

A multi-scale assessment of animal aggregation patterns to understand increasing pathogen seroprevalence

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Citation: Brennan, A., P. C. Cross, M. D. Higgs, W. H. Edwards, B. M. Scurlock, and S. Creel. 2014. A multi-scale assessment of animal aggregation patterns to understand increasing pathogen seroprevalence. *Ecosphere* 5(10):138. <http://dx.doi.org/10.1890/ES14-00181.1>

Abstract. Understanding how animal density is related to pathogen transmission is important to develop effective disease control strategies, but requires measuring density at a scale relevant to transmission. However, this is not straightforward or well-studied among large mammals with group sizes that range several orders of magnitude or aggregation patterns that vary across space and time. To address this issue, we examined spatial variation in elk (*Cervus canadensis*) aggregation patterns and brucellosis across 10 regions in the Greater Yellowstone Area where previous studies suggest the disease may be increasing. We hypothesized that rates of increasing brucellosis would be better related to the frequency of large groups than mean group size or population density, but we examined whether other measures of density would also explain rising seroprevalence. To do this, we measured wintering elk density and group size across multiple spatial and temporal scales from monthly aerial surveys. We used Bayesian hierarchical models and 20 years of serologic data to estimate rates of increase in brucellosis within the 10 regions, and to examine the linear relationships between these estimated rates of increase and multiple measures of aggregation. Brucellosis seroprevalence increased over time in eight regions (one region showed an estimated increase from 0.015 in 1991 to 0.26 in 2011), and these rates of increase were positively related to all measures of aggregation. The relationships were weaker when the analysis was restricted to areas where brucellosis was present for at least two years, potentially because aggregation was related to disease-establishment within a population. Our findings suggest that (1) group size did not explain brucellosis increases any better than population density and (2) some elk populations may have high densities with small groups or lower densities with large groups, but brucellosis is likely to increase in either scenario. In this case, any one control method such as reducing population density or group size may not be sufficient to reduce transmission. This study highlights the importance of examining the density-transmission relationship at multiple scales and across populations before broadly applying disease control strategies.

Key words: aerial survey; *Brucella abortus*; density-dependent transmission; elk feedgrounds; group living; group size distribution; Lloyd's crowding; parasite transmission; seasonal grouping; wildlife disease.

Received 11 June 2014; revised 16 August 2014; accepted 23 August 2014; final version received 18 September 2014; **published** 31 October 2014. Corresponding Editor: A. W. Park.

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INTRODUCTION

There is considerable evidence for a positive relationship between animal density and parasite transmission rates in studies of disease ecology (e.g., Brown and Brown 1986, Davies et al. 1991, Côté and Poulin 1995, Packer et al. 1999, Arneberg 2002, Altizer et al. 2003, Greer et al. 2008, Rifkin et al. 2012), and this relationship is at the root of disease management strategies aimed at reducing the density of susceptible individuals (e.g., Caley et al. 1999, Smith et al. 2001, Wobeser 2002, Lloyd-Smith et al. 2005). However, failing to measure animal density at the spatial and temporal scale relevant to transmission could limit the efficacy of these management strategies and our understanding of disease dynamics. If individuals only interact locally, for example, transmission may be more related to fine scale measures of aggregation (e.g., group size) than broad measures such as regional density. In this case, using regional density as a proxy for the rate of contact among individuals could fail to explain increases in disease prevalence driven by within-group transmission (Cross et al. 2010*b*, Ferrari et al. 2011) and could prevent the development of more productive control measures directed at the group level.

Focusing on group size to understand parasite transmission is common among studies of disease in social animals (Côté and Poulin 1995, Rifkin et al. 2012), but this could also limit our understanding of disease dynamics if aggregation patterns vary over space and time, as frequently occurs among avian and mammalian species (e.g., Brown et al. 1990, Symington 1990, Gerard et al. 2002, Fortin et al. 2009). Thus, learning about the density-transmission relationship may require evaluating how animals aggregate at multiple spatial and temporal scales. However, such data are rare for large mammals where extensive datasets on disease prevalence and animal aggregations are difficult to obtain. In particular, studies of density and transmission may rely on temporal variation in parasite prevalence and density (Begon et al. 1999, Ostfeld et al. 2001, Smith et al. 2009), but few datasets will be long enough to conduct this type of analysis for a long-lived animal and chronic disease. Instead, we focused on the spatial variation in serologic data and elk (*Cervus*

canadensis) aggregation measured at multiple scales across 10 regions in western Wyoming and the Greater Yellowstone Area (GYA) where elk have been exposed to *Brucella abortus*, a bacteria that causes brucellosis. The elk-brucellosis system is an important example for this type of analysis because brucellosis is a significant disease among wildlife and livestock across the globe (e.g., Davis 1990, Gul and Khan 2007) and elk group size distributions and seasonal aggregation patterns are similar to other large herbivores that can harbor chronic diseases (e.g., Talbot and Talbot 1963, Sinclair 1977).

Brucella abortus is widespread, but it has been largely eradicated in the United States and now persists only in the wildlife around the GYA, where it was first introduced by cattle in the early 1900s (Mohler 1917, Meagher and Meyer 1994). Since that time, brucellosis prevalence in the GYA has been highest among bison in Yellowstone and Grand Teton National Park (Cheville et al. 1998) and in elk that are supplementally fed on feedgrounds during the winter in western Wyoming (Scurlock and Edwards 2010). However, recent surveillance efforts have documented increasing brucellosis in some elk populations that previously maintained the disease at lower levels (estimated seroprevalence increased from 0–7% in 1991 to 10–25% in 2010; Scurlock and Edwards 2010, Cross et al. 2010*b*). Several probable causes of these increases have been examined, including increased elk-elk transmission associated with increased density, dispersal of infectious elk from feedgrounds, and changes to an older-age structure. Only elk density, however, explained observed rates of increasing brucellosis seroprevalence, but the data could not differentiate between linear or nonlinear relationships with density and only annual population density at the regional scale was examined (Cross et al. 2010*a*, Cross et al. 2010*b*). Though this is evidence that *B. abortus*-transmission in elk may be weakly density dependent, rates of increasing brucellosis may be better explained by finer scale measures of elk aggregation during the transmission period.

Transmission of *B. abortus* occurs during the months of February through May when elk are aggregated on lower elevation winter range to avoid deep snowfall, or just as they are beginning to migrate to summer range at higher elevations

(Thorne et al. 1991, Roffe et al. 2004). During this period, brucellosis causes roughly half of infected females to abort their first pregnancy post-infection and roughly 10% to also abort their second calf (Thorne et al. 1978). From these abortion events, transmission occurs via uptake of the bacteria through contact with or feeding near the fetus or birthing materials (Cheville et al. 1998), but evidence suggests the duration of exposure may be limited because scavengers typically consume aborted materials within 24 to 48 hours (Cook et al. 2004, Maichak et al. 2009). It is possible for fetuses to remain in the environment for weeks or months in cold, wet and shaded environments with lower scavenger densities (Aune et al. 2012). However, the effects of such long-term contamination on transmission are not known. Other modes of directly transmitting bacteria such as vertical or sexual transmission are not considered important in the spread of *B. abortus* (Thorne et al. 1978).

On the winter range in western Wyoming, elk aggregate into groups ranging in size from one to thousands of individuals and, as with the many other species that aggregate, the group size distribution is right skewed, whereby a majority of groups are small but a majority of the individuals occur in large groups (Reiczigel et al. 2008). With this distribution, disease dynamics may be driven by transmission occurring within groups that are larger than the mean. Therefore, we hypothesized that rates of increasing brucellosis seroprevalence will be more related to group sizes in the tail of the distribution than annual population density or mean group size. In addition, previous work on feedground elk showed brucellosis seroprevalence was higher in feedgrounds that operated later into the spring and for longer periods (Cross et al. 2007). Therefore, we also hypothesized that rates of increasing brucellosis would be related to the tail of the group size distribution observed during the end of the transmission period (end of period elk are found on the winter range) and to the average of monthly elk densities across the transmission period.

To test our hypotheses, we used Bayesian hierarchical models to (1) estimate the rates of increase in brucellosis seroprevalence from 20 years of serologic data across 10 elk populations, and (2) evaluate how these estimated rates of

increasing brucellosis were related to fine (e.g., group size) and broad scale (e.g., annual population density) measures of aggregation. We included broad measures such as annual population and sub-population density in order to compare our results to previous studies that assumed elk were well mixed at these scales, but we also hypothesized that estimated rates of increase in brucellosis would be more related to the density of elk on the winter range (where elk are located during the transmission period) than broader measures of density calculated across winter and summer range. Therefore, this analysis expands upon other empirical studies of the density-transmission relationship by considering density at multiple scales. Furthermore, we retested our hypotheses using only those elk populations having brucellosis seropositive animals for at least two years to investigate the potential importance of aggregation on increasing seroprevalence within places where the pathogen was known to be present (i.e., a lack of increase in seroprevalence may be due to the absence of the pathogen, rather than lower density). Assessing these relationships can help identify where to focus disease management efforts and examining the spatial variation in elk aggregation and brucellosis increases can improve our understanding of how social structure affects disease dynamics.

