REVIEW PAPER

Brucellosis in domestic water buffalo (*Bubalus bubalis*) of Trinidad and Tobago with comparative epidemiology to cattle

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Abstract

The water buffalo is an important domestic animal worldwide and the local Buffalypso variety was developed in Trinidad to have improved beef qualities. Brucellosis was diagnosed in Trinidad and Tobago during 1998 in both cattle and domestic water buffalo (*Bubalus bubalis*) populations. Brucellosis in the latter species is caused by infection with *Brucella abortus,* similar to bovine brucellosis. Control of brucellosis is of paramount importance to preservation of the genetic diversity of these animals in Trinidad, and this has been complicated by differences in the epidemiology of water buffalo and bovine brucellosis. Some diagnostic tests do not have comparable accuracy between the two species, and the RB51 vaccine does not adequately protect against infection in water buffalo. The water buffalo in Trinidad may also be more resistant to infection than cattle. Development of effective vaccination protocols is key to control disease in Buffalypso in Trinidad, and prohibitions on import of virulent *B. abortus* strains for vaccine efficacy studies has impeded progress in this area. These Trinidadian strains are of variable virulence; some might be effective for challenge in vaccine efficacy studies, while other, of lower virulence, may be vaccine candidates for use in water buffalo.

Keywords: Brucellosis, Buffalypso, Cattle, Domestic water buffalo, Epidemiology, Trinidad and Tobago

Introduction

Brucellosis in domestic water buffalo (*Bubalus bubalis*) is generally caused by infection with *Brucella abortus* (Mohan, 1968). Brucellosis is primarily a reproductive disease of cattle, characterized by late-term abortions, retained placentas, epididymitis, and orchitis (Nicoletti, 2001). Abortions in water buffalo infected with *B. abortus* have also been reported (Mathur, 1964). *Brucella*-related abortions usually occur after five months gestation (Polding, 1947), as in cattle, but have also been reported earlier in gestation (Das et al., 1990). Water buffalo herds can also be infected without subsequent abortions (Nicoletti, 1992). Mohan (1968) reviewed the early literature on brucellosis in domestic water buffalo.

The epidemiology of *Brucella* infection has not been studied extensively in domestic water buffalo. These animals shed viable brucellae in milk, but dam-to-calf transmission has not been evaluated directly. However, buffalo calves born to seropositive dams on an infected farm are more likely to become seropositive themselves compared to calves born to seronegative dams (Akhtar and Mirza, 1995). Infected water buffalo expel the bacterium during abortion, and this may serve as a source of infection for herdmates. Experimental studies have demonstrated that ingestion of virulent *B. abortus* causes infection in female water buffalo (Mohan, 1968). Commingling of many animals in a small area facilitates disease transmission, and congregation of water buffalo in wallows may be an important factor for spread of brucellosis (Polding, 1947).

The level of natural genetic resistance, in addition to behavior and management factors, affects the spread of disease in populations. The natural, resistance-associated macrophage protein 1 gene (*Nramp1*) is associated with resistance to brucellosis in cattle (Adams and Templeton,

1998). *Nramp1* alleles A and B have been identified in water buffalo, and there is evidence that the *Nramp1BB* genotype is protective (Borriello et al., 2006). The prevalence of this genotype would therefore be expected to affect the incidence of brucellosis

Trinidad and Tobago, the most southern country in the Caribbean region, was classified "free of bovine brucellosis" by the Office International des Epizooties (OIE) in 1984 (Blajan and Melendez, 1984), and these two Caribbean islands did not have another confirmed case until brucellosis was detected serologically in 1998 and by bacteriologic culture in 1999 (Fosgate et al., 2002a). No other *Brucella* species are known to exist on the islands, and recognition of *Brucella*-infected farms in Trinidad necessitated adoption of a control program, which incorporated limited vaccination with *B. abortus* strain RB51 and test and slaughter. Trinidad and Tobago used the buffered plate agglutination test (BPAT) as the official screening procedure, with competitive ELISA (c-ELISA) for confirmation. These tests have been studied in cattle (Samartino et al., 1999; Stemshorn, 1984) and subsequently evaluated for use in water buffalo (Fosgate et al., 2002b, 2003a).

Water buffalo production in Trinidad and Tobago is distributed among a few large farms and several hundred small holdings located mainly in the sugarcane growing areas of the country (Rastogi et al., 2005). Water buffalo are typically raised under extensive management conditions and farmers, excluding the large farms, generally own only 2 -- 5 head. In Tobago, water buffalo production is limited, with a total population of approximately 200 head (Rastogi et al., 2005), while in Trinidad, the population is estimated to be 5000 animals (Bennett et al., 2007). During

the early years of the brucellosis control program, three large water buffalo producers sold all their stock and closed operations (Rastogi et al., 2005).

