Virulence of *Brucella abortus* isolated from cattle and water buffalo

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Abstract

Brucellosis has been documented in domestic water buffalo (*Bubalus bubalis*) but published literature is limited despite the importance of this species in tropical agricultural systems. The objective of this study was to compare the virulence of *B. abortus* isolates recovered from cattle and water buffalo. Nineteen strains of *Brucella abortus* from cattle and domestic water buffalo in Trinidad were intraperitoneally inoculated into BALB/c mice. Spleens were cultured for *B. abortus* and a histopathological severity scores were calculated based on lymphoid depletion, lymphoid necrosis, splenitis, and macrophage accumulation. A general linear model approach was used to estimate the effect of isolate source (cattle versus water buffalo) on virulence. Isolates of water buffalo origin were significantly less virulent in the mouse model based on recovered *B. abortus* from splenic tissues, spleen:weight ratio, and lymphoid necrosis but not overall histopathological severity scores. Further investigation of isolates recovered from water buffalo might provide the key to the development of procedures for brucellosis control in tropical environments.

Introduction

Infection with *Brucella abortus* has been recognized in domestic water buffalo (*Bubalus bubalis*) of Trinidad (Fosgate et al. 2002a) and other regions of the world (Dhand et al. 2005; Samaha et al. 2008; Capparelli 2009). Reported differences in successful isolation of *B. abortus* from seropositive cattle versus water buffalo (Fosgate et al. 2002a) have been hypothesized to be due to different sensitivity and specificity of serological assays (Fosgate et al. 2002b). Alternative hypotheses include water buffalo being more resistant to infection with *Brucella abortus* (Ramnanan 2010) and differing virulence of isolates circulating within the infected populations of cattle and water buffalo. Mouse models have been used to study the virulence of *Brucella* spp. (Miyoshi et al. 2007) and the BALB/cByJ strain has been determined to be most susceptible to *B. abortus* infection (High et al. 2007). The purpose of this study was to compare the virulence of *B. abortus* isolates recovered from cattle and water buffalo.

Materials and methods

BALB/c mice aged 9 weeks were used in the study after obtaining approval from the Ethics Committee of the Faculty of Medical Sciences, University of the West Indies. A total of 19 strains of *Brucella abortus* were selected to represent all confirmed[1] isolates from cattle and domestic water buffalo in Trinidad in addition to vaccine strains 19 (S19)[2] and RB51 (SRB51).[3] The 21 strains of *B. abortus* were subcultured onto blood agar plates and growth was harvested with 1.0 ml of phosphate buffered saline (pH 7.4). Bacterial suspensions were adjusted spectrophotometrically and each of 20 mice per group was inoculated with 0.1 ml intraperitoneally. Five mice were randomly selected at 3, 6, 9 and 12 weeks post-inoculation. Each selected mouse was anaesthesized and the spleen was aseptically removed. Spleens were

macerated and serially diluted in phosphate buffered saline to determine the number of *B. abortus* per gram of splenic tissues. Portions of spleens from 2 randomly selected mice (out of the total of 5 per time period) were sampled for histopathological studies.

Splenic tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 microns, and stained using a routine haematoxylin and eosin stain. Pathology of the white and red pulp sections was scored. Lymphocyte depletion was graded as 1 for loss of lymphocyte density in at least one follicle and 2 for loss of lymphocyte density in more than one follicle. Lymphoid necrosis was graded as 1 for the presence of lymphocyte necrosis in at least one follicle and 2 for the presence of lymphocyte necrosis in more than one follicle. Splenitis was graded as: 1) neutrophils within splenic tissue or within splenic capsule, 2) neutrophils within splenic tissue and within splenic capsule, 3) granulomas present with or without neutrophils within splenic tissue and capsule, and 4/5) severe manifestations of 1-3. Macrophage accumulation around follicles was graded as 0-5 for normal to severe accumulation.