METHODS

Broad scale density

We used the following broad scale elk density measures: (1) herd unit density, (2) hunt area density, and (3) winter range density. The herd unit and hunt area were two sizes of elk management units delineated by the Wyoming Game and Fish Department (WGFD), where multiple hunt areas were nested within a larger herd unit (Fig. 1). Hunt area boundaries tended to be substantial terrain features (e.g., hydrographic divides and highways) and movement between hunt areas during the winter was possible, but thought to be minor for most hunt areas in our study (WGFD 2011). Each elk herd unit included multiple hunt areas and was more likely to encompass a closed elk population (less than 10% interchange with adjacent herd units throughout the year; WGFD 2011). Therefore, we

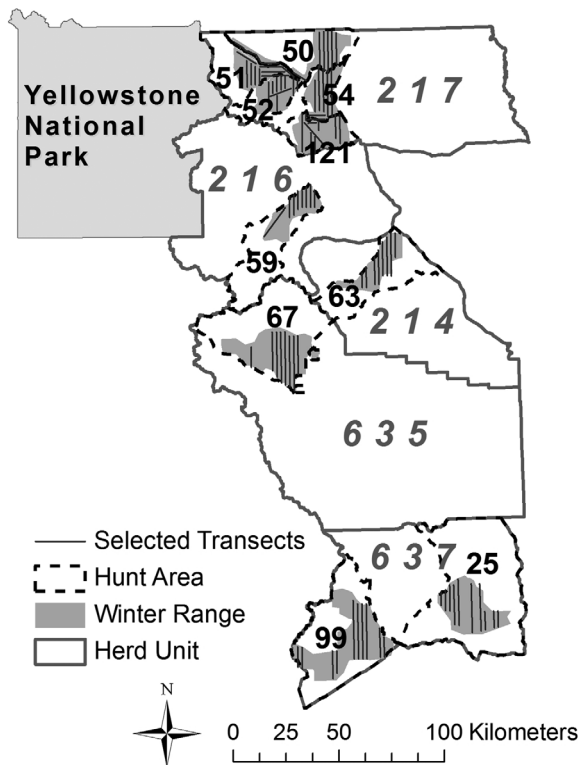


Fig. 1. Map of the study area in western Wyoming, including the five elk herd units (population-level units) of interest (217, 216, 214, 635 and 637), the 10 focal elk hunt areas (sub-population units), elk winter ranges, and aerial transects selected to record elk group location and size.

considered hunt areas to be management units at the sub-population scale and herd units to be management units at the population scale. Our study examined five herd units where brucellosis was endemic in elk, but without supplemental feedgrounds (herd units 217, 216, 214, 635 and 637), and then focused on 10 hunt areas within those herd units (Fig. 1) that represented the range of elk densities (from roughly 0.2 to 3.0 elk/km²) and estimated brucellosis seroprevalence (from roughly 0 to 0.25) in the study area (Cross et al. 2010a, Scurlock and Edwards 2010, WGFD 2011).

We calculated herd unit density (annual population density) and hunt area density (annual sub-population density) as described in Cross et al. (2010a, b) using the most recent aerial elk trend counts obtained by the WGFD up to 2011. Elk trend counts are population counts

conducted within each hunt area by the WGFD from fixed-wing aircraft or helicopters, usually between January and March after recent snowfall (WGFD 2011). Observers and methods for obtaining these counts may vary between hunt areas, and therefore we used these trend counts as indices to demonstrate the spatial variation in density among management units. For a majority of the 10 hunt areas included in the study, the most recent trend count occurred in 2011, but for two hunt areas the most recent trend counts occurred in 2007 and 2009.

The herd unit and hunt area encompass winter range where elk are located during the transmission period, summer range, and other high elevation areas elk avoid in snow (WGFD 2011). To better reflect elk density during the transmission period, each of the 10 hunt areas of interest was refined to only the winter range. To do this, we delineated winter ranges using all elk sightings recorded by the WGFD during the months of January through May, 2005–2009 (Fig. 1). These sightings included elk counted during trend and classification surveys, and any opportunistic sightings made by WGFD biologists or game wardens from the air or ground. Winter range elk density was then calculated by dividing each hunt area's most recent trend count by its winter range area.

Average monthly density and group size

In addition to the three broad scales measures of density, we calculated four finer scale measures of elk aggregation, including: (1) average monthly density, (2) mean group size, (3) Lloyd's crowding (i.e., a weighted average of group size that accounts for the frequency of large groups and the spread of the group size distribution; Lloyd 1967), and (4) Lloyd's crowding in May. To obtain these fine scale measures of aggregation, we recorded elk group sizes from a fixed wing aircraft over the winter range of each hunt area (sub-population unit) once a month from January through May for three years (2010–2012; Fig. 1). These aerial surveys were conducted using transects that were set 2 km apart, for 1-km viewing widths from one side of the plane. Transects were assigned to be non-overlapping, to cover the full extent of each winter range, and to follow a north-south orientation, though in several cases transects were set to follow major

landscape features such as a ridgeline or narrow valley. To increase our chances of seeing elk, four to seven transects were deliberately selected per winter range in order to cover areas with a high probability of elk occurrence, as determined by the WGFD elk sightings (i.e., areas with the highest number of elk sightings were high probability areas for elk occurrence). Three additional transects were randomly selected per winter range, and the viewing direction (east or west) for all transects was also randomly selected. The number of transects selected and length of transects varied by winter range, but consistently these transects covered approximately 35% of each winter range area. The same transects and viewing directions selected at the beginning of the study were used for all subsequent monthly flights.

Transects covered a range of elevations (roughly 1230 to 2800 m), topography (e.g., mountain ridges, valleys, small drainages, and mesas), and habitat (e.g., dense and open forests, grassland, sagebrush steppe, and badlands). On average, we conducted flights at 240 m above ground level and at 150 km per hour. The timing and order of our flights differed each month, as they depended on clear or overcast (high clouds) mornings (before noon), when winds at 2700 and 3600 m above sea level were less than 32 km per hour. From the airplane, we used a camera (D5000 [with 16 megapixels], Nikon, Tokyo, Japan), zoom lens (NIKKOR 70–300 mm, Nikon, Tokyo, Japan), and camera-GPS unit (GP-1, Nikon, Tokyo, Japan) to record elk group size and the GPS coordinates of our location (in Universal Transverse Mercator [UTM]). We examined the photos and coordinates, overlaid on 1-m resolution National Agriculture Imagery Program (NAIP) 2009 imagery data (<http://datagateway.nrcs.usda.gov/>) in a GIS to determine precise locations of elk groups (rather than using the location of the camera when the photo was taken). Using these precise locations, we summed all elk groups within 500 m of one another so that one elk “group” was actually a collection of sub-groups and was defined by distance rather than behavior, which may be subjective or temporary. We used 500 m as the threshold because during several surveys sub-groups within roughly 500 m of one another were observed merging, and on other occasions it

was difficult to assign group membership for groups within 500 m of one another because of their spread (nearly overlapping) and movement (individuals walking and individuals feeding). Also, we did not separate groups by sex or conduct within group sex/age classifications because most groups (combined sub-groups within 500 m) tended to be of mixed sex and age. With these aerial survey methods, we also conducted a double observer study to assess sightability bias (Pollock and Kendall 1987). See Appendix C for a brief description of the double observer survey methods and results.

For our first fine scale measure of elk aggregation, we calculated average monthly elk density per hunt area by averaging across survey months the total number of elk sighted per month divided by the total transect area. We used this measure because elk on a winter range with consistently higher densities throughout the transmission period (February–May) may have a higher probability of infection than elk on a winter range where densities fluctuate because elk are migrating to the winter range late or departing for summer range early (Cross et al. 2007).

For the other measures of aggregation, we constructed the empirical elk group size distribution for each hunt area, and calculated mean group size, Lloyd’s crowding using $c = m + ([s^2/m] - 1)$, where m is the average group size and s is the standard deviation of the empirical group size distribution, and Lloyd’s crowding in May (Lloyd 1967). We used Lloyd’s crowding because it represents the typical group size experienced by a random elk, which will be larger than mean elk group size, and may be a better measure of force of infection than mean group size. We used Lloyd’s crowding in May because recent studies have shown that elk populations remaining on supplemental feedgrounds into May tend to have higher brucellosis seroprevalence than feedgrounds where elk depart the feedgrounds earlier (Cross et al. 2007). We also tested Lloyd’s crowding in March because *B. abortus*-triggered abortion events may peak during this time (WGFD, unpublished data). However, Lloyd’s crowding in March was very similar to Lloyd’s crowding across all months, and therefore we did not include it in the analysis.

Brucellosis datasets

We used a dataset of elk brucellosis status (0 = negative, 1 = positive), consisting of serologic test results from elk blood samples collected by hunters and researchers from 1991 to 2011 within the five herd units of interest (samples were assigned to herd unit and hunt area). We restricted these data to adult female elk to reduce the potential for confounding effects of age on time trends in brucellosis seroprevalence (i.e., log odds of testing positive over time may be higher for adults than for calves or yearlings), and because male elk are not considered important to the spread of *B. abortus* (Cheville et al. 1998).

Serologic test methods have been detailed elsewhere (Scurlock and Edwards 2010). To summarize, seropositivity was determined following current National Veterinary Services Laboratories protocols for the card test, plate agglutination, rivanol precipitation–plate agglutination, fluorescence polarization assay, and complement fixation. Serologic profiles were categorized using the U.S. Department of Agriculture brucellosis eradication uniform methods and rules for cervids (Animal Plant Health Inspection Service 2003), with the exception that all suspect reactors were considered positive. These tests were used to determine brucellosis exposure, not current infection, and they do not address the possibility of titer loss.