Water buffalo production in Trinidad

Historical perspective

Domestic water buffalo (*Bubalus bubalis*) belong to the Class: Mammalia, Subclass: Ungulata, Order: Artiodactyla, Suborder: Ruminantia, Family: Bovidae, Subfamily: Bovinae, Tribe: Bovini, which includes the following three groups; Bovina (cattle), Bubalina (Asian buffalo) and Syncerina (African buffalo). The two general types of domestic water buffalo are the swamp type, with 48 chromosomes, and the river type, with 50 (Borghese and Mazzi, 2005; Mason, 1974). Both water buffalo types are descendents of the Asian wild buffalo (*Bubalus arnee*) and interbreeding yields fertile offspring with 49 chromosomes (Bhat, 1992). Swamp buffalo can be found in the Philippines west to India, and the distribution of the river type extends from India west into Egypt and Europe (Mahadevan, 1992). Swamp type buffalo are predominantly draught animals, but are often slaughtered for meat when no longer productive work animals. Milk production is generally poor in swamp buffalo. River type buffalo are primarily dairy animals but males are often raised for meat and to provide draught power. Another difference is that river buffaloes prefer clear water in which to wallow, whereas swamp buffaloes are often raised in marshy land (Borghese and Mazzi, 2005).

Buffaloes are not indigenous to the Caribbean, and they were initially imported for use as draught animals on sugar plantations (Mahadevan, 1974). The earliest record of importation is of 30 Jaffarabadi breed buffaloes from India between 1900 and 1905 to replace *Bos indicus* cattle

that worked on the plantations (Bennett et al., 2007). Other importations followed, the most recent being 6 purebred Murrah bulls in 1948 (Caroni Limited, 1971).

Descriptive epidemiology of brucellosis on Trinidad.

Brucellosis was first diagnosed in Trinidad and Tobago in 1998 when a Holstein-cross cow that suffered a late-term abortion was serologically positive. The Animal Health Division of the Ministry of Agriculture, Lands and Marine Resources (MALMR) has not developed a policy document for eradication of brucellosis, but control strategies have been implemented. Between 1998 and 2001, MALMR instituted a nationwide testing program. Commercial dairy animals were screened via a milk ring test (MRT) and positive MRT were followed-up by BPAT testing of serum. Large beef herds were screened via BPAT. A test-and-slaughter program was instituted, allowing limited use of a commercial *B. abortus* strain RB51 vaccine. Complete herd vaccination was instituted in one cattle and two domestic water buffalo herds that had seroprevalences >20%. Abattoir surveillance was implemented, but was discontinued after 1999; most seroreactors had been sent to slaughter specifically due to seropositivity, and there were difficulties in performing trace-back investigations due to lack of a national animal identification program.

Test and slaughter effectively reduced the number of premises from 56 in 1999 to 2 in 2002, both of which were water buffalo herds. The policy was adopted on most farms due to the relatively low within-herd animal level seroprevalence (<7%). The single beef cattle farm with a high seroprevalence (27% in 1998) was closed in 2000. Depopulation was not possible in two high-prevalence buffalo herds (60% and 26% in 1999) for economic reasons and potential loss of

genetic variability. In these 2 farms, an RB51 vaccination program focused on calfhood immunization was instituted in addition to test and slaughter. Based on annual reports, ~ 2700 water buffalo were slaughtered due to positive brucellosis status from 1998 to 2008 (MALMR, 1999-2009), when the most recent estimate for the total population was only 5000 animals (Bennett et al., 2007).

During 1998, 4245 livestock (predominantly cattle and water buffalo) were screened for brucellosis using the BPAT and 293 (7%) were serologically positive. Two hundred thirty-nine water buffalo were tested this first year and 17% (41/239) were BPAT positive, which was a significantly higher prevalence than in all other species combined (6%; 252/4006). The proportion of seropositive water buffalo did not decrease over the first four years of the control program (**Fig 1**). A similar trend was observed for farm-level prevalences (**Fig 2**). The majority of water buffalo, however, originated from the two large farms that had high seroprevalences. Two of the large infected cattle herds eliminated new infections during the first four years of the control program (**Fig 3**). One of the other infected farms was closed in 2000. In the remaining infected cattle, herd control was complicated because it housed the only national slaughter facility that handled brucellosis-positive livestock. Successful reduction in new infections in infected water buffalo farms was not achieved until several years later (**Fig 4)**. The large, brucellosis-affected farms tended to be in the traditional sugarcane production regions in southern Trinidad (**Fig 5**).

Brucellosis-positive farms unable to eliminate new infections have been depopulated and closed. A single large brucellosis-positive water buffalo farm (approximately 1200 head) managed by MALMR remains in operation in southeastern Trinidad at the time of this review.

Established research programs

Epidemiology

Diagnostic investigations

The Animal Health Division of MALMR implemented a livestock screening program for brucellosis immediately after recognition during 1998. The BPAT, with positive results confirmed by a complement fixation assay, was chosen as the screening test. In 2000, a competitive ELISA (c-ELISA), based on the work of Nielsen et al. (1994), was implemented for confirmation. Water buffalo were determined to have higher seroprevalence than cattle, and there was a higher proportion of buffalo that were BPAT positive but c-ELISA negative compared to cattle. Seropositive cattle also tended to yield viable *B. abortus* from sampled lymph nodes more frequently than water buffalo (Fosgate et al., 2002a).

Subsequent test validations determined that there were species differences in the accuracy of traditional brucellosis agglutination tests between cattle and water buffalo (Fosgate et al., 2002b). The tests evaluated were BPAT, card agglutination, standard plate agglutination, and standard tube agglutination. The BPAT had greater sensitivity (96.3% versus 88.1%) but lower specificity (90.7% versus 98.1%) in water buffalo compared to cattle, perhaps partially explaining the anecdotal observation that water buffalo had higher BPAT seroprevalences than cattle but also had a higher proportion subsequently confirmed negative by follow-up testing.

