Prevalence of and factors associated with *Tritrichomonas fetus* in bull populations in the state of Florida (USA)

A case review:
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Abstract

The objective of these studies were to report the prevalence of infection with *Tritrichomonas fetus* in bull populations studied in the state of Florida (USA); to assess influence of bull related factors (i.e. age, breed, and grouping); and, to assess effects of trichomonosis on measures of cow performance (pregnancy rate, weaning percentage, weaning weight). The studies were conducted, one, as an epidemiologic study and, the second, as a population survey. The sample population of the case review was 1383 bulls and records for 28,471 cows bred by these bulls in the immediate past breeding season; and, in the Florida survey, 1994 bulls in 54 herds throughout the state of Florida. In both studies, bulls were tested for *T. fetus* colonization by vigorous preputial scraping and culture. Bull infection prevalence within herds was calculated and correlated to bull, herd and management factors and production measurements. In the case review: the mean prevalence for *T. fetus* infected bulls was 11.9% for all bulls (within herd prevalence 0 to 36%). A significant difference was detected between mean age of infected bulls (5.5 years) and non infected bulls (3.9 years). A difference in prevalence among breeds was found, although other factors may have influenced this. Cow performance measurements (weaning percentage, mean weaning weight and adjusted mean weaning weight per exposed cow) for cows exposed by breeding to bull groups with the highest prevalence of infection (36%) were significantly different than mean values for the entire study population. In the Florida survey: an overall prevalence for *T. fetus* infected bulls was 6.0% (within herd prevalence range 0 to 27%). The overall herd prevalence was 30.4%, where 11.1% of herds sampled in North Florida had infected bulls and 39.5% of herds sampled in South Florida had infected bulls. A distinction between herd size was noted, where among medium size herds (100-499 cows) there was a 10.0% herd prevalence, while among large size herds (500+ cows) there was a 53.9% herd prevalence. *T. fetus* infection in natural service beef herds in Florida was associated with bull (age, breed), herd and herd management practices and appears to have had an adverse impact on several production measures.

Keywords

Trichomonosis, *Tritrichomonas fetus*, venereal disease, bull reproduction.

Introduction

Tritrichomonas fetus is responsible for substantial gestational losses in cattle. Bulls with inapparent infection (colonization) of the prepuce, transmit the organism venereally during coitus (1). Susceptible, infected cows may manifest disease by early embryonic death, abortion, pyometra, fetal maceration, or infertility (2,3,4,5). On rare occasions, infected cows may carry infections into the next breeding season (6). Economic losses from trichomonosis in beef cattle are associated with treatment, culling and replacement of infected cattle, and

foremost, the loss of calf crop caused by failure or delay in conception (2). The impact of these losses can be substantial. Wilson et al (7) projected an annual calf loss attributable to *T. fetus* in replacement heifers in Oklahoma of $2.5 million in 1979 ($6.1 million U.S., 1998). In 1958, Fitzgerald et al. (8) estimated that each infected bull in a large herd was responsible for a loss of $800 per year at that time ($4800 U.S., 1998). A simulation model estimated a reduction of 14 to 50% in annual calf crop if prevalence of *T. fetus* in the bull population was 20 and 40%; net return per cow exposed to an infected bull was reduced 5 to 35%, (9).

A survey completed in 1979 indicated that the prevalence of *T. fetus* in beef cattle bulls in Florida was 7.3% (10). In a field investigation (11) *T. fetus* was found in bulls in a Florida herd. The herd was studied with the objective to report a naturally occurring infection of *T. fetus* in a large cow-calf enterprise and to determine the prevalence of infection with *T. fetus* in the bull population; to assess the influence of age, breed, and bull breeding groups; to assess the effects of bull infection on measures of cow performance (pregnancy rate, weaning percentage, weaning weight). Because many questions remained unanswered following the case investigation, a survey of Florida beef cattle herds was conducted with the objective to survey Florida beef cattle herds to estimate the herd and individual bull prevalence of trichomonosis; to perform a descriptive and analytical epidemiological investigation of risk factors for the disease and to assess outcomes of disease.

Materials and Methods

A case review:

Screening of bulls on a large cow-calf ranch suggested that *T. fetus* infection was widespread in the bull population. A ranch-wide test and cull program was begun on the ranch's 11 cattle units. Each cattle unit consisted of 40 to 160 bulls and 1000 to 4000 cows (bull-to-cow ratio, 1:25), and each was subdivided into herds of 150 to 350 cattle each. Cows grazed extensive pastures, containing a variety of native grasses. The breeding season for 1995 was from mid-March to mid-June.

Testing of all bulls for *T. fetus* followed. Between June 1995 and January 1996, 2724 preputial samples were collected from 1383 bulls during 17 ranch visits. Testing was concluded before commencement of the next breeding season. Bulls were not exposed to cows during this time. Bulls were from 1 to 10 years of age and were of 4 breeds or breed-types (Simbrah, Brangus, Braford or Angus). Units with no infected bulls on initial examination were not tested again. For units in which infected bulls were identified, bulls were tested on subsequent visits until no newly detected bulls were identified.