Statistical analysis

We used a two-tier Bayesian hierarchical model, where in the first tier we estimated rates of increase in brucellosis over time. To do this, we used a logit link function, serologic status as the binomially distributed response variable, and year (rescaled so that 1991 was year zero) as the explanatory variable. The slope term measured the log odds of testing seropositive for each one-year increase in time, and was therefore used to describe the rate of increase in brucellosis on the logit scale. We allowed this slope to vary by herd unit (population-level unit) or hunt area (sub-population unit) in order to understand how the rates of increase in brucellosis varied among elk populations and sub-populations. We did not allow the intercept to vary by herd unit or hunt area, because seroprevalence was low in the beginning of the time series across these areas (e.g., during 1991 and 1992 seroprevalence

ranged from 0 to 0.05 across herd units). We assigned this intercept a normal prior distribution with a mean of zero and a precision of 0.0001. We did not account for spatial correlation among hunt areas because the small sample size would limit our ability to estimate this in a meaningful way, and because a previous study of brucellosis in elk found that neighboring hunt areas were relatively uncorrelated with one another and, as a result, models without spatial correlations over time were better representations of the system (Cross et al. 2010a).

In the second tier of the model we assumed that the rates of increase in brucellosis (estimated from first tier of the model) were normally distributed with a mean described by a linear relationship with an aggregation measure (referring to the seven measures of aggregation; see *Methods: Broad scale density* and *Methods: Average monthly density and group size*). We assigned the intercept and slope parameters normally distributed priors with means of zero and precisions of 0.0001. We repeated these steps for each measure of elk aggregation. For a detailed description of the full Bayesian model, see Appendix A.

The goal of the model's second tier was to understand how brucellosis increases were related to each measure of elk aggregation. We acknowledge that this analysis compares rates of increase in brucellosis over 20 years (from 1991 to 2011) to aggregation measured at different time frames, but we used rates of increase in brucellosis rather than seroprevalence in a given year because (1) annual variation in both elk trend counts and seroprevalence were low, (2) there may be complicated time lags between density and seroprevalence, and (3) serostatus reflects cumulative exposure over the life of an elk, but we lacked detailed age information for the animals sampled in our study.

We used trend counts rather than data collected during our aerial surveys to calculate broad scale measures of density because we did not conduct aerial surveys throughout the herd units (population-level units) of interest (see Fig. 1) and we wanted to compare our results to previous work that also used trend counts to calculate density (Cross et al. 2010a). We used only the most recent elk trend counts to calculate hunt area (sub-population unit) and winter range density rather than average density or rate of

increase in density, because all available trend counts between 1991 and 2011 revealed (1) no consistent increases or decreases in density across hunt areas (Appendix B: Figs. B1 and B2) and (2) a fairly stable relative ranking of hunt areas by density across years (i.e., hunt areas with higher densities in the early 1990s also had higher densities at the end of the time series, and likewise for hunt areas with lower densities; Appendix B: Fig. B1). Also, trend counts were not conducted every year and no trend counts were conducted in hunt areas 51 and 99 during the three years we collected group size data (2010–2012). Finally, to calculate the finer scale measures of aggregation, we combined all three years of group size data in order to understand whether brucellosis was increasing at a faster rate in hunt areas with consistently larger groups or more elk on the winter range.

We also conducted a post hoc analysis to understand the relationship between elk aggregation and rates of increase in brucellosis among only those areas where the pathogen was known to be present, because a lack of increase in brucellosis seroprevalence may be due to the absence of *B. abortus*. To do this, we excluded data from the beginning of the time series for years in which seropositivity was zero; in these cases there was no evidence that the disease was present within a herd. Therefore, for each hunt area we included only the first year a seropositive sample was detected and all subsequent years. If a hunt area never had a seropositive sample, we removed it from the analysis. We also removed hunt areas having only one year of seropositive samples, because though there was evidence that *B. abortus* was present once, it may not have become established. We fit models to this truncated dataset following the same two-tier model framework that we fit to the full dataset (as shown in Appendix A).

We also compared models using the deviance information criterion (DIC) as a rough indices to assess whether measures of group size were better supported by the data than broad scale measures of density. All Bayesian hierarchical models were fit using the R2WinBUGS package (Sturtz et al. 2005) to call WinBUGS version 1.4.3 (Lunn et al. 2000) from R version 2.13 (R Development Core Team 2011). For each model, three chains of 100,000 iterations were run and a

burn-in period of 10,000 iterations was used before summarizing the posterior distributions. We checked convergence visually by assessing sample trace plots and by calculating the potential scale reduction factor, \hat{R} (Gelman et al. 2013), to compare within- and among-chain variance, where values of 1 to 1.1 typically indicate convergence (Gelman and Hill 2007). In a separate analysis, we examined the relationships among all aggregation measures using simple linear regression.

RESULTS

Elk aggregation patterns

Across all three years of aerial surveys, we recorded 800 groups ranging in size from 1 to 1952 elk and group size distributions were generally right skewed (Appendix B: Fig. B3). Our flights to and from each hunt area consistently revealed few elk outside the areas we delineated as winter range. Therefore, elk groups that were missed because they were outside the winter range were likely to be rare and small, and would therefore have little effect on estimates of mean group size or Lloyd's crowding (typical group size).

All measures of aggregation varied by herd unit (population-level unit; Appendix B: Table B1) and hunt area (sub-population unit; Appendix B: Table B2) and were positively related with one another (Appendix B: Fig. B4 and Table B3). However, the ranking of hunt areas varied under different measures of aggregation. In hunt area 63, for example, the average elk experienced larger groups (Lloyd's crowding = 554) but lower densities at the hunt area scale (hunt area density = 1.0 elk/km²), whereas in hunt area 52 the average elk experience moderately sized groups (Lloyd's crowding = 261) and higher densities (hunt area density = 2.9 elk/km²) (Fig. 2).

Brucellosis increases over time

The 20-year brucellosis datasets for the five herd units (population-level unit) and 10 hunt areas (sub-population unit) contained serologic results for 4448 and 2765 cow elk, respectively. Brucellosis increased over time in a majority of the herd units (Fig. 3) and hunt areas, but there was little evidence for increasing brucellosis in hunt areas 25 and 121 (Fig. 4). Therefore, across

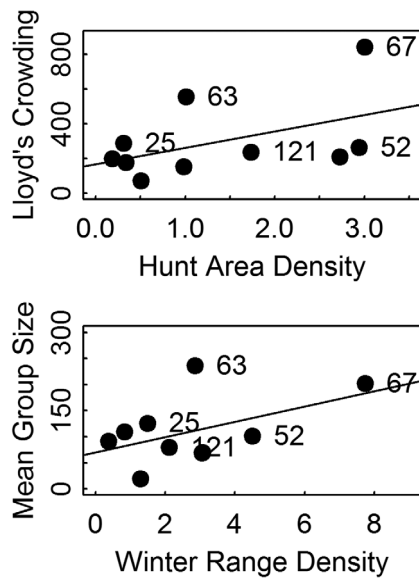


Fig. 2. Example of relationships between different measures of aggregation. Lloyd's crowding (typical elk group size) and mean group size were estimated from empirical group size distributions obtained within each of the 10 elk hunt areas (sub-population units) of interest. Hunt area and winter range elk densities were calculated from the most recent (up to 2011) annual elk trend count conducted by hunt area. The solid line is the fitted relationships estimated from linear regression models. Data labels are shown for five of the hunt areas for reference, including hunt areas 25, 52, 63, 67, and 121. See Fig. 1 for geographic distribution of hunt areas. The units of hunt area and winter range elk density are elk/km².

most of the hunt areas the posterior means and 95% credible intervals of the estimated rates of increase in brucellosis (measuring the log odds of testing seropositive for each one-year increase in time) were positive, but the posterior mean was negative for hunt area 25 and the 95% credible intervals extended below zero for hunt area 121 (Appendix B: Fig. B5). These two hunt areas (25 and 121) contained only one seropositive out of 253 samples, and therefore were excluded from the post hoc analysis that we used to estimate the rate of increase in brucellosis only where the disease was known to be present. For this analysis, we also excluded years at the beginning of the time series when brucellosis seroprevalence was zero. The resulting truncated dataset contained serologic results for 2245 cow elk

