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Estimating probabilities of active brucellosis infection in Yellowstone bison through quantitative serology and tissue culture

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Summary

1. Disease management along the boundaries of wildlife reserves is a growing conservation problem worldwide, as infected wildlife can migrate outside protected areas and pose a threat to livestock and human health. The bison *Bison bison* population in Yellowstone National Park has long been infected with *Brucella abortus*, but culling of Yellowstone bison to prevent transmission to cattle has been ineffective at reducing brucellosis infection. This management strategy is negatively affecting long-term bison conservation because of difficulties in diagnosing actively infected animals.

2. We integrated age-specific serology and *B. abortus* culture results from slaughtered Yellowstone bison to estimate probabilities of active brucellosis infection using a Bayesian framework. Infection probabilities were associated with age in young bison (0–5 years old) and with elevated antibody levels in older bison (> 5 years old). Our results indicate that Yellowstone bison acquire *B. abortus* infection early in life but typically recover as they grow older.

3. A tool was developed to allow bison management to better reflect the probability that particular animals are infective, with the aim of conserving Yellowstone bison while reducing the risk of brucellosis transmission to cattle. Combining selective removal of infectious bison with additional management practices, such as vaccination, has the potential to advance an effective brucellosis reduction programme.

4. *Synthesis and applications.* We conclude that active *B. abortus* infection in Yellowstone bison is age dependent, which allows true infection probabilities to be estimated based on age and quantitative diagnostic tests. These findings have important application to disease management worldwide where accurate diagnostic tests for wildlife are unavailable. Estimation of true infection probabilities can replace culling practices that conflict with wildlife conservation. The ability to identify infective individuals can improve management practices that support conservation, particularly when human health is at risk or endangered wildlife species are involved.

Key-words: age, Bayesian, bison, *Brucella abortus*, brucellosis, conservation, culture, disease, serology, Yellowstone

Introduction

The increasing number of wildlife diseases transmissible to humans has raised worldwide concerns regarding free-ranging wildlife as a source of emerging human pathogens (Daszak, Cunningham & Hyatt 2000). In particular, agents of infectious diseases, such as bovine tuberculosis, brucellosis and salmonellosis, which can establish persistent infections in wild ungulates, are especially difficult to manage (Renter *et al.* 2006; Cross *et al.* 2009; White *et al.* 2011). The limitation of diagnostic tests to accurately identify infectivity of persistent bacterial diseases in wild ungulates has led to disease management practices that are not aligned with wildlife conservation. Traditional test-and-slaughter programmes, which have been

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effective for livestock management, may not be realistic or socially acceptable for wildlife (Bienen & Tabor 2006). However, the sharing of diseases at the human, livestock and wildlife interface is a global problem (Böhm *et al.* 2007), which requires management practices that advance wildlife conservation as well as human and animal health.

During the past century, the livestock industry and wildlife managers in the Greater Yellowstone Area in the western United States have been concerned about the infectious disease brucellosis caused by the bacterium *Brucella abortus*. This zoonosis can infect the reproductive organs of several ungulate species during pregnancy and can lead to the induction of late-term abortions of infected hosts. Aborted foetuses are highly infectious and can serve as a transmission source for the disease (Thorne 2001). This non-native disease was most probably introduced to Yellowstone bison *Bison bison* by European cattle (*Bos* spp.)(Cheville, McCullough & Paulson 1998) nearly a century ago (Mohler 1917). In 1934, a nationwide brucellosis eradication programme was initiated and has since resulted in the elimination of *B. abortus* in most of the United States, with the exception of free-ranging wildlife in the three states surrounding Yellowstone National Park (Yellowstone): Montana, Idaho and Wyoming. Cattle industries in these states have additional economic expense if they lose their brucellosis-free status, as has occurred in all three states because of multiple brucellosis exposures in the past decade (Montana Department of Livestock 2008). As such, this remaining pocket of brucellosis has led to decades of conflict among wildlife managers, environmental groups and the livestock industry over management of Yellowstone bison.

Although brucellosis-infected elk *Cervus elaphus* have been responsible for disease transmissions to cattle (Beja-Pereira *et al.* 2009), Yellowstone bison have long been the focus of brucellosis management in the northern portion of the Greater Yellowstone Area. Yellowstone bison management operates under an Interagency Bison Management Plan (IBMP) aimed at conserving wild bison while reducing the risk of brucellosis transmission to Montana livestock (USDI and USDA 2000). Bison management practices used to prevent brucellosis transmission to local cattle conflicts with the goal of conserving bison and the processes that sustain them (e.g. migration). Severe winter conditions encourage bison movement to low-elevation ranges outside Yellowstone (Geremia *et al.* 2011) where they are not tolerated because of the risk of transmitting brucellosis to cattle. In some years, large numbers of migrating bison are captured and tested for brucellosis, with seropositive animals being shipped to slaughter. Approximately 3200 Yellowstone bison were shipped to domestic slaughter facilities between 2001 and 2010, with 899 shipped during 2006 and 1434 shipped during 2008.

These large-scale bison removals have not been random, because bison social structure and the reproductive demands of pregnancy predispose female bison and their recent offspring (i.e. male and female calves and yearlings) to culling as they move onto low-elevation winter ranges outside the park. The effects of several large, nonrandom culls during the past decade have contributed to a skewed sex ratio in favour of male bison, gaps in the population's age structure and reduced productivity that, if continued over time, could reduce the potential of Yellowstone bison to respond to future challenges (White *et al.* 2011). Additionally, boundary culling has not contributed to a measurable reduction in brucellosis infection in the bison population. The proportion of seropositive adult female bison has increased slightly since 1985 or remained constant at *c.* 60% (Hobbs *et al.* 2009).

Removing brucellosis-infected bison is expected to reduce the level of population infection, but test and slaughter practices may instead be removing mainly recovered bison. Recovered animals provide protection to the overall population through the effect of herd immunity (John & Samuel 2000), thereby reducing the spread of disease. Identifying recovered bison is difficult because serologic tests (i.e. blood tests) detect the presence of antibodies, indicating exposure but cannot distinguish active from inactive infection. In bison, *B. abortus* antibodies are long lived (Rhyan *et al.* 2009); thus, seroprevalence overestimates the level of active infection (Roffe *et al.* 1999) by failing to distinguish between infected and recovered animals (i.e. bison that have cleared the bacteria). Although it is highly probable that bison can have serologic titres and not be infected (Cheville, McCullough & Paulson 1998), all seropositive Yellowstone bison have been treated as actively infected because of potentially chronic or latent forms of the disease.