Preputial specimens for protozoologic culture were collected by vigorous scraping with a clean infusion pipette, and aspiration of smega from the prepuce near the fornix with a syringe attached to the pipette. Scraping technique was adequate if the specimen was mildly blood tinged and the pipette contained smega in approximately one-third of its length. Specimens were placed in commercial transport and culture media[1], transported in a closed, insulated carrier to the microbiology laboratory, and incubated at 37C for 5 days. Culture samples were examined by light microscope at 100 X after 24 to 48 hours and 96 to 120 hours of incubation. Infection was confirmed if one or more *T. fetus* organisms were identified.

Calves conceived during the 1995 breeding season were born from mid-December 1995, to early April 1996, and were weaned in September/October of 1996. To assess the relationship between prevalence of infection in the bull population and cow performance

[1] *In-pouch TF*, BioMed Diagnostics, San Jose, CA.

measurements, ranch records for the period from first potential calf conception (March 1995) to last calf weaning (October 1996; 20 months) were reviewed. Inventory counts of cows and calves by unit were made during September/October of 1995 (at pregnancy examination), in May 1996 (at first calf processing) and during September/October 1996 (at weaning). Calves were weighed and counted at weaning. Weaning weights were reported as total pounds of calf weaned per unit and mean pounds weaned per calf. Pregnancy examination, by transrectal palpation, was done by ranch employees at ∃130 days of gestation.

Pregnancy percentages for the 1995 breeding season were calculated as the number of cows pregnant divided by the number of cows in each unit at the time of pregnancy diagnosis. This was a base line measure of cows exposed to the bull population during the breeding season. Because of variation in cattle numbers over time, an adjustment to the number of exposed cows was made to determine weaning percentage and unit production measures. The adjusted number of exposed cows reflected addition of bred replacement heifers, or loss as a result of death and culling of pregnant cows. Non-pregnant cows remained in the count of exposed cows whether they had been culled or not. Weaning percentage was measured as the number of calves weaned divided by the adjusted number of exposed cows. Since weaning weight is influenced by age at weaning, an adjusted mean weaning weight was calculated to the mean weaning age of all calves (262 d). Mean weaning weight and adjusted mean weaning weight per exposed cow was calculated as the mean weaning weight (or adjusted mean weaning weight) multiplied by the weaning percentage. Data were summarized as descriptive statistics and associations were tested among bull prevalence and production outcomes in the cow population by unit using Pearson correlation and linear regression models[2].

A Florida State Survey:

Sampling- Estimates indicated that there were about 70,000 beef bulls in the State of Florida in 1997 (12), dispersed in 13,590 herds of varying sizes (Table 1). Note that herd-size greater than 500 breeding-age females accounts for 47.6% of the inventory while representing only 2.5% of the total number of operations. A decision was made to survey herds with 50 and greater breeding-age females or a population of approximately 3170 herds. From these, a stratified random sampling based on herd size was used. A pre-survey estimate of trichomonosis prevalence of 10% was selected to calculate the sample size (86 herds, with a 95% Confidence Interval [CI] of detecting a difference) which would be required to estimate, with confidence, the herd prevalence of trichomonosis in Florida beef cattle. To obtain sufficient participants to meet sample size criteria, 1000 of 3170 producers were randomly selected to receive an informational letter and questionnaire, including contact information for those willing to participate in the sampling survey. The State of Florida has a large land mass and considerable variability in management styles and types of cattle operations between North and South Florida. For this reason, the state was evaluated as two geographical areas.

The estimate of individual bull prevalence required a sample size calculation, which was dependent on the desired precision and the prevalence of the disease. Given a 10% estimated prevalence, a 1.5% absolute error, and a confidence level of 95%, 1537 bulls were needed for estimation of the statewide individual bull prevalence.

Sample collection and evaluation- A modified Diamond's media, was prepared every 2-weeks and used for the isolation and cultivation of *T. fetus* (13,14,15). An effort was made to encourage producers to sample all bulls on the premises to allow for a more precise

[2] EpiInfo6, version 6.02, Center for Disease Control and Prevention, Atlanta, GA.

estimate of the within-herd prevalence. When, this was impossible, a subset of at least 20% of bulls was sampled, representing the whole bull population. Ideally, the subset was a random sample, but practically, it was a convenient sample. Each bull was restrained and a sample taken from the prepuce as previously described. Immediately after collection, the specimen was layered on the top of the modified Diamond's media, kept in an incubator and maintained at 37C. Sample evaluation was performed at 24, 48, and 120 hours after sample collection; a 0.1 ml sample drawn from the bottom of each tube was placed on a microscope slide and evaluated at 100X magnification for trichomonads. Suspect organisms were then evaluated at 400X. The diagnostic characteristics of *T. fetus* included: a protozoal organism 20x10 um in size, the presence of three anterior flagella, one trailing posterior flagellum, and a distinct undulating membrane (16).