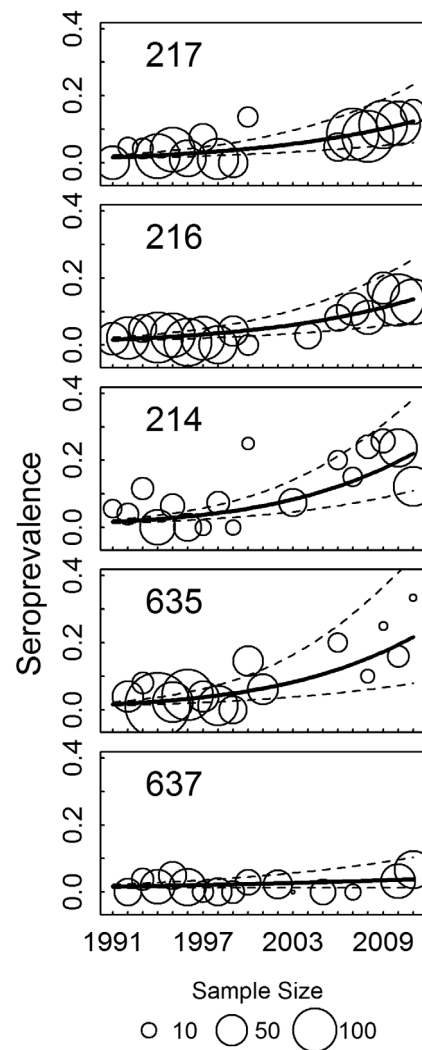


Fig. 3. Increases in brucellosis seroprevalence in elk from 1991 to 2011, estimated by elk herd unit (population-level unit) using a Bayesian hierarchical logistic regression model ($N = 4448$ serologic samples). Herd unit numbers are shown in upper left corner of each panel. See Fig. 1 for geographical distribution of herd units. Size of circles is proportional to sample size, solid black lines are the posterior means of the relationships, and dotted lines display the 95% credible intervals.

among eight hunt areas, and the number of inclusive years ranged from 5 years for hunt area 54 to 15 years for hunt areas 52 and 67. The posterior means and 95% credible intervals of the rates of increase in brucellosis estimated from the truncated dataset were similar to the rates of

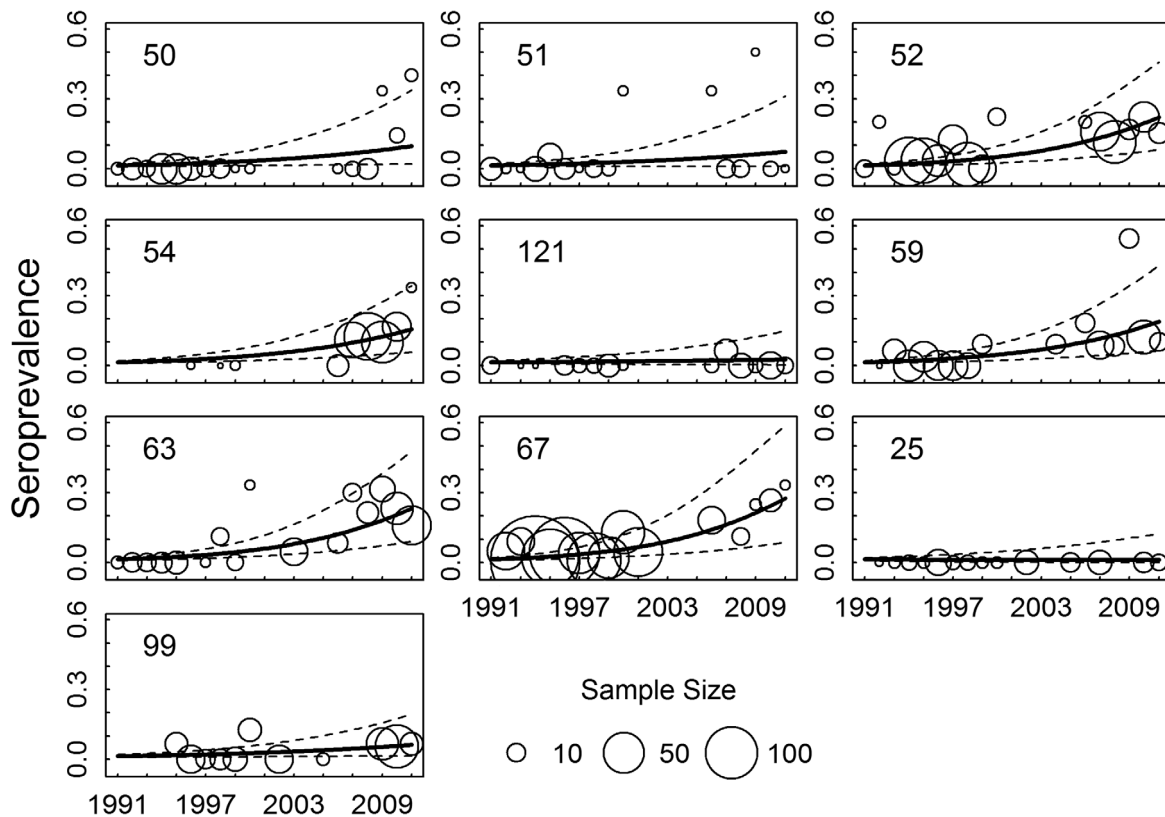


Fig. 4. Increases in brucellosis seroprevalence in elk from 1991 to 2011, estimated by elk hunt area (subpopulation unit) using a Bayesian hierarchical logistical regression model ($N = 2765$ serologic samples). Hunt area numbers are shown in upper left corner of each panel. See Fig. 1 for geographical distribution of hunt areas. Size of circles is proportional to sample size, solid black lines are the posterior means of the relationships, and dotted lines display the 95% credible intervals.

increase in brucellosis estimated from the full dataset (Appendix B: Fig. B5).

Relationship between brucellosis increases and aggregation

The rates of increase in brucellosis tended to increase with increasing aggregation for all measures of aggregation (Fig. 5; Appendix B: Fig. B6 and Table B4). As measured from the posterior distribution, the probability that elk aggregation was positively related to rates of increase in brucellosis was estimated at greater than 0.90 for winter range density, average monthly density and Lloyd's crowding (typical group size) in May (Fig. 6). For herd unit density, mean group size, Lloyd's crowding, and hunt area density, the probability that aggregation was positively related to rates of increase in brucel-

losis was estimated at 0.58, 0.79, 0.81, and 0.85, respectively (Fig. 6). After truncating the data and removing hunt areas 121 and 25, the magnitude of the relationships between aggregation and rates of increase in brucellosis decreased compared to the analysis on the full hunt area dataset (Appendix B: Fig. B7). Contrary to our expectation, DIC scores showed that no measure of aggregation was substantially better than any other at explaining changes in seroprevalence (Appendix B: Table B5).

DISCUSSION

Many species exhibit complex aggregation patterns, and for these animals it is unclear which metric of group size or density may be most relevant to disease transmission. We stud-

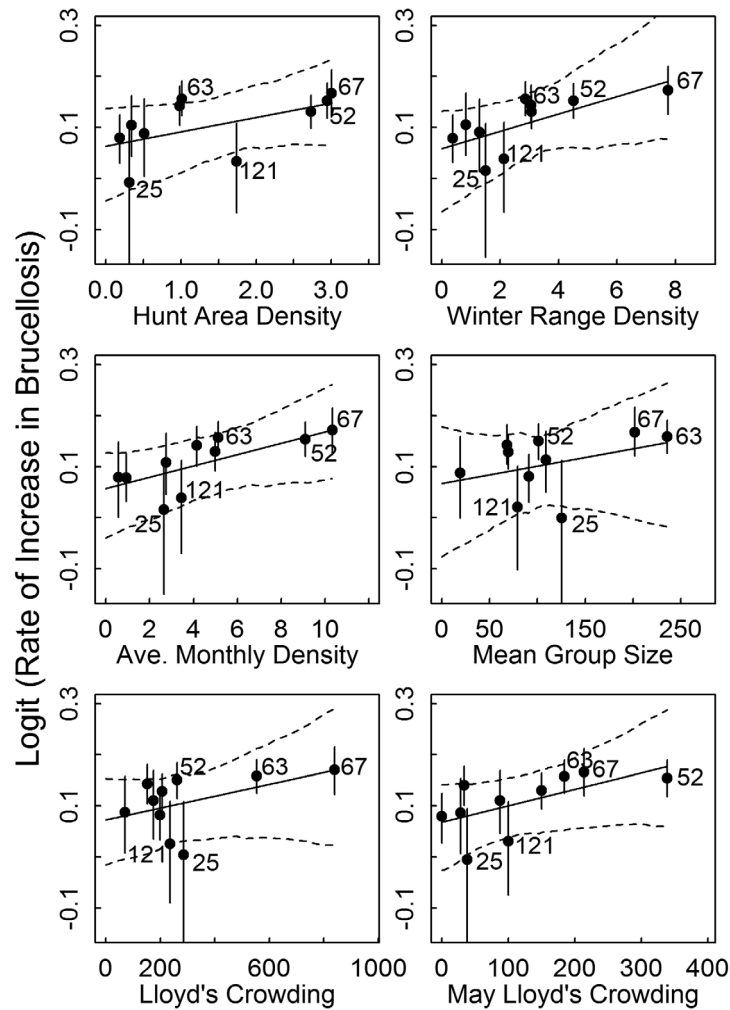


Fig. 5. Linear relationships between elk aggregation and rate of increase in brucellosis seroprevalence (estimated from first tier of hierarchical model, measuring the log odds of testing seropositive for each one-year increase in time). Diagonal solid black lines are the posterior means of these relationships and dotted lines display the 95% credible intervals. Vertical black lines are the estimated 95% credible intervals for each hunt area-specific rate of brucellosis increases. Data labels are shown for five of the hunt areas for reference, including hunt areas 25, 52, 63, 67, and 121. See Fig. 1 for geographic distribution of hunt areas. Panels are organized by the broadest measure of elk density (upper left: hunt area density) to the finest scale of elk group size (lower right: May Lloyd's crowding). The units of all density measures are elk/km².