The ability of *B. abortus* to establish undetectable latent infections in young animals (e.g. heifer syndrome) has been documented in cattle (Lapraik *et al.* 1975; Wilesmith 1978; Catlin & Sheehan 1986), although the occurrence of latency is infrequent (Ray *et al.* 1988; Rhyan *et al.* 2009). Latently, infected animals are typically exposed as calves and do not react on serologic tests until they have calved or aborted their pregnancy following infection. The proportion of adult bison that develop chronic infections (i.e. persistent infection of lymphatic tissue) following acute disease (i.e. reproductive tract infections) is unknown. However, the epidemiology and pathogenesis of brucellosis in chronically infected bison is similar to chronically infected cattle (Rhyan *et al.* 2009), and most infected cattle recover by clearing the infection and exhibiting life-long immunity (Ficht 2003).

The epidemiology of brucellosis in Yellowstone bison appears typical of an endemic disease, with a reduced portion of seropositive animals being actively infected and immune protection increasing with age. *B. abortus* has been isolated from 46% of seropositive Yellowstone bison, with young bison and high antibody titred animals predominantly infected (Roffe *et al.* 1999). Thus, the relationship between bison demography and quantitative serologic responses may be an important association for identifying actively infected animals. We integrated age-specific serology and *B. abortus* culture results from Yellowstone bison shipped to slaughter in 2008 to estimate probabilities of active infection. We then applied this information to brucellosis risk management for the purpose of identifying high-risk animals (i.e. those contributing to brucellosis maintenance) and low-risk animals (i.e. recovered bison contributing to herd immunity). This approach has important

application to the growing problem of disease management along the boundaries of protected reserves (Newmark 2008). The ability to target-specific individuals that disproportionately contribute to the maintenance of infectious disease can improve the effectiveness of disease management programmes while supporting long-term conservation efforts.

Materials and methods

STUDY AREA

Yellowstone bison comprise the largest (3000–5000) wild population of plains bison in North America. Two semi-distinct breeding herds migrate and disperse across an extensive landscape (> 90 000 ha). The central herd occupies the central plateau, which extends from the Pelican and Hayden valleys with a maximum elevation of 2400 m in the east to the lower-elevation and geothermally influenced Madison headwaters area in the west. Winters are often severe, with snow water equivalents (i.e. mean water content of a column of snow) averaging 35 cm and temperatures reaching −42 °C. The northern herd occupies the northern portion of Yellowstone where elevation decreases from 2200 to 1600 m over *c*. 90 km between Cooke City and Gardiner, Montana. The northern range is drier and warmer than the rest of the park, with mean snow water equivalents decreasing from 30 to 2 cm along the east–west elevation gradient. In both breeding herds, bison tend to migrate to lower-elevation ranges in and outside the park as bison density and climatic factors (i.e. snow, drought) interact to limit food availability (Geremia *et al*. 2011).

DATA COLLECTION

During the winter and spring (February–April) of 2008, 1805 migrating bison were captured and held at bison management facilities on the northern and western boundaries of Yellowstone. A total of 1434 bison were consigned to slaughter by the IBMP partner agencies. All bison captured at the west boundary (n = 158) were shipped untested to slaughter. The large number of bison exiting the park's northern boundary (n = 1647) resulted in bison shipped untested to slaughter (n = 860) between 11 February 2008 and 19 March 2008. Bison were transported in trailers from Yellowstone to slaughter houses in Montana and Idaho where blood was collected by state or federal inspectors and transferred to state diagnostic laboratories for serologic testing.

After 19 March 2008, many of the bison (n = 191) shipped to slaughter from the park's northern boundary were animals that tested positive on standard serologic tests conducted at the boundary facility. Test-negative bison were held at the northern boundary facility and released into the park in May 2008. For bison that were tested prior to being shipped to slaughter, whole blood was collected into vacutainer blood tubes, immediately centrifuged, and serum was tested for *B. abortus* antibodies using the standard card test and fluorescent polarization assay (FPA). The quantitative FPA is the diagnostic test of choice for bovine brucellosis because of its high sensitivity (94·5%) and specificity (99·5%), quick diagnosis and ease of use (Gall & Neilsen 2001). FPA is a homogenous assay that measures the rotation time of labelled antigen molecules through a specified angle of polarized light, where slower molecular rotation times indicate larger molecule size because of the binding of *B. abortus* antibodies (Muma *et al*. 2006). Measured rotational times are converted to milli-polarizations (mP) where seropositivity is determined by comparisons to mP values of positive and negative controls. FPA results were obtained from state laboratories for bison that were shipped untested to slaughter and from Yellowstone's northern boundary capture facility for tested bison using the Sentry Fluorescence Polarisation Analyser (Diachemix Sentry TM 100, single tube reader, Diachemix LLC, Wisc.USAFPM-1).

We collected tissue samples from 402 slaughtered bison for culture of *B. abortus*. At receiving slaughter houses, bison were killed by gunshot, and tissues were collected immediately after death. Blood was collected to test for the presence of *B. abortus* antibodies using FPA. Depending on bison sex and age, we collected a section of mammary gland and pairs of the following lymph nodes, which have been identified as the priority tissues for isolating *B. abortus* from Yellowstone bison (Rhyan *et al*. 2001): (i) retropharyngeal, (ii) supramammary, (iii) superficial inguinal and (iv) internal iliac. Tissues were collected into sterile Whirl-Paks (Nasco, Fort Atkinson, WI, USA) and stored frozen (−20 °C) until sent to the National Veterinary Services Laboratories (Ames, IA, USA). Ages of young bison (< 5 years old) were determined by incisor eruption patterns (Fuller 1959) and by cementum annuli analysis of the first incisor for older bison (≥5 years old) with all permanent teeth.