Table 1. Beef cow Inventory, 1997 USDA Census of Agriculture

Herd Size	Number of Operations	Percent of Farms	Number of Cows	Percent of Inventory
1-49 head	10,430	76.7	152,690	15.2
50-99 head	1,448	10.6	95,418	9.5
100-499 head	1,389	10.2	277,597	27.7
500 or more	333	2.5	477,367	47.6
Totals	13,600	100.0	1,003,072	100.0

Herd and Bull Data Collection- At the time of sample collection, an effort was made to interview the person that had access to and knowledge of herd records. A standardized form was used. Bulls were included in the study if they were: of breeding age (~2 years of age and older) and the owner (or agent) agreed to provide herd profile information. Bulls were excluded if they had traumatic lesions to the penis or prepuce that may have interfered with culture, or, if the bulls came from a herd where a diagnosis of trichomonosis had previously been made and bulls removed. Data collected for each herd is listed in Table 2.

Table 2. Data collected for each herd

Herd number	Knowledge level of trichomonosis
Sample identification number	Slightly familiar
Number sampled in the herd	Moderate knowledge
Herd size category	Much Knowledge
Herd size = 50- 99 breeding-age females	Month of commencement of breeding season
Herd size = 100-499 breeding-age females	Length of breeding season (days)
Herd size = 500 and greater breeding-age females	Ratio of bulls-to-cows, i.e. 1: 25
Bull age (in years)	Average number of bulls per herd of breeding females
Bull breed	Number of calves weaned
T. fetus culture status (for each individual bull)	Number of females exposed to bulls to produce these
Number of *T. fetus* culture positive bulls in herd	Weaning rate (calves weaned / females exposed)
Herd status for trichomonosis	Average weaning weight of male calves
Geographical area	Average weaning weight of heifer calves
North Florida	Average weaning weight for all calves
South Florida	Average number of bulls purchased annually
Vaccination status of herd females for T. fetus	Total number of bulls in the herd
None, Yes	Age at purchase
Administered one time per year	Primary purchase source
No, Yes	Raised on ranch
Administered as per manufacturer's recommend	General Livestock Auction Market
No, Yes	Multiple breed sale
Number of years of annual vaccination	Single breed sale
Month of vaccination (January, etc.)	Private treaty (purchased from individual breeder)
Knowledge of trichomonosis prior to survey	Secondary purchase source
None	Tertiary purchase source
Some Knowledge	Previous diagnosis of trichomonosis in herd females

Statistical evaluation was performed using the Stata[3] statistical software. Due to the dichotomous outcome nature of the primary dependent variable, herd and individual *T. fetus* status, the independent variables of interest were evaluated by multi variate logistic regression (Hosmer and Lemeshow, 1989). Each of the independent variables were evaluated individually using two way tabulation with test for independence (Pearson's chi square test or likelihood ratio). Variables that showed evidence of homogeneity were evaluated in models describing *T. fetus* positive and negative herds and individuals, using a forward stepwise selection. The models selected were evaluated for goodness of fit (Hosmer-Lemeshow Statistic).

Results- A case review

One hundred sixty-five bulls were infected with *T. fetus.* Mean prevalence of infection among bulls was 11.9%. Prevalence within unit ranged from 0 and 35.9% (0 to 61 bulls per unit, Table 1). Mean age of all bulls was 4.1 years. Mean age of infected bulls (5.5 ± 1.6 years) was significantly different ($p<0.001$) from the mean age of noninfected bulls (3.9 ± 2.3). A significant ($p<0.001$) difference in prevalence among breed of bull was detected (Simbrah, 58/396, 14.6%; Brangus 76/404, 17.9%; Angus, 20/167, 12%; Braford, 10/394, 2.5%). The relative representation of breed-type in the bull population was Simbrah, 28.6%; Brangus, 30.7%; Angus, 12.1%; Braford, 28.5%.

Table 3. Prevalence of *T. fetus* infection in a population of bulls (a case review).

Ranch Unit	1	2	3	4	5	6	8	9	11	12	16	Total
N (bulls tested)	47	40	133	158	108	192	142	112	170	120	161	1383
T. fetus +	0	0	18	24	1	10	8	8	61	7	28	165
Prevalence (%)	0.0	0.0	13.5	15.2	0.9	5.2	5.6	7.1	35.9	5.8	17.4	11.9*

Ranch Unit = cattle unit identification number. *T. fetus* + = number of culture positive bulls. * Mean prevalence for all cattle units.

Table 4. Regression model for prevalence of *T. fetus* in a bull population and selected performance measurements for cows exposed to the bulls, such that for each 1% rise in prevalence, the performance measure changes by the model coefficient value.