The card agglutination test also had higher sensitivity (90.4% versus 72.6%) in water buffalo than in cattle. BPAT was determined to be the best of the four brucellosis screening tests for use in cattle and water buffalo in Trinidad, due to its higher sensitivity.

The effectiveness of the c-ELISA for confirmation of BPAT-positive cattle and water buffalo was also investigated (Fosgate et al., 2003a). The sensitivity and specificity of the c-ELISA at the usual cutoff of 30% inhibition were 91.4% and 95.4% for water buffalo and 83.9% and 92.6% for cattle. The Youden index (sensitivity + specificity – 1) peaked at a 35% cutoff, and this yielded specificity estimates of 97.4% and 96.2% in water buffalo and cattle, respectively. Therefore, a cutoff of 35% might be more appropriate to confirm positive BPAT results in water buffalo and cattle in Trinidad. Despite the apparent differences in point estimates, overall accuracy of the c-ELISA was the same in water buffalo and cattle, based on comparison of the area under the receiver-operating characteristics curve (0.979 versus 0.935, respectively). The appropriateness of the 35% inhibition cutoff was confirmed in a subsequent analysis (Fosgate et al., 2006b), documenting that the result category of 0.25 – 0.349 had the least amount of influence on updating the prior probability of brucellosis. Test categories above 0.35 proportion inhibition, however, had a greater effect on modification of the prior probability of infection, suggesting more clinical usefulness. The likelihood ratio for the 35% – 49.9% c-ELISA category was estimated to be 3.22 (water buffalo and cattle with results within this category are 3.22 times more likely to have brucellosis).

4.1.2. Field trials

The Animal Health Division of the Ministry of Agriculture, Lands and Marine Resources (MALMR) in 1999 decided to allow limited vaccination with RB51 in herds with high

seroprevalences in effort to preserve the genetic potential of the local water buffalo population. Studies on the effectiveness of RB51 for the prevention of brucellosis in domestic water buffalo were not found in the English language literature and were required to aid in control program design. RB51 at the standard calfhood dose recommended in cattle ($1.0 – 3.4 \times 10^{10}$ colony-forming units) did not protect water buffalo from infection with *B. abortus* under natural exposure conditions (Fosgate et al., 2003b). Vaccination protocols required evaluation in the local water buffalo populations, but standard *B. abortus* challenge strains (e.g. strain 2308) were not available for efficacy studies due to import restrictions.

Field studies for evaluation of vaccination protocols have disadvantages due to long study durations, losses to follow-up, and potentially unequal *B. abortus* exposure across study groups. For example, brucellosis-free water buffalo introduced to an infected herd did not seroconvert after 6 months of natural exposure (Diptee et al., 2007; Fosgate et al., 2003b). There are also difficulties in maintaining identification in water buffalo managed extensively (Fosgate et al., 2006a). Within an infected water buffalo farm in Trinidad, the median ear-tag retention was only 272 days and the rate of loss was 0.0024 per day. It was estimated that, with use of ear tags exclusively for identification (one each in left and right ears), only 21% of water buffalo would be positively identified after a 2-year study period.

Experimental studies

4.2.1. Isolate evaluations

Eighty-six strains of *B. abortus* isolated from lymph nodes of apparently healthy seropositive water buffalo (animals = 9; isolates = 17) and cattle (n = 8; 14), skin lesions (n = 9; 9) and

aborted tissues (n = 16; 46) of water buffalo in Trinidad and Tobago were characterized as to their phenotypic features, phage types and resistance to antimicrobial agents (Adesiyun et al., 2011a). All evaluated *B. abortus* strains were biotype 1 and grew in the presence of penicillin G, i-erythritol and basic fuchsin but none grew in the presence of thionine blue. All were susceptible to bacteriophages TB and BK2 but 95% (82/86) were lysed by bacteriophage Wb. All isolates were previously confirmed as biotype 1 and therefore would be expected to be lysed by all evaluated bacteriophages. The 4 (4.5%) isolates that were resistant to bacteriophage Wb were isolated from 3 water buffalo and 1 cattle and could reflect problems with the lytic activity of the bacteriophage or the presence of atypical biotypes in Trinidad.

Of 8 antimicrobial agents tested by the disc diffusion method, all 86 isolates of *B. abortus* exhibited resistance to one or more of the evaluated antimicrobial agents. Resistance was high to azithromycin (100%; 86/86), sulphamethoxazole/trimethoprim (99%; 85/86) and moxifloxacin (80%; 69/86) and low to streptomycin (7%; 6/86), tetracycline (1%; 1/86) and doxycycline (1%; 1/86). A previous study of isolates recovered from humans and animal products reported susceptibility to fluoroquinolones (ciprofloxacin and monifloxacin) and tetracycline but resistance to rifampicin, streptomycin and sulphamethoxazole/trimethoprim (SXT) (Lopez-Merino, 2004). Resistance to rifampicin in *B. abortus* has been reported by others Baykam, 2004; Turkmani, 2006) and rifampicin-resistant strains of other bacteria have been associated with lower virulence (Moorman, 1981). All evaluated isolates were resistant to azithromycin even though this macrolide is not used in veterinary practice in Trinidad and Tobago and it has been shown to be effective against *B. abortus* in studies performed elsewhere. The prevalence of resistance phenotypes did not vary between isolates recovered from water buffalo and cattle

despite the fact that water buffalo in Trinidad tend to be more extensively managed than cattle and are seldom, if ever, exposed to antimicrobial agents. The prevalence of resistant phenotypes likely represents an intrinsic characteristic of local *B. abortus* strains rather than selection by drug administration.