The long-held gold standard for brucellosis diagnosis is isolation of the bacteria. Although molecular diagnostic techniques are promising [e.g. polymerase chain reaction (PCR) assays], much work still needs to be performed before they can be used in routine brucellosis testing (Yu & Nielsen 2010). Consequently, application of the PCR assay for *B. abortus* testing in bison blood samples has not been accurate, as results have largely been negative in culture-positive animals (Roberto & Newby 2007). Similarly, culture assays may not be sensitive enough to identify low-level infections in all animals sampled without culturing an unrealistic amount of tissue. Based on the large sample size in this study, we chose a subset of tissues demonstrating no statistical difference in isolating *B. abortus* from Yellowstone bison compared with a much larger comprehensive set of tissues (Schumaker *et al*. 2010).

Brucella abortus isolation from tissue samples was conducted according to established standard operating procedures (Doc # MBSOP 1002·04). Trained staff minced and then mixed each tissue separately in phosphate-buffered saline and plated the macerated tissue suspension onto the agar surface of five plates: one tryptose agar plate with 5% bovine serum; one tryptose agar plate with 5% bovine serum and antibiotics; one tryptose agar plate with 5% bovine serum, antibiotics and ethyl violet; one Farrell plate; and one Ewalt plate. Inverted plates were incubated at 37 °C with 10% CO_2 for a minimum of 10 days. Plates were observed at 5 days and 10 days for the presence of morphologically suspect *Brucella* colonies. Suspect colonies were identified as *B. abortus* using traditional biochemical analysis (Doc #MBSOP1003·03).

BAYESIAN ANALYSIS FRAMEWORK

We used a Bayesian analysis to estimate the probability of *B. abortus* presence in targeted bison tissues based on bison age and quantitative serology data collected in 2008. We collected at least partial information from 402 bison, including gender (402), age (401), culture results (397) and serologic status (299). Approximately 41% (n = 165) of the slaughtered bison studied were sampled after brucellosis testing began. This may have biased culture results towards seropositive animals that were more likely to be actively infected and not representative of the infection status of the Yellowstone population. We corrected for this using a two-sample test of proportions and determined that the portion of culture-positive calves was significantly higher once disease screening began (P < 0·0001, n = 49). Thus,

in our analysis, we considered calves prior to disease screening and all results for other ages ($n = 374$). Antibody values specific to *B. abortus* were determined using the FPA based on the difference between the observed mP value and the negative control. We centred and scaled the net FPA difference by subtracting the mean from the raw values and dividing the difference by the mean. Centred and scaled values were between −1·25 and 2·25, which facilitated model convergence. Bison age, determined using incisor eruption patterns and cementum annuli analyses for animals >5 years old, varied between <1 and 15 years. Of the 374 bison with culture results, we had 273 results with FPA and age data and 101 results with only age data. Censoring results when one of multiple predictor values is unknown increases the uncertainty of parameter estimates (Gelman & Hill 2007). The Bayesian framework provides a coherent method for imputation by treating missing values as random variables that arise from the distribution of observed values (Clark 2007), and we imputed missing FPA values within our Monte Carlo Markov chain algorithm.

We hypothesized that Yellowstone bison are exposed to *B. abortus* at a young age and experience acute infection early in their reproductive lives from which they generally recover (i.e. clear *B. abortus*). We expected the probability of active infection to increase rapidly after birth through reproductive maturity followed by a gradual decline with increasing age. The Ricker function, a common phenomenological model used for ecological variables, starts at zero, increases to a maximal value and gradually decreases back to zero. This functional form allowed a process model to be developed that could describe exposure early in life, followed by acute infection at reproductive maturity, and gradual recovery with increasing age. In its simplest form, the Ricker model includes the shape parameters a, the initial linear rate of increase, and b, the reciprocal of the maximum response value. We chose a single functional form for our process model with interpretable parameters based on previous brucellosis studies in Yellowstone bison (Pac & Frey 1991; Roffe *et al.* 1999).

Our response variable, y_i, was active *B. abortus* infection identified by bacterial culture of a single colony-forming unit from tissues collected from each individual bison. Thus, bison were either culture positive or culture negative. We treated y_i as a random Bernoulli variable with probability of isolating bacteria equalling the Ricker equation $aA_ie^{-bA_i}$ where A_i was animal age. This basic model was used with age as the single predictor variable to determine whether the data supported our hypothesis of early infection and recovery with age. A Bayesian analysis seeks the posterior distribution, which is the probability of the parameters conditional on the data. We initially evaluated the posterior distribution as:

$$P(a,b|\mathbf{Y},\mathbf{A}) \propto \prod_{i=1}^{374} Bernoulli(y_i|a,b,A_i) \times gamma(a|0.001,0.001) \times beta(b|1,1)$$

We expected a positive relationship between FPA mP values and active infection status with higher FPA scores relating to higher culture prevalence. However, reproductively immature animals that are responding to active infection may have fairly low FPA values because of competing protein needs (i.e. antibody production vs. growth and maintenance). Thus, we anticipated that the relationship between FPA and culture status would be more pronounced in older bison that have completed growth and achieved sexual maturity. To account for these effects, we again treated y_i as a random Bernoulli variable but with a probability of isolating bacteria equalling $aA_ie^{-bA_i+cF_i+dA_iF_i}$ where F_i was individual FPA mP value. Missing FPA values ($F_{m,i}$) were imputed in our Monte Carlo Markov chain algorithm by treating missing values as arising from the distribution of observed values, where $\bar{u}$ was the mean and σ was the variance of observed FPA values. In this refined analysis, we evaluated the posterior distribution as:

$$P(a,b,c,d,\boldsymbol{F_m}|\boldsymbol{Y},\boldsymbol{A},\boldsymbol{F},\mu,\sigma) \propto \prod_{i=1}^{273} Bernoulli(y_i|a,b,c,d,A_i,F_i) \times \prod_{i=274}^{374} Bernoulli(y_i|a,b,c,d,A_i,F_{m,i}) \times \prod_{i=274}^{374} normal(F_{m,i}|\bar{\mu},\sigma) \times gamma(a|0.001,0.001) \times beta(b|1,1) \times normal(c|0,1000) \times normal(d|0,1000)$$

Existing information on probability models of our parameters, or prior distributions, was unavailable. Thus, we used uninformative probability models, illustrating that we knew little about the values of these parameters. We used normal distributions for the prior parameter distributions for the effects of FPA. [$c \sim NORMAL$ (0,1000)] and age by FPA interaction [$d \sim NORMAL$ (0,1000)]. We used a gamma distribution to estimate the initial linear rate of increase [$a \sim GAMMA$ (0·001, 0·001)] and beta distribution to estimate the reciprocal of the age of maximum infection probability [$b \sim BETA$(1,1)].

