< 0 (0–0.151)	< 0 (0–1)	< 0 (0–1)
Hamblen	16,804	360	14	26 (0)	26 (0)	< 0 (0–0.165)	< 0 (0–0.166)	< 0 (0–0.140)
Overton	32,238	567	23	25 (0)	3 (0)	< 0 (0–0.172)	< 0 (0–0.93)	< 0 (0–0.803)
Warren	33,903	558	27	21 (0)	18 (0)	< 0 (0–0.211)	< 0 (0–0.251)	< 0 (0–0.213)
Monroe	38,436	449	25	19 (0)	18 (0)	< 0 (0–0.223)	< 0 (0–0.237)	< 0 (0–0.2)
Carroll	8654	248	10	19 (0)	5 (0)	< 0 (0–0.236)	< 0 (0–0.733)	< 0 (0–0.632)
Wilson	36,365	769	12	17 (0)	1 (0)	< 0 (0–0.268)	< 0 (0–1)	< 0 (0–1)

N/A: Not applicable.

[a] These 20 counties were either counties in which positive samples were identified (Bedford and Marshall) or top most contributors in bull screening for BT.

herds (≥ 500 cows, 53.9%). Studies have identified several management practices, some implemented in large multi-sire herds, including commingled grazing (Gay et al., 1996), high bull-to-cow ratio, and large number of bulls per breeding unit (Rae et al., 1999), to be positively associated with risk of infection with *T. foetus*. According to the National Agricultural Statistics Service (USDA and NASS, 2014), there were approximately 33,556 beef cow herds in TN in 2012, and approximately 53% of all beef cows and 87% of all beef cow herds were associated with herds with ≤ 49 beef cows. The risk of venereal disease transmission tends to increase with an increase in herd size, which corresponds to multi-sire breeding groups. As the number of sires increase in a herd, the potential risk of a *T. foetus* infected bull spreading BT to other bulls increases because of extensive breeding overlap. Therefore, in multiple-sire pasture breeding, as many as 80% of cows will be bred by two or more bulls during one estrous period (Barth, 2007). In the laboratory record review, all 3 positive samples originated from the southern-middle TN in an area with a greater population of beef cattle and a higher proportion of larger beef cattle herds (≥ 100 cows per farm). Moreover, the 3 positive samples originated from neighboring counties (Bedford and Marshall). Besides the use of natural service for breeding, other risk factors for BT include neighboring a *T. foetus*-positive herd (Jin et al., 2014) and commingling of cattle (Mardones et al., 2008). Herds are at greater risk for introduction of *T. foetus* based on a relatively high local prevalence of the disease and the use of management practices that increase the risk of the introduction of BT into the herd.

It is common for the prepuce of bulls to be contaminated with feces or other debris, which may affect the accuracy of test results. One of the major strengths of the present study was the use of microscopic examination of diagnostic culture and qPCR testing. Diagnosis of *T. foetus* has traditionally relied solely upon microscopic identification of key morphological characteristics in culture media. The presence of other, morphologically similar trichomonadid protozoa interferes with diagnosis of *T. foetus* infection by microscopic examination of diagnostic culture (Cobo et al., 2003; Hayes et al., 2003). To our knowledge, this was the first large-scale active study of BT that utilized data generated from the use of qPCR and microscopic examination of diagnostic culture of all preputial smegma specimens. Non-*T. foetus* trichomonads, typically of fecal origin, complicate culture-based testing, but it can be overcome through the use of *T. foetus*-specific PCR testing (BonDurant et al., 1999). Regardless of efforts to avoid contamination of preputial smegma samples, the two suspect specimens observed in the active bull screening study were likely the result of fecal contamination. PCR-based assays were utilized during BT diagnostic testing for the bull screening study, in part to avoid false-positive results (Cobo et al., 2007). In that study, misclassification of the two suspect samples was avoided because these specimens yielded negative results upon subsequent PCR testing. In the laboratory record review, 559 (28%) of the samples were screened with microscopic examination of diagnostic culture, and 1972 (99%) of the samples (including all samples screened with microscopic examination of diagnostic culture) were screened with PCR-based diagnostics. Of the 3 positive BT samples, 2 were initially identified by microscopic examination of diagnostic culture and subsequently confirmed by PCR, and the other was only ever diagnosed by PCR [a subsequent (sample collected 7 days after the initial sample) PCR confirmed the first test result]. Contrarily, a recent study found that bacteria that is not inhibited by growth media may interfere with *T. foetus* identification by culture and PCR and adversely affect the diagnostic sensitivity of these tests (Clothier et al., 2015). Therefore, surveys utilizing grossly contaminated preputial smegma samples would erroneously underestimate the prevalence of BT. In realization of potential pitfalls in BT diagnostic testing for the active bull screening survey, we utilized more than one diagnostic test (microscopic examination of a diagnostic culture and qPCR), collected preputial smegma while avoiding overaggressive scraping and blood contamination, avoided fecal contamination of the preputial smegma samples, and ensured appropriate specimen handling before diagnostic testing. Thus, we are more confident that the true prevalence of BT in TN beef bulls is < 0.01%.

Testing bulls to determine their *T. foetus* infection status is a basic component of BT control programs and prevalence surveys. Although it seems to be straightforward, 3 phases of any diagnostic testing process must be properly completed to arrive at a correct diagnosis. The pre-analytical phase (collection and handling of samples before performing an analysis) accounts for approximately 62% of all diagnostic errors (Plebani, 2010). While optimizing diagnostic tests for BT is helpful, more attention to pre-analytical factors is necessary for increasingly accurate determination of the *T. foetus* infection status of individual animals and herds (Mukhufhi et al., 2003). For instance, in order to achieve an accurate diagnosis of trichomonosis in cattle, appropriate measures should be taken to protect diagnostic samples from temperature extremes (maintain samples at temperatures of 4–37 °C), and samples should arrive at the diagnostic laboratory within 24–48 h of collection (Clavijo et al., 2011). When a limited concentration of *T. foetus* is present in diagnostic specimens, as may occur in naturally infected bulls, extremes in temperature and/or excessive duration of time between sample collection and arrival at the laboratory will likely give negative results by culture as well as PCR (Clavijo et al., 2011). In the laboratory record review reported here, no date of collection was provided for 185 (9%) of the sample submissions, and 76 (4%) of samples with a date of collection took more than 2 days to arrive at the diagnostic laboratory. While the pre-analytical phase of diagnostic samples utilized for the active BT screening survey reported here were optimal (ideal temperature and timing) pre-analytical conditions applied to samples for the laboratory record review were often undetermined. Therefore, a lower estimated prevalence of BT in TN may be in part because of the pre-analytical phase of diagnostic samples utilized in the laboratory record review. Veterinarians and other individuals involved with overseeing specimens should be reminded of the importance of proper specimen collection and handling to ensure accurate BT test assessment in the laboratory.

5. Conclusion

The prevalence of BT in TN is low. Therefore, current surveillance efforts towards BT control in TN are acceptable. Future efforts should focus on educating TN cattle stakeholders on the importance of optimal specimen collection and handling as well as routine testing for BT before cattle movement. In addition, cattle producers should be reminded of leading risk factors associated with trichomonosis in cattle.

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