Mouse infection models

Nineteen strains of *B. abortus* isolated from lymph nodes of apparently healthy water buffalo (n = 6) and cattle (n = 7), and from skin lesions (n = 3) and aborted tissues (n = 3) of water buffalo in Trinidad and Tobago have been evaluated for virulence in BALB/cByJ mice (Adesiyun et al., 2011b). Mice experimentally inoculated with *B. abortus* strain 19 (S19) and strain RB51 were the basis of comparison, and virulence was based upon the number of colony forming units of *B. abortus* recovered from each gram of splenic tissue, the spleen:weight ratio, and histopathological severity scores. The last was calculated as the sum of individual scores for lymphoid depletion, lymphoid necrosis, splenitis, and macrophage accumulation. Virulence measures were quite variable among evaluated isolates but colony forming units of recovered *B. abortus* per gram of splenic tissue, spleen:weight ratio, and lymphoid depletion were significantly lower for isolates from water buffalo than for those from cattle. An overall virulence score assigned to each isolate was used to identify isolates that had significantly higher virulence than S19, but this evaluation was used to aid in future studies since data are not available in the peer-reviewed publications. This study demonstrated that a mouse model could be used to compare virulence of isolates from different sources and potentially identify virulent challenge strains for future evaluations. The observed heterogeneity in virulence also suggests

that some isolates could be candidates for the development of new vaccines for protection of water buffalo.

Ruminant studies

Resistance to *B. abortus* infection of water buffalo and cattle

Cattle and water buffalo calves aged 3-6 months (n = 7 each) were inoculated intraconjunctivally with counts ranging from 1.5 x 10^7 to 1.7 x 10^{10} colony forming units (cfu) of a local *B. abortus* strain (Adesiyun et al., 2010). Animals were monitored over an 8-week period for clinical manifestations and serological and hematological evidence of *B. abortus* infection. At slaughter, lymph nodes were collected for bacteriological and histopathological examination. Overall, 2 (29%) of 7 water buffalo and 3 (36%) of 7 cattle seroconverted as detected by either BPAT or c-ELISA. Only the 1.7 x 10^{10} cfu dose caused seroconversion of water buffalo (2/2 animals), while in cattle, both 1.8 x 10^9 cfu (1/2 animals) and 1.7 x 10^{10} cfu (2/2 animals) caused seroconversion. The 2 seropositive water buffalo were both BPAT-positive but c-ELISA-negative, while all 3 cattle that seroconverted were positive by BPAT and c-ELISA. The number of culture-positive lymph nodes was statistically significantly greater in cattle than in water buffalo. In both species, seropositive animals were lymph node culture-positive. In addition, 1 seronegative water buffalo and 2 seronegative cattle also had viable *B. abortus* recovered from sampled lymph nodes. Histopathological lesions in lymph nodes varied between water buffalo and cattle. Hematological parameters were not different between species.

The small numbers of water buffalo and cattle that seroconverted, coupled with the low numbers of brucellae recovered from sampled lymph nodes and the absence of significant dose-related

hemotological changes, are indications that the virulence of the local strain is comparatively lower than reported for standard challenge strain 2308.

Based on seropositivity in inoculated animals and recovery proportions from sampled lymph nodes (reflective of more rapid clearance of brucellae or localized infection), water buffalo appear to be more resistant than cattle to infection by the evaluated local strain.

Evaluation of RB51 vaccine in domestic water buffalo

B. abortus RB51 vaccine does not induce in water buffalo serum antibodies that react in the BPAT and c-ELISA (Diptee et al., 2006, 2007; Fosgate et al., 2003b). Both complement fixation (CF) and dot-blot ELISA can be used to monitor antibody response to RB51 vaccination in water buffalo, but CF has higher sensitivity. The sensitivity and specificity at 12 weeks post-vaccination for the CF test were 92% and 100%, respectively, and for the dot-blot assay were 91% and 95%, respectively. RB51 is cleared from vaccinated water buffalo calves between 6 and 12 weeks post-vaccination at the recommended dose (Diptee et al., 2005), similar to clearance in bovine calves. Lateral transmission of RB51 and environmental shedding of RB51 antigen from mucosal surfaces does not occur (Diptee et al., 2006). Eight percent (2/24) of vaccinated water buffalo are persistently infected with RB51 through the first year after inoculation. These water buffalo, however, received a larger dose than currently recommended for calfhood vaccination of cattle (Diptee et al., 2007).

Twenty-four female water buffalo calves aged 6 to 10 months were given the standard calfhood dose of RB51 vaccine at either 4 (group 1) or 18 (group 2) week intervals, with 8 unvaccinated

controls (group 3) (Ramnanan, 2010). Animals were bred, confirmed pregnant, and challenged intravenously with a local strain of *B. abortus* at approximately 180 days of pregnancy.