Monte Carlo Markov chain procedures were implemented using the rjags package to call JAGS (Plummer 2009) version 2·1·0 from R (R Development Core Team 2010). We ran each of the three models for 25 000 iterations using three different Monte Carlo Markov chains. The first 5000 iterations were excluded to allow for burn-in. Convergence was assessed visually and when the potential scale reduction factor was <1·1 for all parameters (Gelman & Hill 2007).

BAYESIAN APPLICATION

We used the described Bayesian analysis framework to develop a management tool for making probabilistic statements about the true infection status of bison based on age and net positive FPA values. The objective of the tool is to increase the effectiveness of a brucellosis reduction programme, such as vaccination by selectively removing bison with a specified probability of being infectious.

Results

Brucellosis seroprevalence in bison shipped untested to slaughter ($n = 237$) increased with age in bison <6 years old and decreased in older age classes (Table 1). Active *B. abortus* infection measured by culture prevalence increased rapidly in bison <3 years old, closely matching seroprevalence in young bison, and then decreased in age classes following reproductive maturity (Table 1). The highest culture prevalence was observed in 2·75-year-old bison, just before the age of first parturition. The portion of sampled seropositive bison that were actively infected was highest in calves and decreased with age (Fig. 1). FPA values in seropositive bison were more variable across ages for culture-positive bison compared with culture-negative animals (Fig. 2), indicating that seropositive bison that were culture negative may maintain reduced levels of antibodies, while active infection raises this level because of repeated exposure to *B. abortus* antigen.

The magnitude of estimated parameters in a simple form of the Ricker model, which included only bison age as a predictor variable, indicated the probability of active *B. abortus* infection increased in young bison (median $a = 0{\cdot}29$, 95% credible

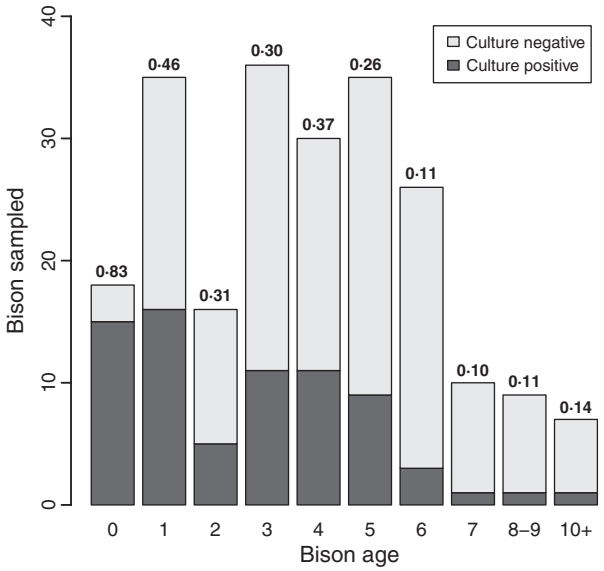

Fig. 1. Age distribution of active *Brucella abortus* infection in seropositive Yellowstone bison shipped to slaughter during 2008. Values above bars indicate the proportion of culture-positive bison for each specified age.

Table 1. Age-specific seroprevalence and culture prevalence of *Brucella abortus* in Yellowstone bison shipped untested to slaughter during the winter of 2008

Bison age	Culture prevalence	Seroprevalence	Seroconversion rate*
0	0·11 (3/27)	0·10 (3/29)	0·10
1	0·28 (17/61)	0·37 (22/59)	0·21
2	0·43 (6/14)	0·46 (6/13)	0·19
3	0·11(3/27)	0·65 (17/26)	0·23
4	0·16 (4/25)	0·48 (11/23)	0·12
5	0·27 (6/22)	0·81 (17/21)	0·24
6+	0·16 (4/25)	0·54(13/24)	0·10

*Age-specific seroconversion rates were estimated using the formula: $p = 1-(1-P_y)^{1/y}$, where p = annual rate of seroconversion, y = age in years and P_y = seroprevalence at age y.

interval = 0·19–0·43) and peaked at 2·6 years of age (median $1/b$ = 2·63, credible interval = 2·08-3·45), which coincided with middle to late gestation during the age of first pregnancy (Fig. 3). These results suggest that active *B. abortus* infection in Yellowstone bison occurs early in life and peaks during the time when female bison would have their first opportunity to transmit the bacteria.

Posterior distributions from the process model which included covariate values for age and FPA allowed for testing whether higher antibody levels were associated with active infection in older bison. Posterior probabilities indicated that active infection was greatest in young bison while active infection in older bison was associated with higher FPA values (Fig. 4). The posterior distributions of model parameters suggested that active infection increased with age in young bison

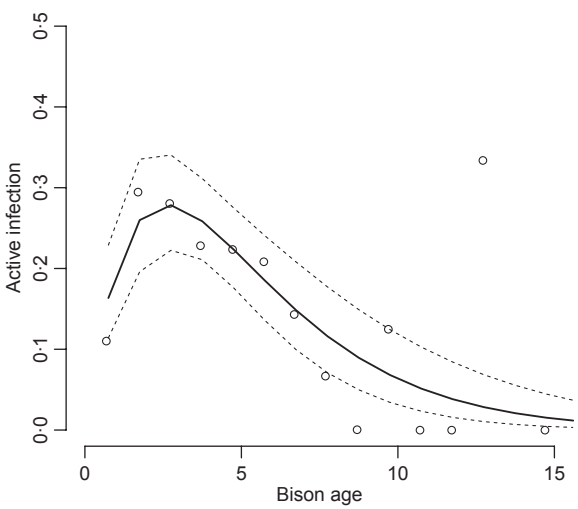

Fig. 3. Estimated posterior probabilities of active *Brucella abortus* infection based on shape parameter (p) estimates of a Bernoulli distribution. The shape parameter is the probability of observing active infection with bison age related by $p = aA_i e^{-bA_i}$. The solid line represents the median, and dotted lines represent the 95% credible interval. Points represent observed proportions of culture-positive bison for specified ages.