ied this issue among elk populations that have been exposed to *Brucella abortus* because elk have a wide range of group sizes and exhibit seasonal aggregation patterns similar to many other large herbivores, and because studies of brucellosis dynamics have broad implications due to the disease's global impact on wildlife and livestock. In our study, rates of increase in brucellosis seroprevalence were positively related to all

measures of aggregation we considered, and all models were equally supported by the data. Therefore, mean and larger (Lloyd's crowding) group sizes measured at different points during the transmission period did not explain rates of increase in brucellosis any better than broad scale measures of annual population or sub-population density. These results suggest that patterns of aggregation related to both within- and

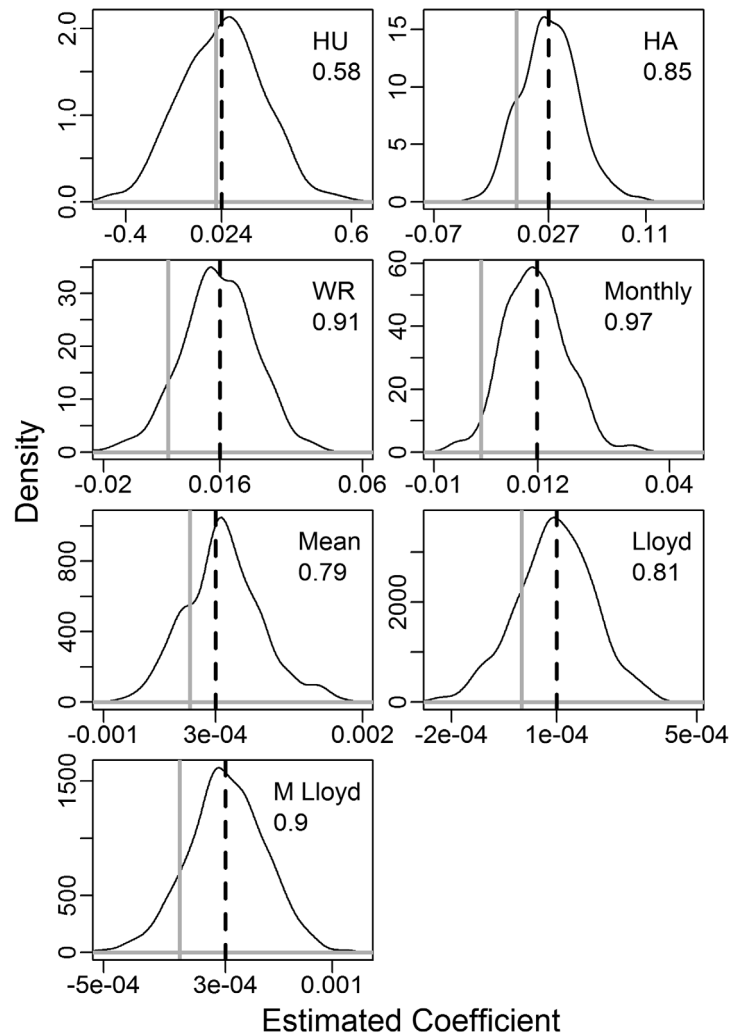


Fig. 6. Posterior distributions of the relationships estimated between measures of elk aggregation and rates of increase in brucellosis seroprevalence. Dotted black lines are the means of the posterior distributions and the grey lines mark zero along both axes. The upper right of each panel contains the aggregation measure and the probability that each measure was positively related to the rates of increase in brucellosis (where the posterior distributions were greater than zero). HU = herd unit density, HA = hunt area density, WR = winter range density, Monthly = average monthly density, Mean = mean group size, Lloyd = Lloyd's crowding, M Lloyd = Lloyd's crowding in May.

between-group transmission may be increasing the spread of *B. abortus* among elk. Such complex transmission mechanisms may occur, for example, in animals with distinct social groups that are highly connected due to group-group contact and between-group movement of certain individuals (e.g., Craft et al. 2011), and may explain wildlife systems where the prevalence of disease is not described well by a linear relationship with

host density (e.g., Smith et al. 2009, Storm et al. 2013). However, few studies have evaluated and compared the effects of density and group size measured at multiple scales or examined the relationship between density of a large mammal and rate of increase in pathogen seroprevalence.

The similarity in fit among the models we used to examine the relationship between rates of increasing brucellosis and each measure of

aggregation was not surprising because these measures of aggregation were positively related to one another, though with varying degrees of association. For example, 61% of the variation in Lloyd's crowding (typical group size) and 49% of the variation in May Lloyd's crowding was explained by the variation in winter range density (Appendix B: Fig. B4 and Table B3), and therefore larger elk groups were found more often in higher density winter ranges. However, much of the variation in group size was not explained by winter range density. We found that among hunt areas (sub-population units) with similar rates of increase in brucellosis there were some areas with high densities and large groups, some areas with high densities and smaller groups, and some areas with low densities and larger groups. Moreover, another study of elk in the GYA also found that the tail of the group size distribution was only weakly related to population density (Proffitt et al. 2012), and comparisons of mean group size and population density have produced mixed results (Hebblewhite and Pletscher 2002, Proffitt et al. 2012). This suggests there may be more than one way for elk to be "dense" and spread diseases such as brucellosis. In this case, even though large elk groups are likely to increase within-group transmission, between-group transmission may be less frequent if those large groups are distributed across a large area. On the other hand, as elk densities increase, an area with many small groups may facilitate between-group transmission via fetuses that persist on the landscape for several days to weeks. Thus, we expect that directly transmitted pathogens that do not survive long in the environment may be more correlated with group size and within-group transmission, whereas pathogens that are primarily transmitted by prolonged environmental contamination may be more correlated with broader measures of density and between-group transmission.

For *B. abortus* in elk, most transmission probably occurs shortly after abortion events, but transmission via prolonged environmental contamination may also be possible under certain conditions (cold, wet areas with few scavengers; Aune et al. 2012). When modelling disease dynamics for pathogens that are transmitted primarily by contact with a contaminated environment, it is often assumed that transmission

rates will be more related to the proportion of infected individuals (frequency-dependent transmission) than population density (density-dependent transmission) (May and Anderson 1978, Getz and Pickering 1983). This distinction may also depend on the scale of the model, where transmission is better explained by density-dependent models at local scales and frequency-dependent models across populations (Ferrari et al. 2011). However, for diseases such as chronic wasting disease (CWD) that are both directly transmitted among individuals and indirectly transmitted by contact with contaminated soil or water, transmission dynamics may be better represented by models intermediate to classic frequency- or density-dependent models (Schauber and Woolf 2003, Storm et al. 2013). This may also be the case for *B. abortus*-transmission and could explain the weak relationships between measures of aggregation and rates of increasing brucellosis. It is also possible that the weak relationships were because larger groups have a greater proportion of juveniles than small groups, and juveniles have a lower probability of testing positive than adults. We were unable to test this however, because we did not classify groups by age.

Our findings suggest that brucellosis management strategies aimed at only reducing the total elk population size may not be effective at reducing transmission. Across our study region for example, we found elk group sizes of greater than 500 elk in populations with relatively high hunt area densities (3.0 elk/km²) and in populations with relatively low hunt area densities (0.3 elk/km²), suggesting that large groups and within-group transmission could occur even when high density populations are reduced by tenfold. Moreover, brucellosis appeared to be increasing over time across a wide range of densities and conditions. As a result, our model estimates suggest that substantial and unrealistic decreases in density or group size would be needed to prevent brucellosis increases. For example, winter range densities of 7.7 elk/km² would need to be reduced to about 0.5 elk/km² in order to reduce the odds of testing positive over one year from 1.17 (a 17% increase in the odds of testing positive with each additional year, and increases in seroprevalence from 0.01 to 0.24 over 20 years) to 1.07 (a 7% increase in the odds of

testing positive with each additional year, and increases in seroprevalence from 0.01 to 0.05 over 20 years), but this level of offtake would not be practical given other elk management priorities (WGFD 2011).

By examining measures of group size, our study builds upon previous work that examined the relationship between rates of increasing brucellosis and only hunt area elk density (Cross et al. 2010a). We compared our analysis to the results of this other study, which used 6458 serological tests from 1991 to 2008 across 34 hunt areas and a similar Bayesian hierarchical logistic regression model, and found that the magnitude of the relationship between hunt area density and logit-scale rate of increase in brucellosis seroprevalence was similar across the two studies (the posterior mean was 0.028 in our study vs. a posterior mean of 0.024 in the previous study). However, uncertainty in the estimated posterior mean was greater in our study (the posterior standard deviation was 0.027 in our study vs. a posterior standard deviation of 0.015 in the previous study), probably due to the smaller sample size (2765 serological tests in our study vs. 6458 in previous study).

Our study also differs from this previous work because we refined our analysis to include only the eight elk populations where brucellosis was known to be present. We found that when including only these populations, the relationship between aggregation and rates of increase in brucellosis was much weaker (posterior mean decreased by roughly half) compared to the relationship estimated across all 10 populations (Appendix B: Table B4). A possible explanation is that there is more movement into areas with higher elk densities as animals search for similar grazing opportunities or escape from predation. As a result, these areas have a higher probability of repeated introduction of *B. abortus* that could lead to disease establishment within the population. Once the disease is established, however, it is likely to spread at similar rates under a wide range of densities and group sizes (Fig. 5).