Animals were lost during follow-up and only 9, 7, and 5 finished the study in groups 1, 2, and 3, respectively. Vaccination did not affect the time to abortion or normal delivery of calves. It did not protect against abortion with 50% abortion proportion in vaccinates (5/9 in group1 and 3/7 in group 2) versus 40% (2/5) in the unvaccinated controls.

Conclusions and future directions

The domestic water buffalo (*Bubalus bubalis*) is an important livestock species worldwide because they are a source of high-quality protein and traction power, in addition to being able to survive on marginal land and subsist on low-quality forages. However, published water buffalo-specific research is limited because it is not an important animal in many countries that have the necessary resources to perform large projects and ensure publication of results in international journals. Brucellosis affects domestic water buffalo, but despite similarities to cattle, differences in disease epidemiology between these species are important in establishing disease control measures.

The brucellosis control program in Trinidad and Tobago was unable to identify the original source of *B. abortus*, but an appealing hypothesis is that brucellosis was endemic in water buffalo populations at the time of its recognition in 1998. Evidence for this includes high seroprevalence with no recent history of abortion storms. Furthermore, the index brucellosis case was a dairy cow raised on a facility that slaughtered water buffalo from farms subsequently identified as being infected with *B. abortus*.

During the first 10 years of the brucellosis control program, 3255 ruminants were slaughtered due to brucellosis in Trinidad and Tobago (MALMR, 1999-2009). Brucellosis control has had a devastating effect on local water buffalo numbers, and the 1200 animals in the remaining infected farm continue to be at risk of infection and culling. The current control program has been more effective in reducing brucellosis in cattle than in water buffalo. Research efforts must, therefore, focus on the differences between water buffalo and cattle in order to prevent the further loss of genetic potential.

The accuracy of various tests for brucellosis is different between water buffalo and cattle. This difference is important when designing screening and test-and-slaughter programs. The water buffalo of Trinidad and Tobago also appear to be more resistant to *B. abortus* infection than cattle, and tend to be infected with strains of lower virulence. At the time of first recognition, infected water buffalo herds had significantly higher seroprevalences than cattle, likely a result of differences in test accuracy and epidemiology. The possibility that the observed difference was due exclusively to the presence of infection in water buffalo over a longer time period cannot be excluded.

Vaccination is an important method of control in populations with a high prevalence when depopulation is not an option. Strain 19 has been used in domestic water buffalo to prevent brucellosis (Afzal et al., 2000) but systematic studies of its efficacy in this species are lacking. This vaccine is not currently an option for control programs in Trinidad and Tobago due to import restrictions. Results of a recent study in Italy suggest that a modified RB51 administration schedule will protect water buffalo from infection secondary to experimental

exposure to a challenge strain of *B. abortus* (Caporale et al., 2010). Doses were 3 times the typical calfhood dose, and might prove effective for use in Trinidad. Local isolates are also possibilities for vaccine development, based on the large variability observed in virulence, but further evaluations are required.

The survival of the local Buffalypso depends upon a concerted effort to control brucellosis. Brucellosis also threatens other water buffalo populations and the estimated 168 million buffalo in the world (Borghese and Mazzi, 2005) will benefit from research being performed in Trinidad and Tobago.