Measurement	*Variable constant*	*Model coefficient*	*R^2 value*	*P value*
Pregnancy (%)	*0.758*	*-0.002*	*0.093*	*0.390*
Weaning (%)	*0.762*	*-0.006*	*0.575*	*0.029*
Mean weaning weight (lb)	*487.7 (222 kg)*	*-1.03*	*0.142*	*0.253*
Adjusted mean weaning wt (lb)	*507.3 (231 kg)*	*-2.79*	*0.355*	*0.053*
Mean weaning wt (lb) / exposed cow	*374.6 (170 kg)*	*-3.35*	*0.564*	*0.032*
Adj mean weaning wt (lb) / exposed cow	*368.4 (167 kg)*	*-3.52*	*0.589*	*0.026*

Pregnancy (%) = the pregnancy percentage by breeding unit. Weaning (%) = number of calves weaned / adjusted number of exposed cows (reflects addition of heifers or transfer of cows and loss or sale of pregnant cows). Mean weaning weight = gross calf weight per unit in pounds (kg) / number of calves weaned. Adjusted mean weaning weight = 262 day adjusted weaning weight. Mean weaning weight / exposed cow = gross weaning weight/ adjusted number of exposed cows. Adj mean weaning wt / exposed cow = 262 day adjusted weaning weight per exposed cow.

Cow performance was measured by pregnancy percentage, weaning percentage, weaning weight and adjusted weaning weight. The within herd regression model for the variables was determined (Table 4); such that, for each 1% rise in within herd prevalence, the

[3] StataCorp, College Station, TX 77840

performance measure changed by the model coefficient value, i.e. weaning percentage was reduced by 0.6% or adjusted mean weaning weight per exposed cow was reduced by 3.5 lb. (1.6 kg). The estimated single-test sensitivity was approximately 73% and the negative predictive value was 96.4%.

A Florida State Survey:

Description of the Survey Population- The survey was performed over a period of 23 months (November 1997 to October 1999). A total of 1984 preputial samples were obtained from bulls in 59 herds, located in 27 counties. Only data from 3 herds from herd size 50-99 head were obtained. The sampling frame in herd size 100-499 was 30 herds and herd size greater than 500 was 26 herds, each providing reasonable survey representation (Table 5). The number of samples tested per herd ranged from 2 to 212 bulls. The 1984 bulls that were cultured for T. fetus represented 81.8% of all bulls (2425) in the surveyed herds, and approximately 2.8% of the population of bulls in Florida in 1997 (12; Table 5).

The age of the bulls in the surveyed herds ranged from 2 to 15 years of age. The mean age was 5.0 years (4.9 to 5.1 years, CI); 92.5% of bulls were 2-8 years of age. There were 22 different breeds represented in the survey including *Bos taurus, Bos indicus,* and crosses of *Bos taurus X Bos indicus*. About 91% of the bulls were in 7 breeds- Angus, Hereford, Brangus, Charolais, Beefmaster, Braford and Brahman (Table 10).

Table 5. Number of ranches (and bulls) sampled by geographical area and herd size.

	Herd Size			
	50-99 head	100-499 head	500+ head	Total
North Florida	3 (9)	13 (166)	5 (225)	21 (400)
South Florida	0 (0)	17 (192)	21 (1392)	38 (1584)
Total	3 (9)	30 (358)	26 (1617)	59 (1984)

The survey respondents purchased 372 bulls (6.3 bulls/herd, 4.6-7.9 bulls CI 95%) in the breeding season prior to the survey. Over fifty percent (52.5%) of survey herds purchased bulls from an individual breeder. The bulls in the survey were from ranches with an inventory of breeding- age females representing 55,211 animals (55 to 6,000 animals, a mean of 936 animals or approximately 7.5% of the population of cattle in beef herds having greater than 50 head of breeding females (12). The exposed females weaned 42,179 calves or a mean of 715 (44 to 4680) calves. The calculated weaning rate (number calves weaned/number females exposed) was 76.8% (74.3 to 79.3% CI, range 50 to 93.1%). The mean calf weaning weight for survey herds was 222 kg (215 to 229 kg, CI). Weaning weight expressed as kilograms of calf weaned per exposed female was 172 kg (162 to 179 kg CI, range 115 to 179 kg) per exposed female. Ten of the 59 herds (16.9%) used a vaccine that included antigens for *T. fetus*. Only 2 of 10 herds used the vaccine as recommended by the manufacturer, i.e., two injections in the initial immunization year, 2 to 4 weeks apart, with the second injection given less than 30 days before the beginning of the breeding season. Generally, only a single injection immunization schedule was used. Three surveyed herds indicated that a diagnosis of trichomonosis had been made through submission of aborted specimens to a diagnostic lab, but no prior testing of bulls had been performed.

Of the 1984 bulls cultured for *T. fetus*, 119 bulls (6.0%) were culture positive. These 119 bulls were from 17 of the 59 surveyed herds. This represented an unstratified herd prevalence (sem) of 28.8 ± 0.06% (16.9 to 40.7%, CI). Due to the inadequate sampling of herds with 50-99 head, this category was not included in the analysis by herd size. Thus, the

survey estimated an unstratified herd prevalence for trichomonosis in Florida beef cattle herds with 100 and greater breeding-age females to be 30.4% (18 to 43%, CI). Two of 18 herds were positive in North Florida (11.1% prevalence; -5 to 27%, CI, Table 6) and 15 of 38 (39.5%; 23 to 55.8%, CI) in South Florida.

Herd size of 100-499 head had a herd prevalence of 10% (3/30, Table 7), while herd size of 500+ head had a 53.9% herd prevalence. The univariate unadjusted odds ratio of a herd size of 500 or greater breeding-age females having at least one culture positive *T. fetus* bull in the herd was 10.5 times more likely than that of a herd of 100-499 breeding-age female herd ($p<0.001$).