Data were described by calculating medians, ranges, means, and standard deviations. The number of colony forming units (CFU) per gram of splenic tissue was $\log_{10}$ transformed for presentation and statistical analysis. A histopathological severity score was calculated as the sum of the individual scores for lymphoid depletion, lymphoid necrosis, splenitis, and macrophage accumulation and natural log transformed for statistical analysis. A general linear model approach was used to estimate the effect of isolate source (cattle versus water buffalo) while adjusting for the effects of inoculating dose, week of sampling, and whether or not the isolate was recovered from an animal with clinical signs of brucellosis. Evaluated outcomes included CFU per gram of splenic tissue, spleen:weight ratio, histopathological severity score, and inoculating dose. Mann-Whitney U tests were used to estimate the effect of isolate source

on individual histopathological lesion scores. Statistical analyses were performed in commercially available software[4] and interpreted at the 5% level of significance.

Results

Thirteen isolates of water buffalo origin and 6 isolates from cattle were evaluated in addition to the 2 vaccine strains. Three of the evaluated isolates from water buffalo were recovered from aborted tissues and 3 others from skin lesions of naturally infected animals. All remaining isolates (including all those from cattle) were recovered from apparently healthy seropositive animals sampled at slaughter. Animals yielding isolates originated from 2 herds each of cattle and water buffalo. However, 3 of the herds were originally managed by the same corporation and the other cattle farm was the location of the only water buffalo abattoir in Trinidad. All cattle isolates and 1 isolate from an apparently health water buffalo were recovered in 1999. Three isolates from apparently healthy water buffalo were isolated in 2001 and the remaining was recovered over the subsequent years.

The CFU/gram of splenic tissue, spleen:weight ratio, and lymphoid necrosis scores were significantly lower for isolates from water buffalo (**Table 1**). Descriptively, the CFU/gram did not change over time for isolates from water buffalo compared to isolates originating from cattle (**Fig. 1**). Spectrophotometrical adjustment of bacterial suspensions was not precise and inoculating dose varied by isolate but the mean dose was not significantly different for mice receiving water buffalo and cattle origin isolates.

Discussion

The water buffalo (*Bubalus bubalis*) is an important domestic species in many tropical countries but there is limited peer-reviewed literature concerning brucellosis in this species. This report provides evidence suggesting that *B. abortus* isolates recovered from domestic water buffalo of Trinidad have lower virulence compared to cattle. The reason for this lower virulence is uncertain and it is unknown if the population of isolates available for study accurately reflects the population of all isolates circulating among livestock of the island. However, this recognition could have theoretical and practical implications. *Brucella abortus* infection might be more host-adapted to water buffalo and passage within this species could be the cause of the lower virulence. It is also theoretically possible that the original source of infection was different between cattle and water buffalo and management might limit transmission between species.

The strains of *B. abortus* circulating within populations have importance when designing control programs. Vaccination studies have not been able to identify protocols that protect water buffalo from infection with field strains of *B. abortus* (Diptee et al. 2007). However, a recent report (Caporale et al. 2010) identified a similar vaccination strategy that protects water buffalo from infection with a laboratory strain of *B. abortus*. An unanswered hypothesis is that water buffalo have greater natural resistance to brucellosis and further investigation of isolates recovered from water buffalo could address this question and provide other important information for better understanding and controlling brucellosis within tropical regions.

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Footnotes

[1]Confirmed as *B. abortus* biovar 1 by the Central Veterinary Laboratory, Weybridge, UK

[2]Kindly provided by Drs. J. Payeur and B. Martin, National Veterinary Services Laboratories, Ames, IA, USA

[3]Colorado Serum Company, Denver, CO, USA

[4]SPSS version 17.0, SPSS Inc, Chicago, IL, USA

Competing interests

The authors declare that there are no competing interests.

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Table 1. Descriptive statistics and comparisons of virulence measures using an experimental mouse model for *Brucella abortus* isolates from cattle (n = 6) and domestic water buffalo (n = 13) from Trinidad.