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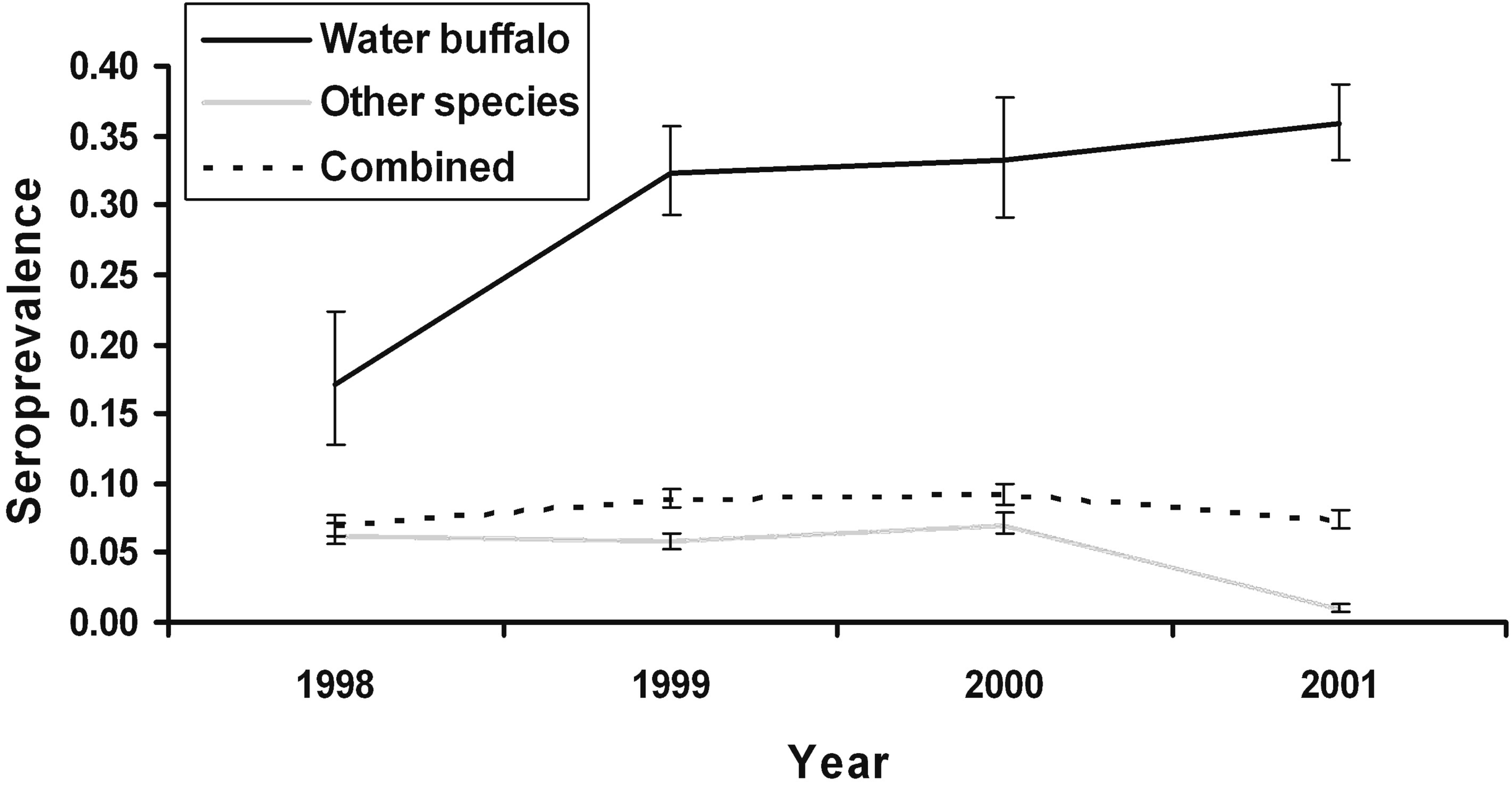
Fig 1. Animal-level buffered plate agglutination test (BPAT) seroprevalence for domestic animals in Trinidad and Tobago during the first 4 years after recognition of brucellosis. Other species were predominantly cattle but also included tests performed on sheep, goats, swine, and dogs. Error bars correspond to mid-P exact 95% confidence intervals for the prevalence.

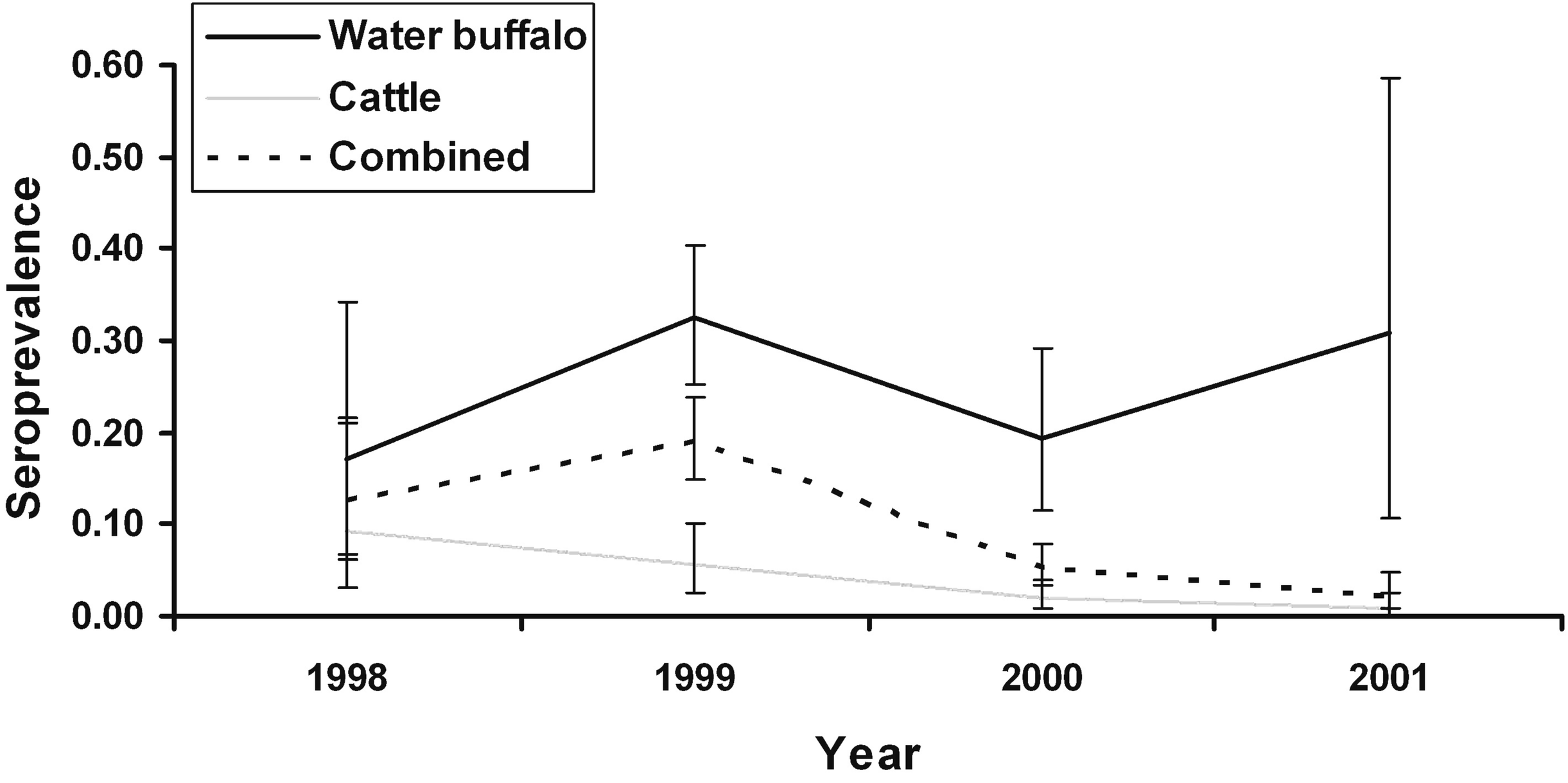
Fig 2. Herd-level buffered plate agglutination test (BPAT) seroprevalence for domestic water buffalo and cattle farms in Trinidad and Tobago during the first 4 years after recognition of brucellosis. Error bars correspond to mid-P exact 95% confidence intervals for the farm-level prevalence.