Table 6. Survey herds with culture positive *Tritrichomonas fetus* bulls by geographical area.

	Geographical Area		
Herd Status[1]	North Florida	South Florida	Total
Negative (N)	16	23	39
Positive (N)	2	15	17
Prevalence (%)	11.1%	39.5%	30.4%
Totals	18	38	56

[1] Herd Status Negative Status = No bulls with *T. fetus* positive cultures.
Positive Status = One or more bulls with *T. fetus* positive cultures.

Table 7. Survey herds with culture positive *Tritrichomonas fetus* bulls by herd size.

	Herd Size		
Herd Status[1]	100-499 head	500+ head	Total
Negative (N)	27	12	39
Positive (N)	3	14	17
Prevalence (%)	10.0%	53.9%	30.4%
Totals	30	26	56

[1]Herd Status Negative Status = No bulls with *T. fetus* positive cultures.
Positive Status = One or more bulls with *T. fetus* positive cultures.

Estimates of within herd prevalence ranged from 1.8% to 27%, with a mean of 11.9%. Herds with less than 500 breeding females (3 culture positive herds) had a mean within herd prevalence of 24.4%; herds with over 500 had a mean within herd prevalence of 9.2%. North Florida had a mean within herd prevalence of 17.9% and South Florida 11.1%.

The surveyed herds used primarily multiple sire breeding groups (mean, 7.6 bulls per unit). The 9% of herds that used single sire breeding groups were all *T. fetus* negative herds. The 91% of herds that used more than one bull per breeding group (range 3 to 31, mean 8.6 per unit) had a mean within herd prevalence of 11.9%. Of herds with multiple sire breeding groups, the mean number of bulls per unit in *T. fetus* negative herds was 7.7 bulls per group, whereas *that of T. fetus* positive herds was 10.3 bulls per unit. The surveyed herds purchased 0 to 30 bulls annually (mean of 6.3 bulls per year). *T. fetus* culture negative herds purchased a mean 4.0 bulls per year, while *T. fetus* positive herds purchased a mean 11.9 bulls per year.

Logistic Regression of the Herd Prevalence Data- The herd level logistic regression model (Table 8) found herd size (500 and greater breeding-age females) and bull-to-cow ratio (less than 24 breeding-age females to one bull), to be significant risk factors for trichomonosis in Florida beef herds. This model indicated that large herds or a greater bull-

to-cow ratio, each resulted in a 12.8 times greater likelihood of a bull within the herd being culture positive for *T. fetus* (p= 0.004 and 0.039, respectively).

Individual Bull Prevalence- The survey's unstratified individual bull prevalence (± sem) was 6.0 ± 0.005 % (4.9 to 7.0%, CI). Evaluation of the differences in prevalence by geographical area indicated that there were significant differences (p=0.006, Table 9). Bull age was also a significant risk factor (p< 0.0001). Bulls culture negative for *T. fetus* had a mean age of 5.0 years (range 2-15 years), whereas culture positive bulls had a mean age of 5.8 years (range 2 -12 years). Breed of bull appeared to be associated with *T. fetus* culture status (Table 10) and was evaluated for such a predilection. Surveyed bulls were initially divided into *Bos taurus, Bos indicus,* and hybrid crosses of *B. taurus and B. indicus* and a resultant logistic regression model suggested that *Bos taurus* bulls were about 3.8 times more likely to be culture positive for *T. fetus* (p = 0.09) than *Bos indicus* bulls. Breed was therefore included as a model variable.

Table 8. Mutivariable logistic regression model of herd prevalence of trichomonois in Florida beef cattle (odds ratios, p values, and confidence intervals).

Logit estimates				Number of observations		56
				F (3, 53)		3.89
				Prob > F		0.0138
Herd Status	Odds Ratio	Std Error	t	P>|t|	95% CI	
Herd size	12.78	10.68	3.04	0.004	2.39	68.27
Bull to Cow Ratio = 1: 25	3.46	4.12	1.04	0.302	0.31	37.75
Bull to Cow Ratio = 1: <25	12.82	15.50	2.11	0.039	1.13	144.54

Number of Covariate Patterns = 6; Regression Fit using Pearson X^2 (4) = 1.42; Probability > X^2 = 0.8412

Table 9. Individual bulls sampled for *T. fetus* culture status by geographical areas

	North Florida	South Florida	Totals
T. fetus culture negative bulls	348	1477	1865
T. fetus culture positive bulls	12	107	119
Prevalence (%)	3.1	6.8	6.0
Total Bulls	391	1584	1975

Multi-variable Logistic Regression Models for Individual Bull Prevalence- Herd effect was recognized as important and was specified as the primary sampling unit in the statistical model. The model was determined by a forward stepwise selection procedure. *Bos taurus* bulls were 6 times more likely to culture positive than *Bos indicus* bulls. Within the *Bos taurus* group, several breeds were more likely be culture positive for *T. fetus* (Table 11). Simmental bulls had the greatest odds (13.7, p < 0.001) when compared to the reference group, *Bos indicus*. The prevalence in Simmental bulls was 18.9% (Table 10); as a breed, they accounted for 1.9% of all bull surveyed. Charolais bulls had an odds ratio of 12.5 (p< 0.001) and a *T. fetus* prevalence of 6.8%. Angus bulls were most represented in the survey (22.9%) and had a *T. fetus* prevalence of 12.3% and an odds of infection 11.8 times that of the *Bos indicus* reference group. Other breeds were not statistically significant in the model.