Outcome	Cattle isolates		Water buffalo isolates		P value
	Mean (SD)	Median (range)	Mean (SD)	Median (range)	
Log_{10} CFU recovered	4.89 (1.22)	5.11 (<2, 7.78)	3.98 (1.91)	4.70 (<2, 7.53)	<0.001[a]
Spleen : weight ratio	7.7E-3 (4.6E-3)	6.0E-3 (1.9E-3, 2.4E-2)	6.1E-3 (3.1E-3)	5.1E-3 (2.4E-3, 2.0E-2)	<0.001[a]
Lymphoid depletion	0.77 (0.88)	1 (0, 4)	0.59 (0.76)	0 (0, 2)	0.296[b]
Lymphoid necrosis	0.54 (0.74)	0 (0, 3)	0.27 (0.53)	0 (0, 2)	0.033[b]
Spleen inflammation	2.03 (0.99)	2 (0, 4)	2.15 (0.86)	2 (1, 4)	0.528[b]
Macrophage accumulation	1.26 (0.70)	1 (0, 3)	1.12 (0.68)	1 (0, 3)	0.302[b]
Overall (sum) histopathological score	4.60 (1.93)	4 (2, 9)	4.14 (1.84)	4 (1, 10)	0.273[a]
Log_{10} CFU inoculating dose	3.93 (1.59)	4.17 (<2, 6)	3.90 (0.61)	4.11 (2, 5)	0.715[a]