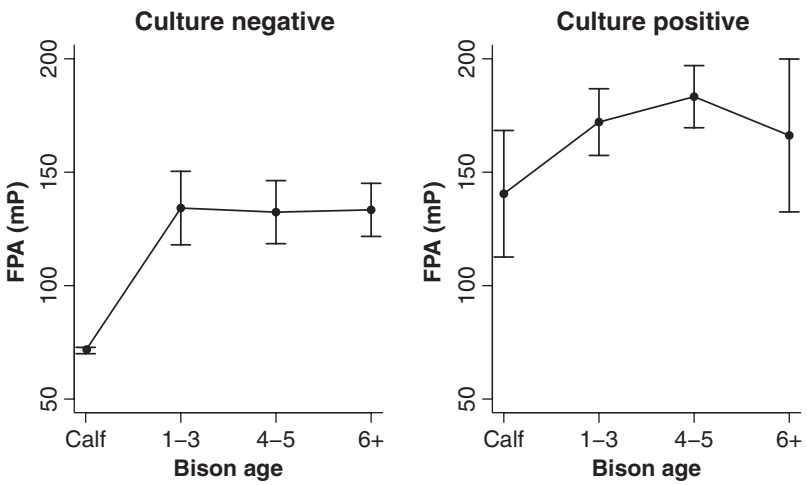

Fig. 2. Age-stratified fluorescent polarization assay values (mP) based on *Brucella abortus* isolation from culture assays.

(median a = 0·32, 95% credible interval = 0·20–0·49) and peaked just before age of first parturition (median 1/b = 2·22, 95% credible interval = 1·72–3·03). FPA antibody levels had a positive effect on our ability to culture *B. abortus* (median c = 0·32, 95% credible interval = −0·04–0·64). There was a high probability (0·82) that the interaction of age and FPA was positively associated with active infection (median d = 0·05, 95% credible interval = −0·05–0·15), indicating the probability of active infection increased at higher FPA values in older bison.

We developed a management tool that forecasts the true infection status of bison based on age (0·75–14·75 years old) and net FPA values (−25–250 in 25 mP increments). Posterior distributions of the probability of infection for each age and net FPA combination were recovered. We used the empirical cumulative distribution function to determine age and net FPA combinations such that the probability of infection was >95%, >85%, >75%, >65% and >55% (Table 2).

Discussion

The data supported our hypothesis that *B. abortus* in bison behaves much like an endemic disease, with infection occurring primarily in young animals and recovery increasing with age. Bison age was an important predictor of active infection, in which active infection increased rapidly in young bison and peaked during the age of first pregnancy. These findings are in agreement with Rhyan *et al.* (2009), which found seroconversion rates in Yellowstone bison to be highest in calves and juveniles (20%) compared to adult females (10%). Bison social

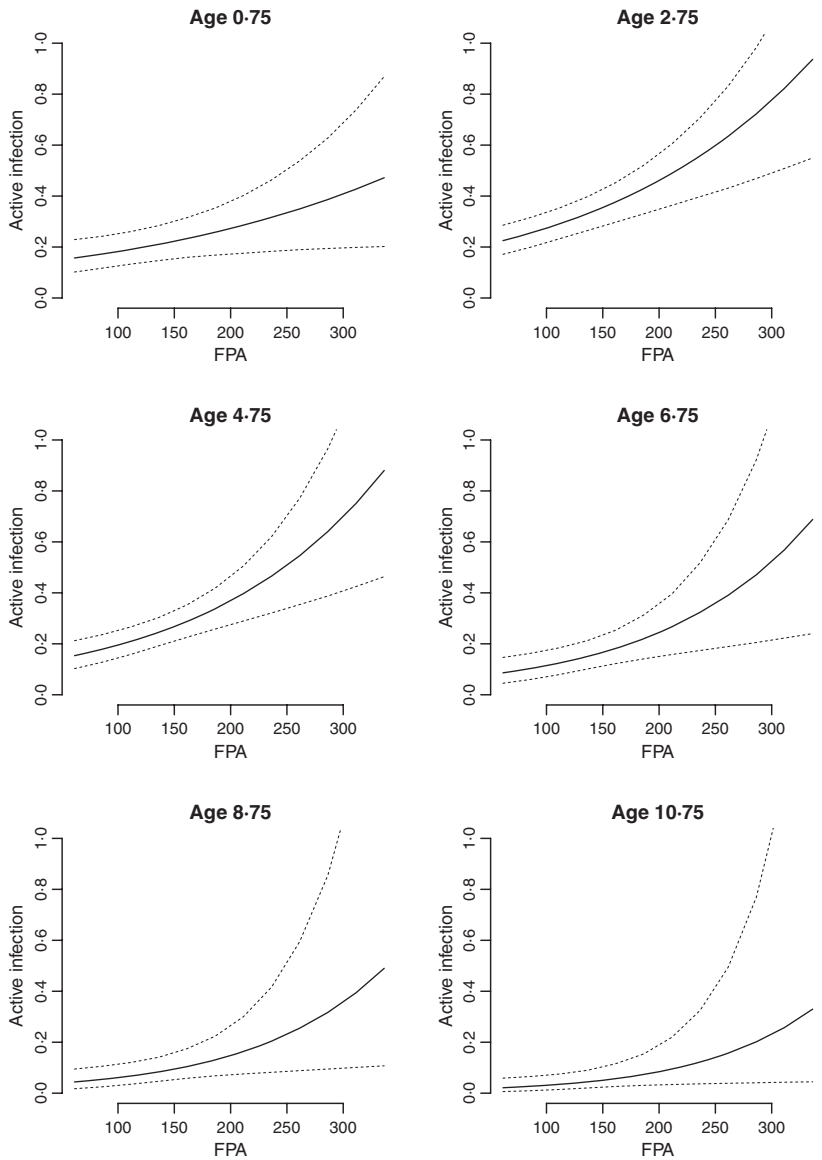

Fig. 4. Estimated posterior probabilities of active *Brucella abortus* infection based on shape parameter (p) estimates of a Bernoulli distribution. The shape parameter is the probability of observing active infection with age and fluorescent polarization assay related by $p = aA_i e^{-bA_i + cF_i + dA_iF_i}$. The solid line represents the median, and dotted lines represent the 95% credible interval.