Where greater than ten bulls per unit were used, there was an increased likelihood of an individual bull being *T. fetus* culture positive, and as the bull-to-cow ratio increased (lower cow numbers per bull, i.e., 1 to 15-24), the risk (OR = 2.1) for an individual bull to be culture positive also increased.

Table 10. Breed representation in the survey population of Florida beef bulls and prevalence of *T. fetus* by breed..

Breed	# of Bulls	# of Herds	% of Population	# Culture Positive	Prevalence *(%)*
Angus	454	14	23.0	56	12.3
Brangus	247	10	12.5	7	2.8
Braford	155	3	7.8	5	3.2
Beefmaster	166	4	8.4	5	3.0
Brahman	135	8	6.8	3	2.2
Charolais	235	9	11.9	16	6.8
Hereford	414	14	21.0	18	4.3
Limousin	42	3	2.1	1	2.7
Simmental	37	4	1.9	7	18.9
Other	90	17	4.6	1	1.1
Totals	1975			119	6.0

Table 11. The odds ratios, p values, and confidence intervals as determined by a multivariable logistic regression for the model of individual bull prevalence of trichomonosis in Florida beef cattle.

Survey Logistic Regression					Number of observations	1876	
PSU[8]: herd number					Number of PSU	56	
					F (7, 49)	26.88	
					Prob > F	0.000	
T.fetus. Culture Status[1]		Odds Ratio	Std. Error	t	P-value	95% CI	
Breed[2]	Angus	12.673	9.177	3.507	0.001	2.968	54.095
	Brangus	2.200	1.780	0.975	0.334	0.434	11.138
	Braford	2.650	2.634	0.981	0.331	0.361	19.425
	Beefmaster	2.994	2.224	1.477	0.145	0.675	13.267
	Charolais	13.641	8.498	4.194	0.000	3.914	40.540
	Hereford	2.788	1.292	2.214	0.031	1.101	7.058
	Limousin	3.076	3.652	0.947	0.348	0.285	33.213
	Simmental	13.413	8.734	3.987	0.000	3.637	49.465
Bulls/unit[3]		3.868	1.612	3.478	0.002	1.677	8.920
Age[4]		2.213	0.744	2.090	0.022	1.128	4.342
Bull:cow ratio[5]		2.169	0.752	2.241	0.030	1.083	4.347
Knowledge *of T. fetus.*[6]		0.317	0.116	-2.916	0.003	0.151	0.662
GeographicalArea[7]		4.430	1.869	3.238	0.001	1.901	10.321

[1]T. fetus culture status = positive or negative, [2]Breed=bull breed (Note: the reference group for bull breed is *Bos indicus* (Brahman) bulls), [3] Bulls/unit= Bulls per breeding group (<10 or ∃ 10 bulls), [4]Age = bull age (2-5 year of age, 5+years), [5]Bull:Cow = Bull to Cow ratio(<1:25 or ∃1:25), [6]Knowledge of *T. fetus = yes or no*, [7]Geographical area = North or South Florida, [8]PSU=Primary sampling unit.

Bulls over age 5 were 3.8 times more likely to have trichomonosis when compared to bulls 5 years or under, where all other factors were held constant ($p < 0.02$). An evaluation of age by culture status, sorted by geographical area (Table 12) indicated that the mean bull age was younger in South Florida where the prevalence of trichomonosis was significantly higher (prevalence of 6.8% and 2.2 times more likely to be culture positive for *T. fetus* compared to North Florida's estimated 3.1% prevalence). Bull age, although a significant ($p<0.0001$) risk

factor for infection with *T. fetus*, was also very likely influenced by the prevalence of disease in the population.

Table 12. Mean bull age by *T. fetus* culture status, the geographical areas of the survey and the herd case reviewed.

Geographical area	Culture Status	Observations	Mean Age (years)	p-value
North Florida	Negative	379	5.4	
	Positive	12	7.7	0.0009
South Florida	Negative	1477	4.9	
	Positive	107	5.6	0.0007
The case review	Negative	1224	3.9	
	Positive	165	5.5	0.001

Owner knowledge of trichomonosis was associated with *T. fetus* culture status in a bull within the herd; owners/agents with knowledge of the disease were about 70% less likely to have the disease in their herd. Herd owner/agents without knowledge of the disease were about 3.3 times more likely to have a bull with trichomonosis, all other factors being constant. A bull in South Florida was 4 times more likely to be culture positive than a bull from North Florida. Herd size was not statistically significant in the individual bull prevalence model.