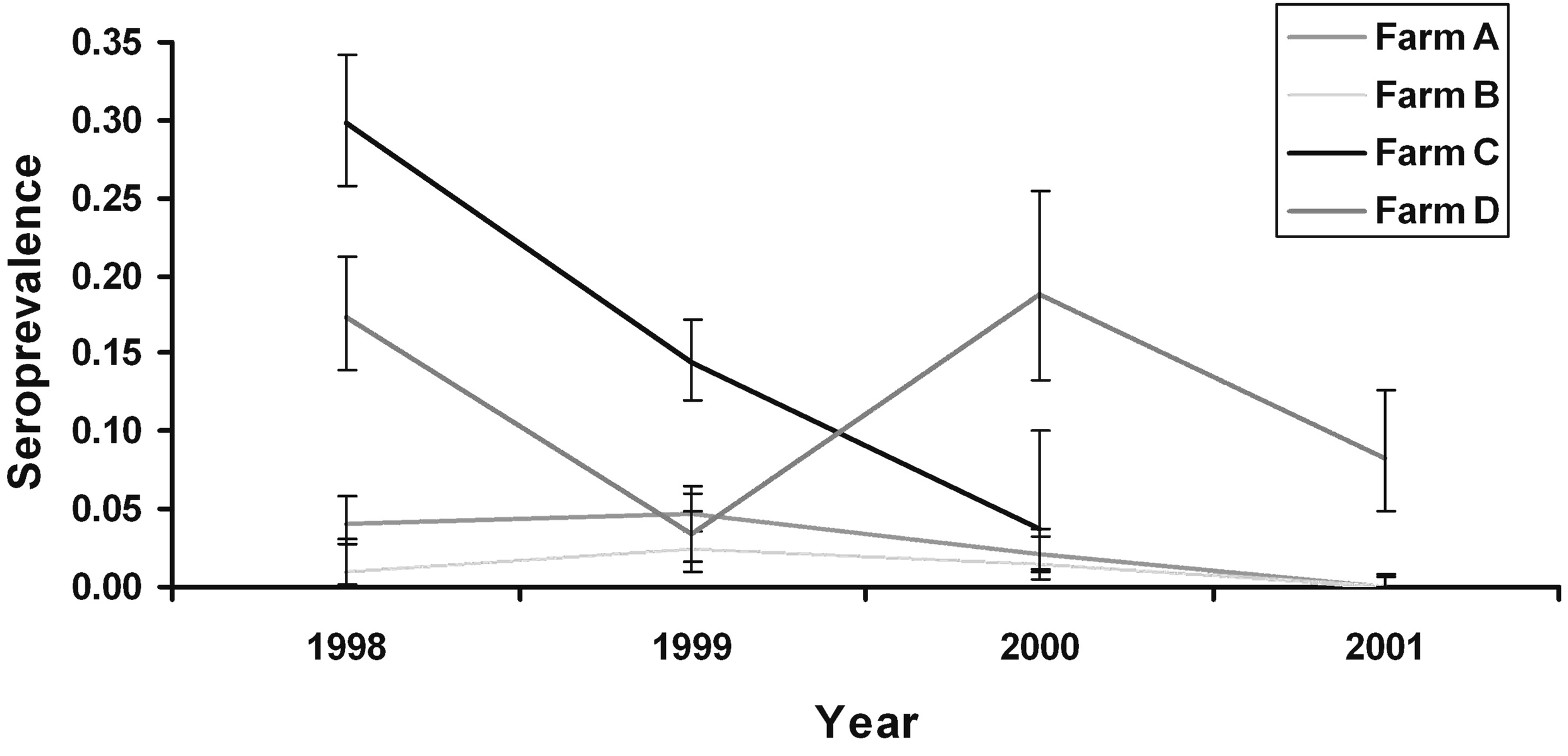
Fig 3. Animal-level buffered plate agglutination test (BPAT) seroprevalence for 4 infected cattle farms in Trinidad and Tobago during the first 4 years after recognition of brucellosis. Error bars correspond to mid-P exact 95% confidence intervals for the prevalence.