Table 2. Minimum net fluorescent polarization assay values for corresponding probabilities of active infection and bison age

	Probability of active *Brucella abortus* infection				
Bison age	> 95%	> 85%	> 75%	> 65%	> 55%
0·75	200	175	175	150	125
1·75	175	150	125	100	50
2·75	150	150	125	100	75
3·75	150	150	125	100	75
4·75	175	150	150	125	100
5·75	175	150	150	125	125
6·75	175	150	150	125	125
7·75	175	150	150	125	125
8·75	150	150	150	125	125
9·75	150	150	150	125	125
10·75	150	150	150	125	125
11·75	150	150	150	125	125
12·75	150	150	150	125	125
13·75	150	150	150	150	125
14·75	150	150	150	150	125

structure may predispose newborns and reproductively immature bison to *B. abortus* exposure. In Yellowstone, pregnant and barren females tend to associate with females in similar states of pregnancy (Rutberg 1984). Young bison in these groups have been observed licking birth tissues shed by calving females (Jones, Treanor & Wallen 2009). The high seroprevalence observed in reproductively immature bison may result from close associations with infectious, pregnant females. Additionally, neonates born to infected mothers may become infected at birth or through *B. abortus* in milk; nursing calves receive maternal antibodies, but these wane after 5–6 months of age (Rhyan *et al.* 2009). In our study, calves were *c.* 9 months old when sampled, and antibodies were highly associated with active infection (Table 1), suggesting that active *B. abortus* infection begins early in life.

The highest probability of active infection was in bison *c.* 2·75 years old. Seroprevalence (0·46) and culture prevalence (0·43) were also similar in this age class, which may be caused by systemic infection following reproductive maturity. Ingestion of *B. abortus* into the gastrointestinal tract is the most common route of exposure from where infection spreads to local lymph nodes before colonization of the uterus during pregnancy (Carvalho Neta *et al.* 2010). The proliferation of *Brucella*, necessary for transmission, is influenced by the stage of gestation (Nicoletti 1980), with increasing replication in placental cells during late gestation (Carvalho Neta *et al.* 2010). Female bison in Yellowstone typically experience their first fertile oestrous early (27 months of age) as 2 years old. In the present study, all reproductively mature female bison sampled were in middle to late stages of gestation, suggesting that this large, recently infected age group may be the primary source of infection for the overall population.

Seroprevalence tracked culture prevalence in young animals but diverged after reproductive maturity (age 2, Table 1). Similarly, the proportion of seropositive bison found to be culture positive decreased as age increased, with low levels of active infection prevalence after the age of five (Fig. 1). These results suggest that bison exposed to *B. abortus* early in life may begin to recover from acute infection after their first pregnancy following seroconversion. However, *B. abortus* exposure, measured in seroprevalence, increased until bison were older than 5 years of age. Rhyan *et al.* (2009) found the greatest potential for positive tissue culture occurred within 2 years following seroconversion, which suggests a high risk of transmission during this period. In our study, seroconversion began early in life, prior to reproductive maturity, and active infection peaked at the age of first parturition with a steady decrease in the probability of active infection beyond the age of three. This suggests that young bison (< 3 years old) may be a vulnerable age class to *B. abortus* infection in comparison with older animals, where a large proportion has experienced *B. abortus* infection earlier in life.

The protective effect of the humoral immune response (i.e. serum antibodies) in brucellosis is mild or questionable (Carvalho Neta *et al.* 2010) because the *Brucellae* are intracellular pathogens able to hide from humoral defences. Accordingly, the positive association between bacterial isolation and high serologic titres (Roffe *et al.* 1999; Thorne 2001) may indicate reactivation of persistent infection or recent exposure. In the absence of *B. abortus* antigen, antibody levels are expected to decrease. Yellowstone bison identified with low antibody titres have converted back to seronegative status (Rhyan *et al.* 2009). This finding may explain the delayed decline in seroprevalence after the age of five in comparison with declines in culture prevalence after the age of three. If bison are able to clear the infection with age, then we may observe a slower decrease in seroprevalence because of circulating antibodies (i.e. long-lived humoral IgG responses), high test sensitivity and spikes in antibodies resulting from re-exposure.

We found a positive relationship with FPA values and active infection (Figs 2 and 4), supporting the view that active infection is associated with increased antibody production. Bison that have cleared the infection will still react positive on serologic tests until antibodies have decreased below detectable levels. The FPA is a sensitive test for detecting antibodies specific to *B. abortus*, and the high rates of seroprevalence in older bison include animals that seroconverted at younger ages because of antibody persistence. These bison may have undergone acute infection early in life and have developed some level of immunity against re-exposure.

Although *B. abortus* antibodies may not be protective, they do play an important role in decreasing the number of bacteria upon subsequent exposure. Opsonized *Brucellae*, which are extracellular bacteria marked for destruction by host antibodies, are less likely to survive and establish intracellular infections (Carvalho Neta *et al.* 2010). Once *B. abortus* establishes intracellular infection, bacterial clearance requires effective cell-mediated immune responses induced by activation of specialized T cells (Oliveira, Soeurt & Splitter 2002). Memory T cells persist after the infection has been cleared and allow for secondary responses to low concentrations of *B. abortus* antigen that are faster and of greater magnitude than the primary response (Bernard & Tough 2002). Effective cell-mediated immune responses may clear the initial infection and quickly

respond to secondary infections, thereby reducing antibody levels as a result of eliminating *B. abortus* from bison tissues. Thus, high antibody levels may indicate ineffective cell-mediated immune responses, and conversely, effective cell-mediated immune responses may clear bacteria and reduce *B. abortus* antibody production.