Beyond studies of brucellosis, a key component of our study was to examine the density-transmission relationship across multiple scales of density (or aggregation), because often only one scale is examined (e.g., Cavanagh et al. 2004, Ezenwa 2004, Smith et al. 2009). Similar to our

use of hunt area density, annual population densities are often calculated within defined management units even when these unit-boundaries include areas not used by the animal. For this reason, we refined hunt area density to winter range density in order to focus on the area used by elk during the transmission period. We found that both densities were similarly related to rates of increase in brucellosis, which was not unexpected due to the relationship between these two density measures. However, these measures may not be highly associated for other animals, and relating disease prevalence to animal density at particular places and times may be an important consideration, particularly where seasonal differences in habitat use (i.e., resulting in differences in area occupied) cause densities to change within a year even though population totals remain relatively constant (Altizer et al. 2006, Cross et al. 2007). It may also be important in social species to describe the group size distribution and how it relates to population density and disease prevalence. If the group size distribution remains constant even as abundance changes (e.g., Cross et al. 2009, Cross et al. 2013), for example, disease control strategies aimed at reducing the total number of individuals may not reduce transmission.

In conclusion, social behaviors can affect how individuals contact one another and spread disease. We provide evidence that the spread of disease may be driven by both within- and between-group transmission in species with complex aggregation patterns. If these patterns were influenced by the effects of aggregation on disease-establishment within a population, preventing brucellosis from reaching other disease-free populations may require understanding the drivers of dispersing infectious animals and the factors promoting high densities of elk. *B. abortus*-transmission dynamics may also be affected by exposure to long-term environmental contamination, but we do not know how the probability of contacts changes with the time since abortion event on native winter range. The variability in density, group size, and rates of increase in brucellosis among hunt areas highlights the importance of evaluating spatial differences in disease prevalence and aggregation patterns. As many avian and mammalian species have complex aggregation patterns, considering

the multiple spatial and temporal scales that animals aggregate could lead to a better understanding of pathogen transmission dynamics in social species.

ACKNOWLEDGMENTS

We thank the Wyoming Game and Fish Department for collecting, analyzing, and compiling the serologic data. We thank D. Stinson for his flying around the mountains of Wyoming and for keeping us safe in the air. We also thank K. Overfield and S. Ard for their dedicated flying, and C. Butler for his hard work as an observer. We are grateful to Teton and Cody Interagency Dispatch for keeping up with our progress in flight. This work was supported by the National Science Foundation and National Institutes of Health Ecology of Infectious Disease (grant number DEB-1067129) and the U.S. Geological Survey. Any mention of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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SUPPLEMENTAL MATERIAL

APPENDIX A

Bayesian model description

Table A1. Description of the Bayesian hierarchical models used for estimating rates of increase in brucellosis seroprevalence, and for estimating the linear relationship between these rates of increase and each measure of elk aggregation.

Equation	Description
$y_{ij} \sim \text{Bin}(p_{ij})$	Let y_{ij} be the exposure status determined by serology for individual elk i in region j (either herd unit 1, 2, ..., 5 or hunt area 1, 2, ..., 10). We modeled y_{ij} as a binomially distributed response variable, where p_{ij} was the probability of testing positive.
$\text{logit}(p_{ij}) = \alpha + \beta_j \times \text{year}_{ij}$	We used a logit link to relate p_{ij} to year (rescaled so that 1991 was zero), and we allowed the slope (β) to vary by region j (elk herd unit or hunt area). Year_{ij} refers to the year from which the i th elk came.
$\alpha \sim N(0, 0.0001)$	We estimated a common intercept term (not forced through zero, but not allowed to vary by region j). We assumed the intercept came from a normal distribution with mean of zero and precision of 0.0001.
$\beta_j \sim N(\mu_j, \tau)$	We assumed the region-specific slopes came from a normal distribution with a mean (μ) estimated from the linear model below, and a standard deviation (τ). In the text of this article, we referred to these herd unit or hunt area-specific slopes as rates of increase in brucellosis seroprevalence, which measure the log odds of testing seropositive for each one-year increase in time.
$\mu_j = \delta + \psi \times \text{agg}_j$	We hypothesized that the rates of increase in brucellosis seroprevalence were linearly related to measures of aggregation (agg = herd unit density, hunt area density, winter range density, cumulative density, mean group size, Lloyd's crowding, or Lloyd's crowding in May).
$\sigma \sim \text{Uniform}(0, 20) \quad \tau = 1/\sigma$	We assumed the standard deviation was uniformly distributed from 0 to 20 on the prior distribution.
$\delta \sim N(0, 0.0001)$	We assumed the intercept term came from a normal distribution with mean of zero and precision of 0.0001.
$\psi \sim N(0, 0.0001)$	We assumed the slope term came from a normal distribution with mean of zero and precision of 0.0001.

Note: For each model (separate model for each measure of aggregation), three MCMC chains of 100,000 iterations were run with a burn-in period of 10,000 iterations.

APPENDIX B

Supporting tables and figures

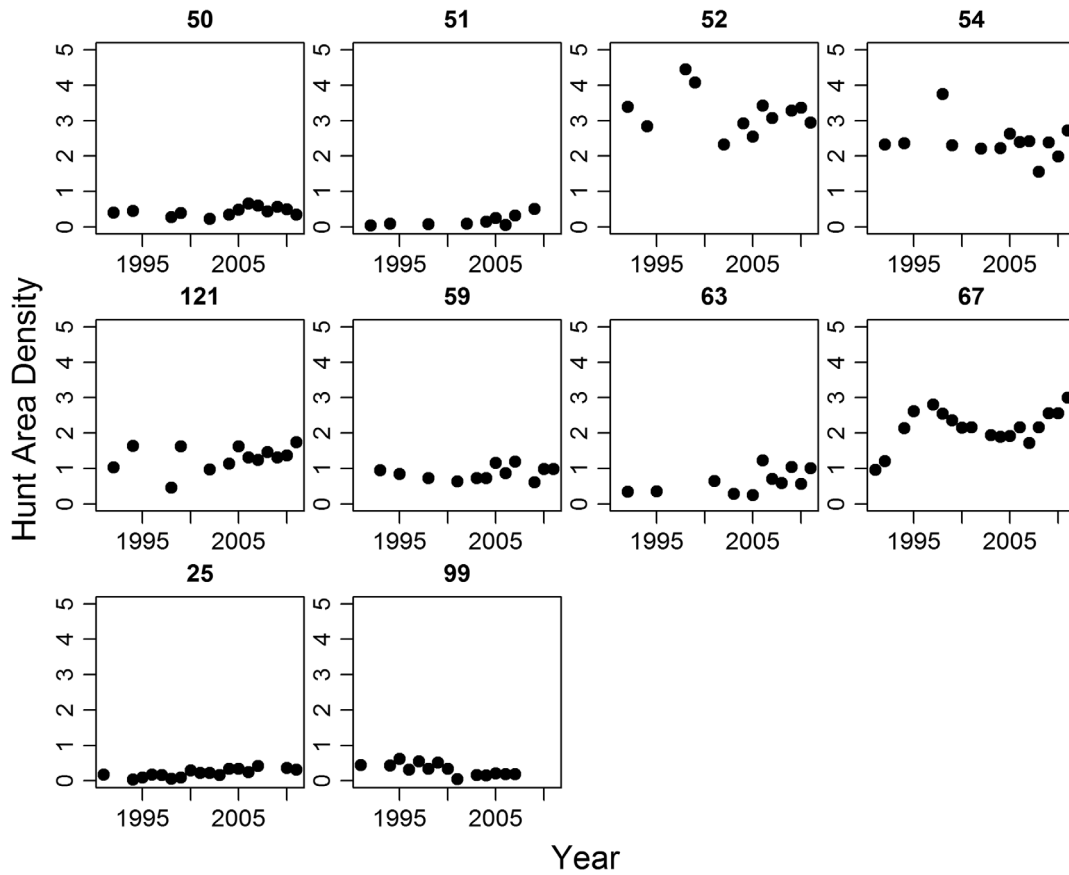


Fig. B1. Hunt area elk density (elk/km²) by year (from 1991 to 2011) for the 10 hunt areas arranged from north (upper left) to south (lower middle). See Fig. 1 for geographical distribution of hunt areas. Hunt area densities were calculated using annual elk trend counts obtained from the Wyoming Game and Fish Department (WGFD 2011).

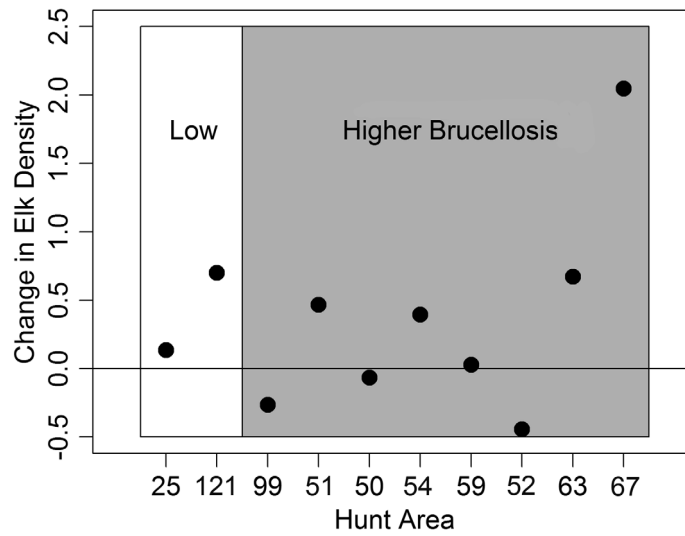


Fig. B2. The change in hunt area elk density (elk/km²) between the earliest and most recent trend counts conducted from 1991 to 2011 across the 10 hunt areas of interest (change in elk density = most recent trend count minus the earliest trend count). From left to right, elk hunt areas are arranged from lowest to highest rate of increasing brucellosis. The “Low” box indicates hunt areas that had fewer than two years of brucellosis seropositive animals. The “Higher Brucellosis” box indicates hunt areas that had two or more years of brucellosis seropositive animals.