Discussion

The within herd prevalence of *T. fetus* in the case review was between 0 and 35.9%, (ranch-wide prevalence, 11.9%) which is greater than, but similar statistically to the prevalence reported in the statewide survey (30.4% herd prevalence and a 6.0% individual bull prevalence) and in bull populations of other studies (range, <1 to 38.5%). Three such reports describe bulls tested in livestock markets or abattoirs; prevalence in these populations was between 0.2 and 7.8% (7,10,17). In 1979, Abbitt and Meyerholz (10) reported a 7.3% prevalence, where 8 of 109 cull Florida beef bulls were culture positive for *T. fetus*. A comparison of the prevalence proportions between the 1979 survey and the current survey suggests that there is not an apparent change in prevalence of trichomonosis from 1979 to 1998 in the state of Florida. The 1979 survey, however, does not report herd of origin and is thus difficult to make direct correlations. Two studies reported herd investigations involving compromised reproductive performance in cows bred by infected bulls. Skirrow et al (18) reported a 38.5% prevalence of *T. fetus* infection in 195 beef bulls. Kimsey et al. (19) reported a prevalence of 5.8% in 328 bulls. In a survey of 729 bulls in 57 herds in California, the prevalence of infection in sampled bulls was 4.1% (20); of herds surveyed, 15.4% had at least 1 infected bull and a within herd prevalence from 4.0 to 38.5%. These studies validate the need for epidemiological investigation to identify the risk factors associated with disease and that would assist in improved prevention and control. A principal strength of the current survey was the scope of the study. Sampling herds throughout the state gave a broad representation of risk factors and the large number of observations improved the likelihood that the population sample truly represented the whole population.

Individual Bull Prevalence- The mean age of all bulls in the case review was 4.1 years, the mean age of *T. fetus* infected bulls was 5.5 years. This finding agrees with other reports of a predilection for colonization in older bulls (i.e., >4 years of age; 2,13,21). The survey model also reaffirms bull age as a factor; as bull age increased beyond 4 years, the likelihood of having a culture positive result increased four times. Older bulls may have deeper and more numerous crypts on the mucosal surface of the prepuce than younger bulls, providing a more conducive environment for development of the permanent carrier state

which increases the probability of transfer of the organism (1,5). Use of young bulls in *T. fetus* infected herds has been a management tool to control losses associated with the disease. Older bulls are more likely to become infected and remain so, but infection is clearly possible in younger bulls (18), perhaps associated with increased herd prevalence of disease. In the case review, only 17 of 165 infected bulls were less than 4 years of age (1.8%, 5.8% and 14.3% of 2-, 3- and 4-year-old bulls, respectively).

The highest prevalence of *T. fetus* infection by breed in the case review was in Brangus bulls (17.9%) and lowest prevalence was in Braford bulls (2.5%), both *Bos taurus* x *B indicus* breeds. In the survey, *Bos taurus* bulls were 6 times more likely to culture positive than *Bos indicus* bulls. Within the *Bos taurus* group, several breeds were more likely be culture positive for *T. fetus*, Simmental, Charolais and Angus. Simmental bulls had the greatest odds ratios (13.7, $p< 0.001$) when compared to the reference group *(Bos indicus)*. The *T. fetus* prevalence in Simmental bulls was 18.9%, while accounting for only 1.9% of all bulls surveyed. Charolais bulls had an odds ratio of 12.5 ($p< 0.001$) and a *T. fetus* prevalence of 6.8%. Angus bulls had a *T. fetus* prevalence of 12.3%, an odds ratio of 11.8 times greater than the *Bos indicus* reference group, representing 22.9% of the bulls surveyed. Other breeds were not significant in the model. Several studies have examined breed predilections, but a clear association has not been reported (10,13). BonDurant et al. (13) showed a greater prevalence among *Bos taurus* breeds than *B indicus* or crossbred bulls, but could not attribute biological significance to the finding because of a lack of equal distribution of breeds across all herds. Similarly, in the case review and the survey, there was not an equal distribution of breeds across breeding units/herds, and additionally, information regarding source of bulls was not available to help clarify the reason for the breed differences observed. Breed related anatomical differences could contribute to a greater prevalence of infection in one breed than another. As described for the age association, one breed or breed-type may develop a preputial environment more conducive to establishment and maintenance of colonization than another. Further investigations are warranted to delineate breed association with *T. fetus* infection.

Owner familiarity with trichomonosis seemed to be associated with a reduced risk of disease in the individual bull. Owners/agents in the survey with knowledge of the disease were about 70% less likely to have the disease in their bulls and conversely, those without knowledge of the disease were about 3.3 times more likely to have an individual bull with trichomonosis, all other factors held constant. In the survey results, the probability of an individual bull being culture positive in North Florida decreased from 6.9% to 3.7% when owners had knowledge of the disease. In South Florida, where the disease was more prevalent, the predicted probability of individual bulls being *T. fetus* positive where owners had no knowledge of trichomonosis was 19.9%, in contrast to 5.4% where they had knowledge of the disease. Owner knowledge of the disease had a protective effect. Education concerning this disease would likely aid in its control.