A limitation of our work may be that we failed to detect low-level chronic infections in adult bison. *Brucella abortus* is known for its intracellular hiding ability which can facilitate long-term persistence (Spera *et al*. 2006). Consequently, the association of high serologic responses with active infection in older bison may indicate recent exposure or recrudescence of chronic infection. Chronically infected female cattle have been observed to periodically shed *B. abortus* in genital infections (Manthei, DeTray & Goode 1950; Lambert *et al*. 1960). However, in our study, the relationship between antibody levels and active infection in older bison suggests that low serologic responses may be indicative of recovery rather than chronic infection. The reactivation of infection is expected to occur from stress and favourable conditions for *B. abortus* proliferation during late gestation (Cheville, McCullough & Paulson 1998). In 2008, bison were sampled in middle to late gestation during one of the most stressful years in Yellowstone's recorded history (based on high bison population density and winter severity). Thus, we would expect a high rate of recrudescence (i.e. active infection) in older animals if chronic infection was the typical progression of brucellosis in Yellowstone bison.

MANAGEMENT APPLICATIONS

The objective of the present study was not to identify every actively infected bison, but to provide managers with a reliable tool for identifying FPA cut-off values based on specified active infection probabilities and bison age (Table 2). This allows managers to take a conservation approach to bison management by targeting infectious animals based on active infection probabilities, which can be adjusted to different phases of a brucellosis reduction programme. Currently, brucellosis seroprevalence is high (0·45, White *et al*. 2011), with bison diagnosed as seropositive when net FPA values typically exceed 10–20 mp. This tool allows managers to reduce removals when seroprevalence is high by identifying infectious bison (e.g. probability of active infection $>0{\cdot}95$) with a high level of certainty. Similarly, as immune protection increases against *B. abortus* through vaccination and seroprevalence declines, removal efforts can focus on the majority of *B. abortus*-exposed bison (e.g. probability of active infection $>0{\cdot}55$) that are less likely to be actively infected. This targeted approach allows for removing high-risk individuals and increasing herd immunity through vaccination, while promoting bison conservation without large-scale culling.

Brucellosis in the greater Yellowstone area is one of the most challenging issues facing wildlife managers, livestock producers and the concerned public in the western United States. High genetic diversity, unique alleles and lack of introgression of domestic cattle genes (Halbert 2003) make the Yellowstone bison population critical to the conservation of the species in North America. Because definitive brucellosis tests do not currently exist, the threat of spreading brucellosis has prevented translocation of Yellowstone bison for purposes of genetic augmentation of smaller herds elsewhere in North America. Yellowstone bison management has long relied on serologic tests, which overestimate the level of brucellosis infection, the continuation of which may lead to management decisions that reduce bison numbers and reduce long-term viability of this unique population. We have presented a novel method for estimating active brucellosis infection in bison that will allow managers to better assess the relative risk of individual animals and influence culling decisions in a way that should reduce annual losses from this practice. Our approach has broad application to balancing disease management with wildlife conservation, such as situations where migratory wildlife poses a risk to livestock and human health or when endangered wildlife may be impacted by infectious disease. The ability to identify infectious individuals, which disproportionately contribute to disease maintenance, increases management options beyond traditional culling practices.

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References

Beja-Pereira, A., Bricker, B., Chen, S., Almendra, C., White, P.J. & Luikart, G. (2009) DNA genotyping suggests recent brucellosis outbreaks in the greater Yellowstone area originated from elk. *Journal of Wildlife Diseases*, **45**, 1174–1177.

Bernard, M. & Tough, D.F. (2002) Qualitative differences between naïve and memory T cells. *Immunology*, **106**, 127–138.

Bienen, L. & Tabor, G. (2006) Applying an ecosystem approach to brucellosis control: can an old conflict between wildlife and agriculture be successfully managed? *Frontiers in Ecology and the Environment*, **4**, 319–327.

Böhm, M., White, P.C.L., Chambers, J., Smith, L. & Hutchings, M.R. (2007) Wild deer as a source of infection for livestock and humans in the UK. *The Veterinary Journal*, **174**, 260–276.

Carvalho Neta, A.V., Mol, J.P.S., Xavier, M.N., Paixão, T.A., Lage, A.P. & Santos, R.L. (2010) Pathogenesis of bovine brucellosis. *The Veterinary Journal*, **184**, 146–155.

Catlin, J.E. & Sheehan, E.J. (1986) Transmission of bovine brucellosis from dam to offspring. *Journal of the American Veterinary Medical Association*, **188**, 867–869.

Cheville, N.F., McCullough, D.R. & Paulson, L.R. (1998) *Brucellosis in the Greater Yellowstone Area*. National Academy Press, Washington, D.C.

Clark, J.S. (2007) *Models for Ecological Data: An Introduction*. Princeton University Press, Princeton, New Jersey, USA.

Cross, P.C., Heisey, D.M., Bowers, J.A., Hay, C.T., Wolhuter, J., Buss, P., Hofmeyr, M., Michel, A.L., Bengis, R.G., Bird, T.L.F., DuToit, J.T. & Getz, W.M. (2009) Disease, predation and demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. *Journal of Applied Ecology*, **46**, 467–475.

Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science*, **287**, 443–449.

Ficht, T.A. (2003) Intracellular survival of *Brucella*: defining the link with persistence. *Veterinary Microbiology*, **92**, 213–223.

Fuller, W.A. (1959) The horns and teeth as indicators of age in bison. *Journal of Wildlife Management*, **23**, 342–344.

Gall, D. & Neilsen, K. (2001) Evaluation of the fluorescent polarization assay and comparison to other serological assays for detection of brucellosis in cervids. *Journal of Wildlife Diseases*, **37**, 110–118.

Gelman, A. & Hill, J. (2007) *Data Analysis Using Regression and Multilevel Hierarchical Models*. Cambridge University Press, New York, NY, USA.

Geremia, C., White, P.J., Wallen, R.W., Watson, F.G.R., Treanor, J.J., Borkowski, J., Potter, C.S. & Crabtree, R.L. (2011) Predicting bison migration out of Yellowstone National Park using Bayesian models. *PLoS ONE*, **6**, e16848.

Halbert, N. (2003) The utilization of genetic markers to resolve modern management issues in historic bison populations: implications for species conservation. PhD thesis, Texas A&M University, College Station.