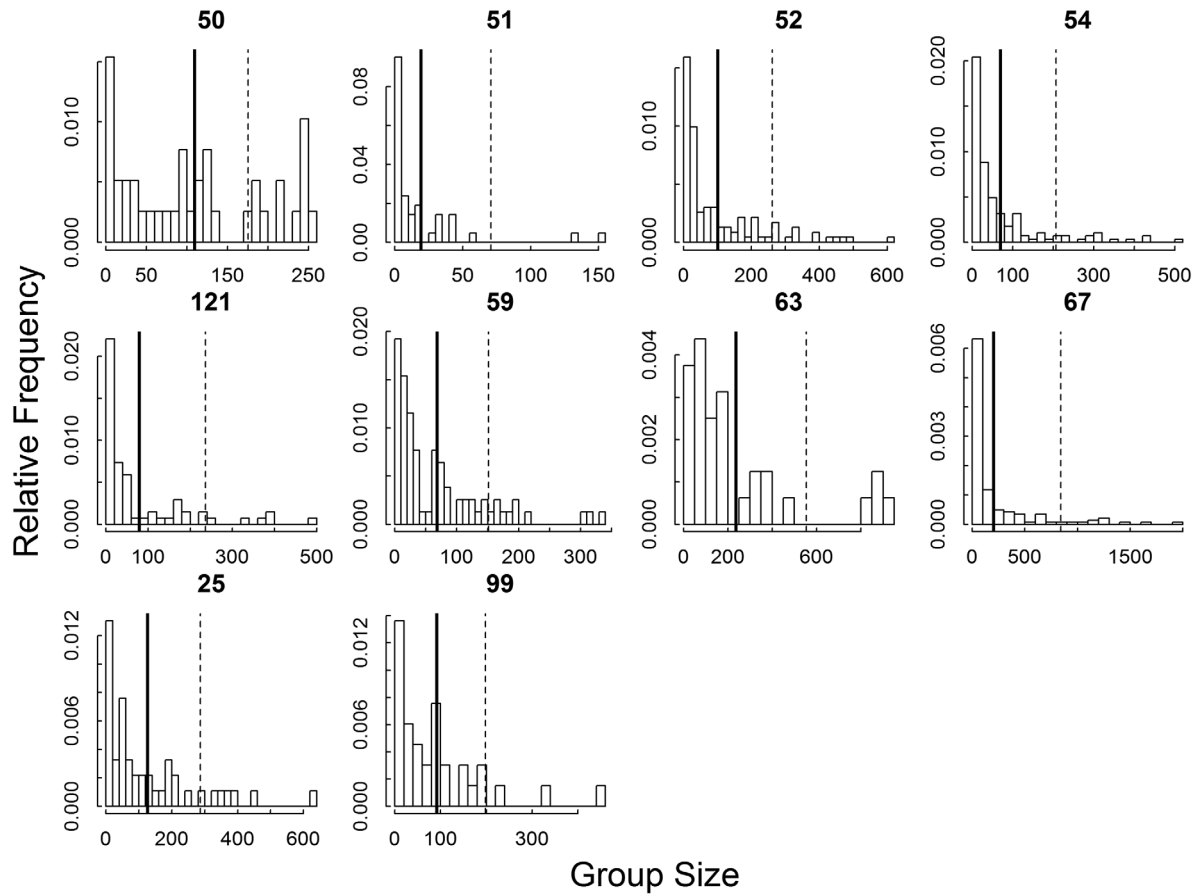


Fig. B3. Elk group size distributions for the 10 hunt areas arranged from north (upper left) to south (lower middle). The distributions are plotted as relative frequencies, where each histogram has a total area equal to 1. Hunt area numbers are listed above each histogram (see Fig. 1 for geographical distribution of hunt areas); solid black lines are mean group size and dotted lines are Lloyd's crowding.

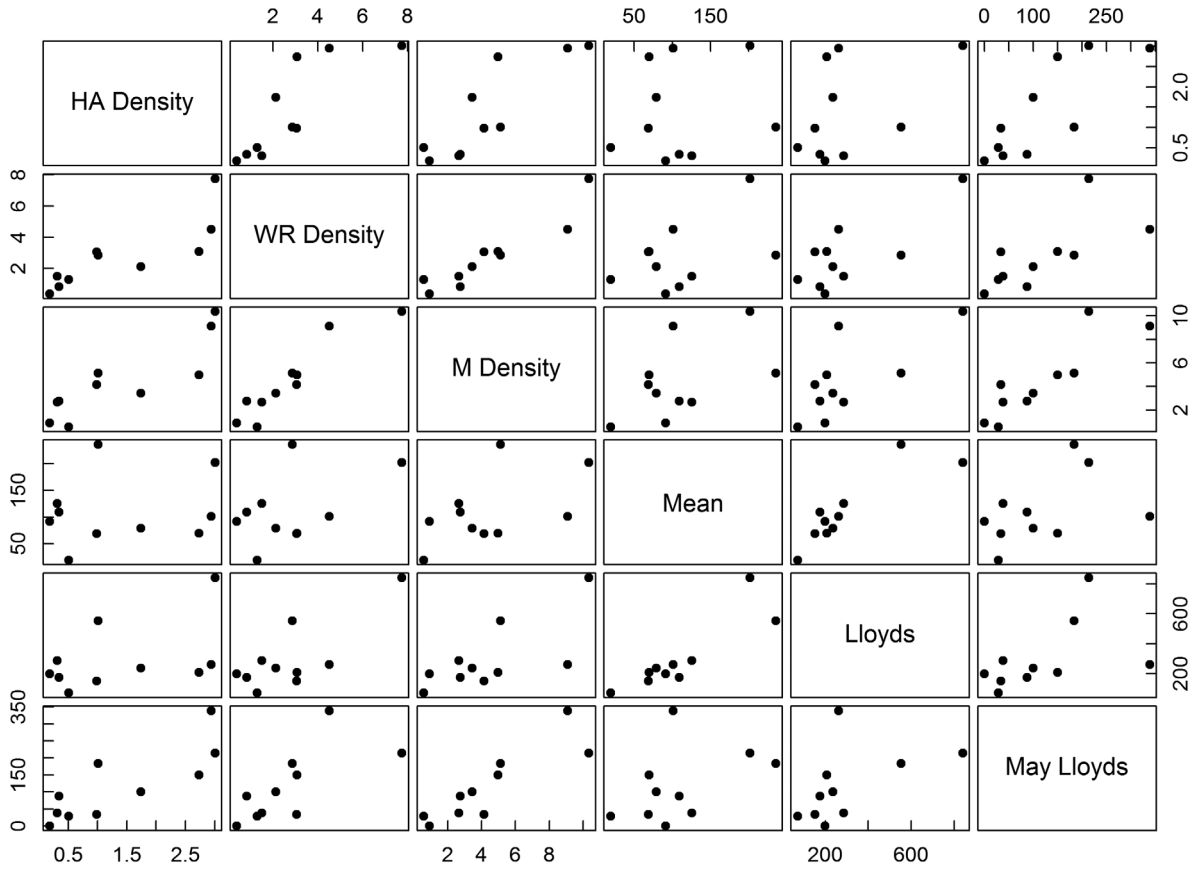


Fig. B4. Scatterplot matrix of six elk aggregation measures. HA = hunt area, WR = winter range, M = average monthly, Mean = mean group size, Lloyds = Lloyd's crowding, May Lloyds = Lloyd's crowding in May. The units of all density measures are elk/km².

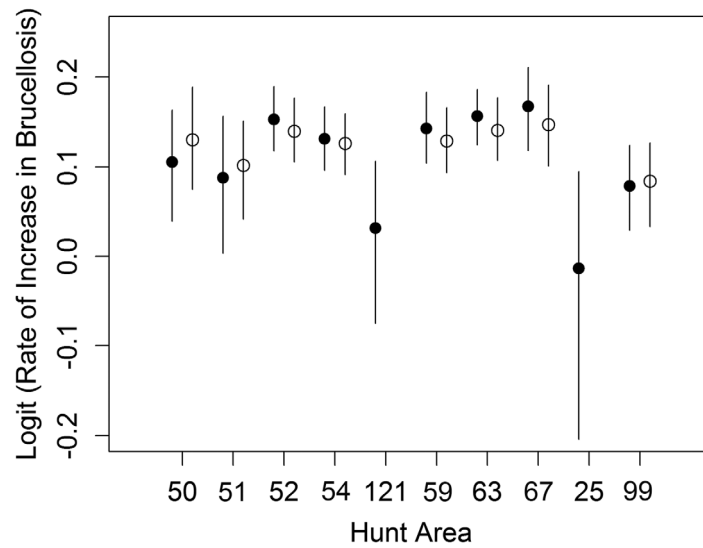


Fig. B5. Comparison of the rates of increase in brucellosis seroprevalence. Closed symbols are posterior means of the rates of increase in brucellosis estimated from all 10 elk hunt areas and open symbols were estimated from the eight elk hunt areas where brucellosis was known to be present (which excluded initial years of zero seropositives, and excluded hunt areas 121 and 25). Bars are estimated 95% credible intervals.