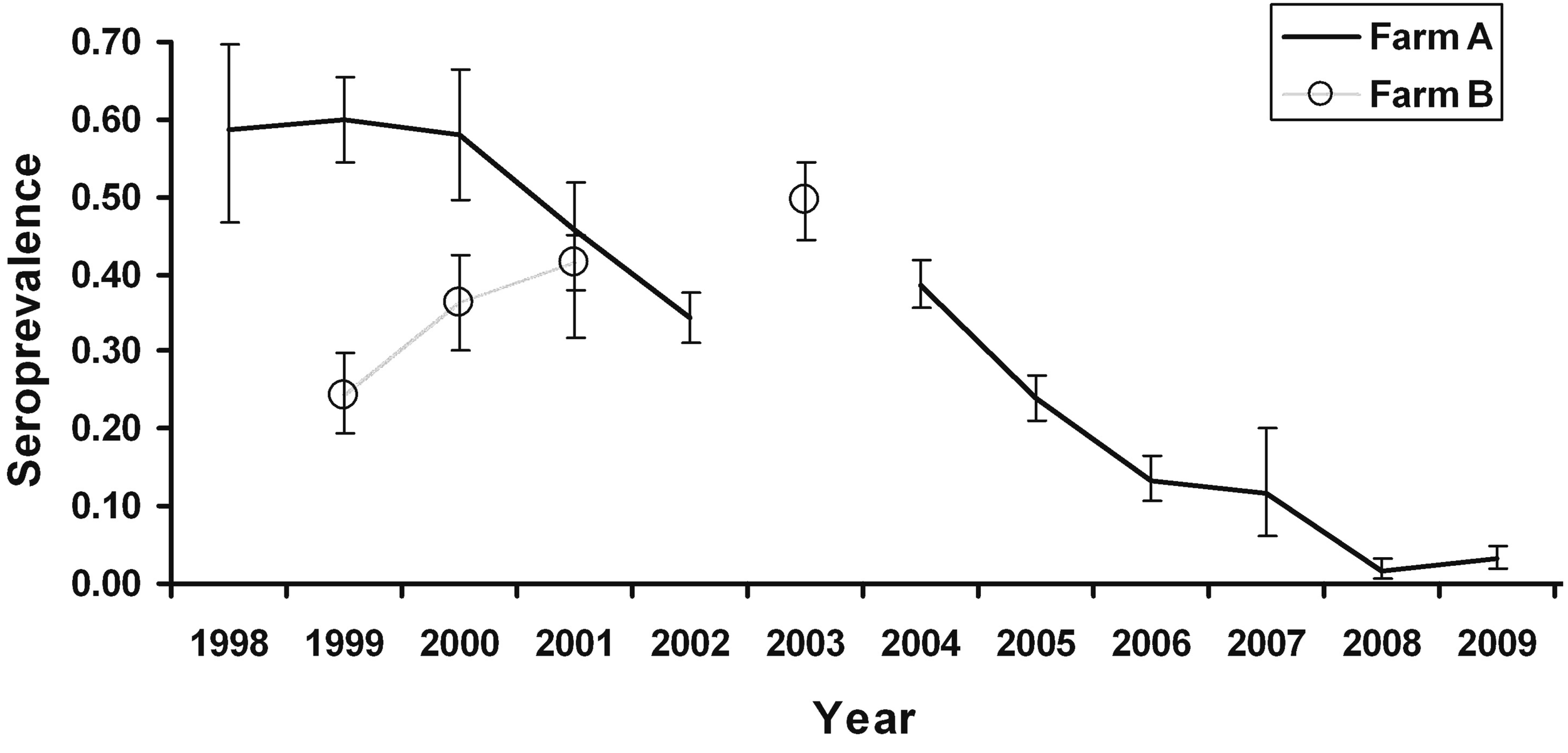
Fig 4. Animal-level buffered plate agglutination test (BPAT) seroprevalence for 2 infected water buffalo farms in Trinidad and Tobago after recognition of brucellosis. The second farm was closed in 2004 and animals were transferred to the remaining farm. Error bars correspond to mid-P exact 95% confidence intervals for the prevalence.

Fig 5. Distribution of brucellosis-affected large cattle (circles) and domestic water buffalo (triangles) farms in relationship to administrative regions of Trinidad and Tobago.


Water buffalo
Other species
Combined
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Seroprevalence
1998
1999
2000
2001
Year


Water buffalo
Cattle
Combined
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Seroprevalence
1998
1999
2000
2001
Year


Farm A
Farm B
Farm C
Farm D
Seroprevalence
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1998
1999
2000
2001
Year


Farm A
Farm B
Seroprevalence
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
Year


N
W
E
S
Caribbean Sea
Tobago
San Juan/ Laventille
Diego Martin
Venezuela
Port of Spain
Tunapuna/ Piarco
Sangre Grande
Chaguanas
Couva/ Tabaquite/ Talparo
North Atlantic Ocean
Gulf of Paria
Mayaro/ Rio Claro
San Fernando
Princes Town
Point Fortin
Siparia
Penal/ Debe
Trinidad
Kilometers
0 3.75 7.5 15 22.5 30