Hobbs, N.T., Wallen, R., Treanor, J., Geremia, C. & White, P.J. (2009) *A Stochastic Population Model of the Yellowstone Bison Population*. Colorado State University, Fort Collins.

John, T.J. & Samuel, R. (2000) Herd immunity and herd effect: new insights and definitions. *European Journal of Epidemiology*, **16**, 601–606.

Jones, J.D., Treanor, J.T. & Wallen, R.L. (2009) Parturition in Yellowstone bison. *Report YCR-2009-01*. Yellowstone Center for Resources, National Park Service, Mammoth Hot Springs, Wyoming.

Lambert, G., Amerault, T.E., Manthei, C.A. & Goode, E.R. Jr (1960) Further studies on the persistence of *Brucella abortus* in cattle. *Proceedings of the United States Livestock Sanitary Association*, **64**, 109–117.

Lapraik, R.D., Brown, D.D., Mann, H. & Brand, T. (1975) Brucellosis: a study of five calves from reactor dams. *Veterinary Record*, **97**, 52–54.

Manthei, C.A., DeTray, D.E. & Goode, E.R. (1950) Brucella infection in bulls and the spread of brucellosis in cattle by artificial insemination. I. Intrauterine injection. *Proceedings of the Annual Meeting of the American Veterinary Medical Association*, **87**, 177–184.

Mohler, J.R. (1917) *Report of the Chief of the Bureau of Animal Industry, Pathologic Division*. Annual Reports of the Department of Agriculture, Washington, D.C.

Montana Department of Livestock (2008) *Preliminary Epidemiology Report*. Helena, Montana.

Muma, J.B., Samui, K.L., Siamudaala, V.M., Oloya, J., Matope, G., Omer, M.K., Munyeme, M., Mubita, C. & Skjerve, E. (2006) Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. *Tropical Animal Health and Production*, **38**, 195–206.

Newmark, W.D. (2008) Isolation of African protected area. *Frontiers in Ecology and the Environment*, **6**, 321–328.

Nicoletti, P. (1980) The epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine*, **24**, 69–98.

Oliveira, S.C., Soeurt, N. & Splitter, G.A. (2002) Molecular and cellular interactions between *Brucella abortus* antigens and host immune responses. *Veterinary Microbiology*, **90**, 417–424.

Pac, H.I. & Frey, K. (1991) *Some Population Characteristics of the Northern Yellowstone Bison Herd During the Winter of 1988–1989*. Montana Department of Fish, Wildlife, and Parks, Bozeman, Montana.

Plummer, M. (2009) rjags: Bayesian graphical models using MCMC. R package version 1.0.3-9. URL http://CRAN.R-project.org/package = rjags.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna Austria.

Ray, W.C., Brown, R.R., Stringfellow, D.A., Schnurrenberger, P.R., Scanlan, C.M. & Swann, A.I. (1988) Bovine brucellosis: an investigation of latency in progeny of culture-positive cows. *Journal of the American Veterinary Medical Association*, **192**, 182–186.

Renter, D.G., Gnad, D.P., Sargeant, J.M. & Hygnstrom, S.E. (2006) Prevalence and serovars of *Salmonella* in the feces of free-ranging white-tailed deer (*Odocoileus virginianus*) in Nebraska. *Journal of Wildlife Diseases*, **42**, 699–703.

Rhyan, J.C., Gidlewski, T., Roffe, T.J., Aune, K., Philo, L.M. & Ewalt, D.R. (2001) Pathology of brucellosis in bison from Yellowstone National Park. *Journal of Wildlife Diseases*, **37**, 101–109.

Rhyan, J.C., Aune, K., Roffe, T., Ewalt, D., Hennager, S., Gidlewski, T., Olsen, S. & Clarke, R. (2009) Pathogenesis and epidemiology of brucellosis in Yellowstone bison: serologic and culture results from adult females and their progeny. *Journal of Wildlife Diseases*, **45**, 729–739.

Roberto, F.F. & Newby, D.T. (2007) Application of a real-time PCR assay for *Brucella abortus* in wildlife and cattle. *U.S. Animal Health Association*, **110**, 196–199.

Roffe, T.J., Rhyan, J.C., Aune, K., Philo, L.M., Ewalt, D.R., Gidlewski, T. & Hennager, S.G. (1999) Brucellosis in Yellowstone National Park bison: quantitative serology and infection. *Journal of Wildlife Management*, **63**, 1132–1137.

Rutberg, A. (1984) Birth synchrony in American bison (*Bison bison*): response to predation or season? *Journal of Mammalogy*, **65**, 418–423.

Schumaker, B.A., Corso, B.A., Rhyan, J.C., Philo, L.M., Salman, M.D. & Gardner, I.A. (2010) Evaluation of the fluorescence polarization assay for the detection of *Brucella abortus* antibodies in bison in a natural setting. *Comparative Immunology, Microbiology and Infectious Diseases*, **33**, e119–e125.

Spera, J.M., Ugalde, J.E., Mucci, J., Comerci, D.J. & Ugalde, R.A. (2006) A B lymphocyte mitogen is a *Brucella abortus* virulence factor required for persistent infection. *Proceedings of the National Academy of Sciences*, **103**, 16514–16519.

Thorne, E.T. (2001) *Brucellosis. Infectious Diseases in Wild Mammals* (eds E.S. Williams & I.K. Barker), pp. 372–395. Blackwell Publishing, Ames, Iowa.

United States Department of the Interior, National Park Service (USDI) and United States Department of Agriculture, Forest Service, Animal and Plant Health Inspection Service (USDA). (2000) Final environmental impact statement for the interagency Bison management plan for the state of Montana and Yellowstone National Park. Washington, D.C.

White, P.J., Wallen, R.L., Geremia, C., Treanor, J.J. & Blanton, D.W. (2011) Management of Yellowstone bison and brucellosis transmission risk – expectations and realizations. *Biological Conservation*, **144**, 1322–1334.

Wilesmith, J.W. (1978) The persistence of *Brucella abortus* infection in calves: a retrospective study of heavily infected herds. *Veterinary Record*, **103**, 149–153.

Yu, W.L. & Nielsen, K. (2010) Review of detection of *Brucella spp* by polymerase chain reaction. *Croatian Medical Journal*, **51**, 306–313.

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