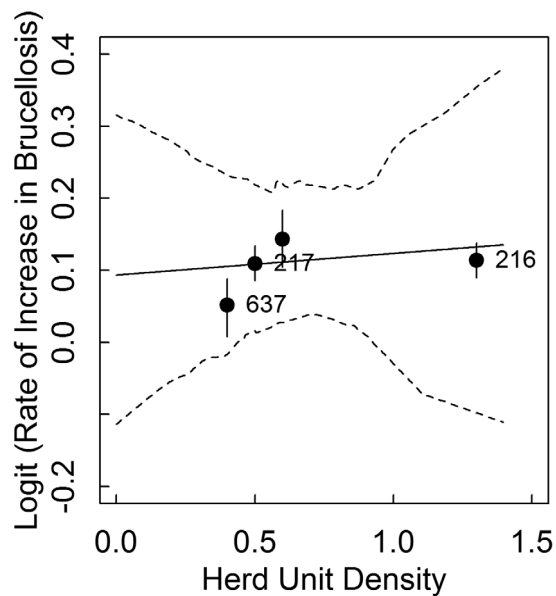


Fig. B6. The linear relationship between herd unit elk density (population-level density; elk/km²) and rates of increase in brucellosis seroprevalence. Points are labeled by several herd units for reference. See Fig. 1 for the geographical distribution of herd units. Diagonal solid black lines are the posterior means of these relationships and dotted lines display the 95% credible intervals. Vertical black lines are the estimated 95% credible intervals for each herd unit-specific rate of brucellosis increases.

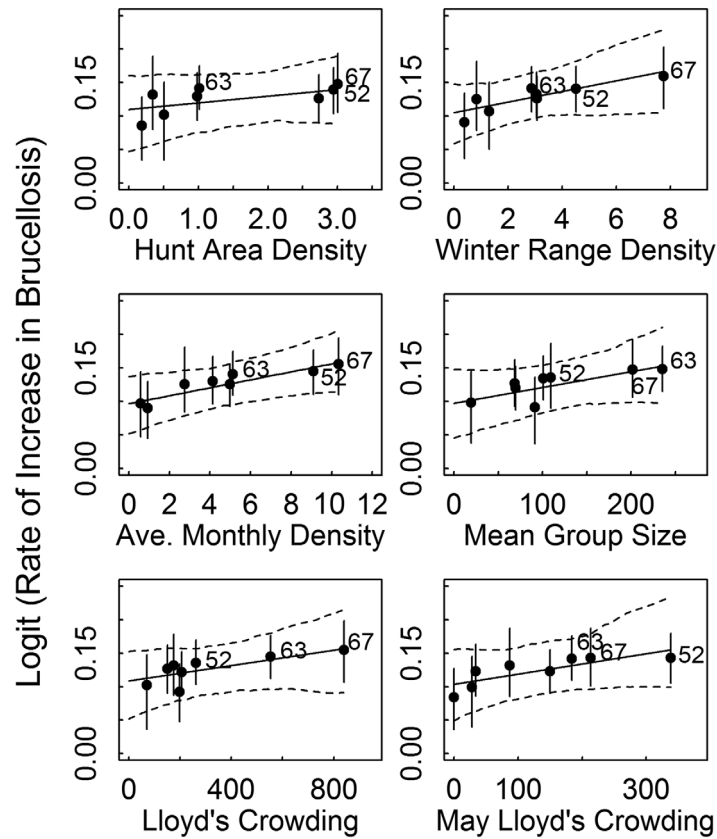


Fig. B7. Linear relationships between elk aggregation and rates of increase in brucellosis seroprevalence fitted to the truncated dataset, which included only the eight elk hunt areas with at least two years of seropositive test results and excluded early years in the time series when brucellosis seroprevalence was zero. Diagonal solid black lines are the posterior means of the relationships, dotted lines display the 95% credible intervals, and vertical black lines are the estimated 95% credible intervals of the hunt area-specific rates of brucellosis increases. Data labels are shown for three of the hunt areas for reference, including hunt areas 52, 63, and 67. See Fig. 1 for geographic distribution of hunt areas. Panels are organized by the broadest measure of elk density (upper left: hunt area density) to the finest scale of elk group size (lower right: May Lloyd’s crowding). The units of all density measures are elk/km².

Table B1. Herd unit (HU) elk densities.

Herd unit	HU density (elk/km ²)
217	0.5
216	1.3
214	0.6
635	0.6
637	0.4

Table B2. Six measures of elk aggregation for the 10 hunt areas of interest.

Hunt area	HA density (elk/km ²)	WR density (elk/km ²)	Average monthly density (elk/km ²)	Mean group size	Lloyd's crowding	May lloyd's crowding
50	0.3	0.8	2.7	109	175	87
51	0.5	1.3	0.6	19	70	28
52	2.9	4.5	9.1	101	261	339
54	2.7	3.1	5.0	69	207	150
121	1.7	2.1	3.4	79	236	100
59	1.0	3.1	4.1	68	151	34
63	1.0	2.9	5.1	235	554	184
67	3.0	7.7	10.3	201	840	214
25	0.3	1.5	2.7	125	286	38
99	0.2	0.4	0.9	91	198	0

Note: HA = hunt area; WR = winter range.

Table B3. Correlation matrix (Pearson's *r*) of six measures of elk aggregation.

Measure of elk aggregation	HA density	WR density	M density	Mean	Lloyd's	May Lloyd's
HA density	1.00
WR density	0.83	1.00
M density	0.86	0.94	1.00
Mean	0.19	0.49	0.54	1.00
Lloyd's	0.47	0.78	0.72	0.88	1.00	...
May Lloyd's	0.81	0.70	0.88	0.44	0.50	1.00

Note: Coefficients in bold are values greater than 0.70. HA = hunt area; WR = winter range; M = average monthly; Mean = mean group size; Lloyds = Lloyd's crowding; May Lloyds = Lloyd's crowding in May.

Table B4. Estimated posterior means (coefficient) and standard deviations (SD) for the relationships between elk aggregation and rates of increase in brucellosis seroprevalence.

Measure of elk aggregation	Full dataset	Truncated dataset
HU density	0.036 (0.16)	...
HA density	0.028 (0.024)	0.010 (0.013)
WR density	0.017 (0.011)	0.0079 (0.0055)
Average monthly density	0.012 (0.0073)	0.0059 (0.0034)
Mean group size	0.00033 (0.00046)	0.00022 (0.00019)
Lloyd's crowding	0.00010 (0.00011)	0.000060 (0.000052)
May Lloyd's crowding	0.00031 (0.00025)	0.00015 (0.00011)

Note: Full dataset = all years and the five herd units of interest (for HU density) or all years and the 10 hunt areas of interest (for all other measures of aggregation); truncated dataset = only the eight elk hunt areas with two or more years of seropositive test results and excludes early years in the time series where brucellosis seroprevalence was zero. HU = herd unit; HA = hunt area; WR = winter range. An ellipsis indicates that HU density was not calculated for this dataset.

Table B5. Comparison of estimated deviance information criteria (DIC) for the relationships between elk aggregation and rates of increase in brucellosis seroprevalence.

Measure of elk aggregation	Full dataset		Truncated dataset	
	DIC	ΔDIC	DIC	ΔDIC
HA density	1130.6	0.2	1082.8	1.1
WR density	1131.5	1.1	1082.7	1.0
Average monthly density	1132.0	1.6	1081.7	0
Mean group size	1130.9	0.5	1082.3	0.6
Lloyd's crowding	1131.6	1.2	1082.6	0.9
May Lloyd's crowding	1130.4	0	1082.2	0.5

Note: Full dataset = all years and elk hunt areas; truncated dataset = only the eight elk hunt areas with two or more years of seropositive test results and excludes early years in the time series where brucellosis seroprevalence was zero; ΔDIC = difference from the lowest DIC value; HA = hunt area; WR = winter range. The DIC for herd unit density was estimated from a different dataset and therefore not included in this comparison.

APPENDIX C

Double observer methods and results

We used a double observer method to assess sightability bias (Pollock and Kendall 1987). To do this, two observers flew in separate airplanes in tandem over three winter ranges (one winter range in March 2012 and two winter ranges in April 2012) and recorded elk group sizes as described in the Methods section of the manuscript. We determined the difference in total number of groups and total elk seen between the two observers, and estimated the difference in our group size measures of interest.

The two observers recorded 33 and 32 elk groups, and 3084 and 3067 total elk, respectively. Observer one missed three of the groups recorded by observer two, and observer two missed four of the groups recorded by observer one. These missed groups were all small, ranging in size from three to 24 elk with an average of 11 elk. The mean group size recorded by the two observers was 138 and 123, respectively, and the Lloyd's crowding (typical elk group size) determined by each observer was 651 and 648, respectively.