Bulls per breeding unit and the bull-to-cow ratio are each management factors that could affect exposure to *T. fetus*. Where owners used greater than ten bulls per unit, the likelihood that an individual bull would have a culture positive *T. fetus* status increased (OR=3.8). As the ratio of bull-to-cows increased (lower cow numbers per bull), the risk (OR = 2.1) for an individual bull to be culture positive for trichomonosis increased. These practices were used by producers to increase conception rates in large operations where cow density per acre was low and acreage was large. These practices seem to lend themselves to increasing the within herd prevalence of the disease. It is assumed that each of these practices increased the number of potential sexual contacts and thereby, increased the probability that an individual bull might be positive or become positive.

Geographical differences in prevalence were noted. An individual bull in South Florida was 4 times more likely to have a *T. fetus* culture positive result than a bull from North Florida. Differences in management and herd dynamics were presumed factors. Herd size, however, was not statistically significant in the individual bull prevalence model.

Herd Prevalence- Evaluation of herd prevalence indicated that there were significant levels of trichomonosis in Florida beef cattle herds. The results suggest that herds with 500 or more breeding-age females, which are common to South Florida, are at significant risk of having trichomonosis. The herd and cattle density in South Florida makes *T. fetus* more of a threat to the South Florida beef industry.

One objective of the survey was to evaluate production data for effects that a *T. fetus* positive bull or herd status might have on production outcomes. The weaning weights and adjusted weaning weights collected as a result of the survey did not indicate that there was a significant differences regardless of herd status for trichomonosis ($p< 0.87$). Unfortunately, weights could not be adjusted to day of age at weaning, which would have better reflected differences. Only a few operations had production data summarized, (4 of 56 herds); all were *T. fetus* negative. Weaning percentage (number of calves weaned per exposed females) analysis, as reported, indicated that there were no significant differences by herd status ($p< 0.27$). The long breeding seasons common to Florida allows for compensatory increases in weaning rate, so, decreases associated with *T. fetus* exposure, may be difficult to recognize. Clark et al (2) reported herd calving percentages of 30% for heifers infected with *T. fetus* during their first bull exposure, and prolonged calving intervals that were significantly different from those of non-infected heifers; overall, calf production was reduced by 18%. Skirrow et al (18) recorded an increase in pregnancy percentage from 74% to 85% following resolution of infection in a herd infected with *T. fetus*. In the case reviewed here, pregnancy percentage and mean weaning weight did not have a significant association with prevalence of infection in herd bulls. The lack of association with pregnancy percentage may have resulted from either inaccuracies in pregnancy examination, or cow infection early in the 90 day breeding season, which cleared, permitting a pregnancy to be established, so that, only the time-to-conception within the breeding season was affected. Mean weaning weight was clearly not an appropriate measurement, because calves on different units were weaned at different times. The adjusted mean weaning weight provided a time-referenced value across units. Significant association was observed for the measures of weaning percentage, mean weaning weight per exposed cow and adjusted weaning weight per exposed cow with prevalence of infection of bulls in breeding units. The use of mean weaning weight and adjusted mean weaning weight per exposed female measured the combined influence of conception and time-to-conception within the breeding season. That is, as a result of being infected with *T. fetus* during the breeding season, cows that conceived late in the breeding season, or not at all, contributed a lesser proportion to this measure due to a failed conception or a delay in conception and depressed weaning weight, all other factors being equal. The regression model for prevalence of infection in bulls and the production measures of cows in the case review showed a linear, inverse relationship. The authors acknowledge that limitations existed in the accuracy of production data in the study herd because of the nature and size of the enterprise.

The effect of vaccination on herd status and within herd prevalence was another important focus of the survey. The low numbers of surveyed herds with a history of vaccination made statistical significance an improbability. Only 10 of the 56 herds had used any vaccination protocol. Of these, only two administered the vaccine as per manufacturer's recommendations. Of the ten herds that had a history of at least one vaccination five herds were positive for *T. fetus*. The mean within herd prevalence of the 12 culture positive but

unvaccinated herds was 13%, whereas, the mean within herd prevalence of the 5 culture positive, vaccinated herds was 8% ($p>0.10$). Further investigation is needed.

Survey Limitations- The primary limitation of this study was the inability to obtain a true random sample. Surveys are a convenience sampling because they depend on an acceptance or willingness to participate that is often out of the control of the investigator. An effort was made by personal contact to encourage the prompt return of the questionnaire and a commitment to present bulls for sampling. Multiple dates and opportunities were provided. There was also a potential for geographical bias. The inherent regional differences within the State may have influenced the level of knowledge of disease and owners perception of the disease's importance. The direction of the bias was likely to overestimate the overall herd prevalence, since those with some knowledge of or concern for disease were more likely to agree to present their animals for sampling. Another important limitation was an apparent lack of accuracy relative to herd production data. Many producers did not have production records and were unable to accurately estimate production parameters; particularly, weaning numbers, exposed females, pregnancy percentages and weaning weights. It is suspected that this would impose a bias to lessen the expected differences in weaning rates and weaning weights of trichomonnosis infected herds compared to those without the disease. As a result no clear outcome effect was evident from the survey data.

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