SD = standard deviation

[a]Based on general linear model

[b]Based on Mann-Whitney U tests

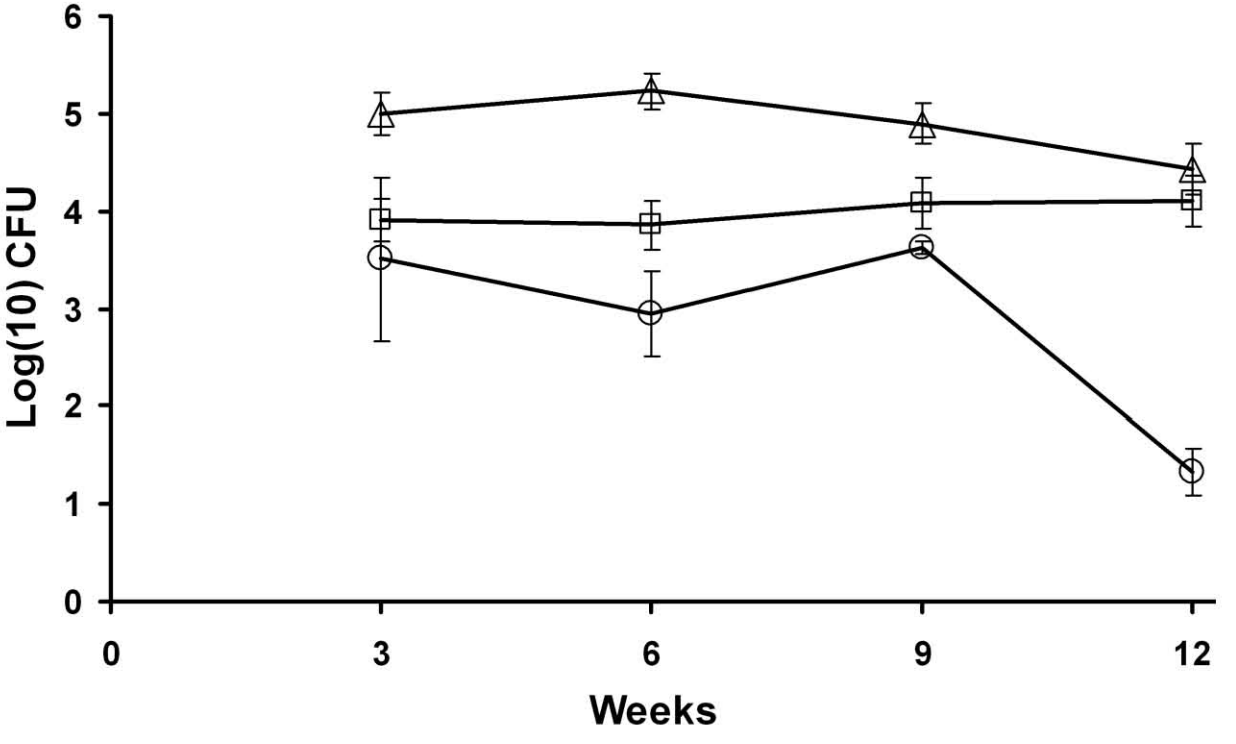
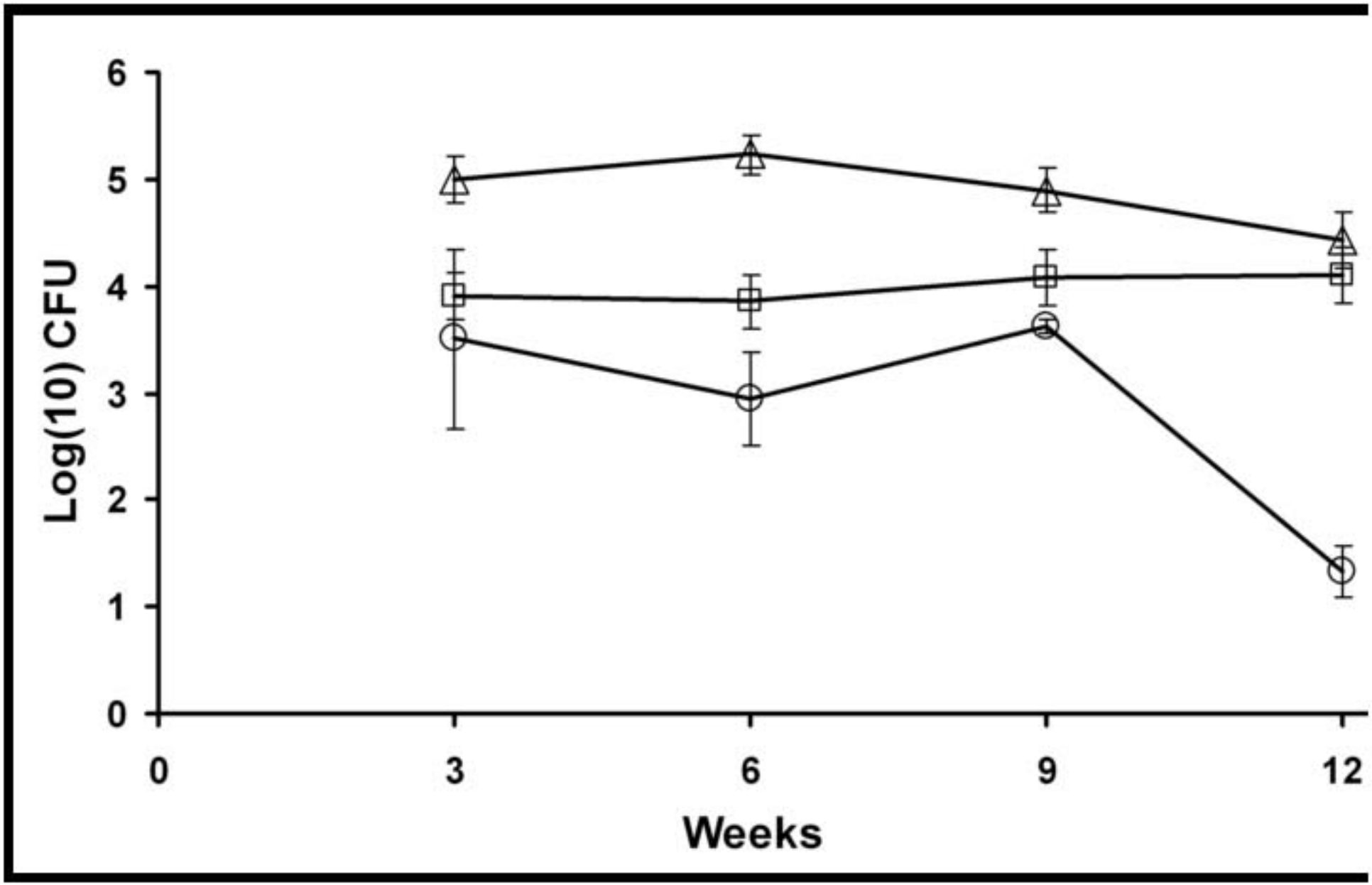


Fig. 1. Mean colony forming units (CFU)/gram recovered from the spleens of experimentally inoculated mice with *Brucella abortus* vaccine strains (circles) and isolates from cattle (triangles; n = 6) and domestic water buffalo (squares; n = 13) from Trinidad. Error bars correspond to the standard error of the mean.


Log(10) CFU
6
5
4
3
2
1
0
0
3
6
9
12
Weeks