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Herd-level risk factors for *Campylobacter fetus* infection, *Brucella* seropositivity and within-herd seroprevalence of brucellosis in cattle in northern Nigeria

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ABSTRACT

Brucellosis and campylobacteriosis are economically important diseases affecting bovine reproductive efficiency in Nigeria. A questionnaire-based survey was conducted in 271 cattle herds in Adamawa, Kaduna and Kano states of northern Nigeria using multistage cluster sampling. Serum from 4745 mature animals was tested for *Brucella* antibodies using the Rose-Bengal plate test and positives were confirmed in series-testing protocol using competitive enzyme-linked immunosorbent assay. Preputial scrapings from 602 bulls were tested using culture and identification for *Campylobacter fetus*. For each disease, a herd was classified as positive if one or more animals tested positive. For each herd, information on potential managemental and environmental risk factors was collected through a questionnaire administered during an interview with the manager, owner or herdsman. Multiple logistic regression models were used to model the odds of herd infection for each disease. A zero-inflated Poisson model was used to model the count of *Brucella*-positive animals within herds, with the number tested as an exposure variable. The presence of small ruminants (sheep and/or goats) on the same farm, and buying-in of >3 new animals in the previous year or failure to practice quarantine were associated with increased odds of herd-level campylobacteriosis and brucellosis, as well as increased within-herd counts of *Brucella*-positive animals. In addition, high rainfall, initial acquisition of animals from markets, practice of gynaecological examination and failure to practice herd prophylactic measures were positively associated with the odds of *C. fetus* infection in the herd. Herd size of >15, pastoral management system and presence of handling facility on the farm were associated with increased odds, and gynaecological examination with reduced odds of herd-level *Brucella* seropositivity. Furthermore, the zero-inflated Poisson model showed that borrowing or sharing of bulls was associated with higher counts, and provision of mineral supplement with lower counts of *Brucella*-positive cattle within herds. Identification of risk factors for bovine campylobacteriosis and brucellosis can help to identify appropriate control measures, and the use of zero-inflated count model can provide more specific information on these risk factors.

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1. Introduction

Brucellosis and campylobacteriosis are economically important bacterial diseases of cattle that are prevalent in Nigeria (Mai et al., 2012; Mshelia et al., 2010a). Brucellosis is a zoonotic disease that is caused by *Brucella abortus* and *B. melitensis*. Although serology alone cannot identify the *Brucella* spp. (Godfroid et al., 2013), only *B. abortus* has been isolated from cattle, sheep and horses in Nigeria (Ocholi et al., 2004a). Although *B. melitensis* is the causative agent of brucellosis in small ruminants, outbreaks of *B. melitensis* in cattle have become a worldwide emerging problem (Álvarez et al., 2011). Brucellosis is transmitted from animals to man through ingestion of raw milk or unpasteurized cheese or direct contact with infected materials via abraded skin (Solorio-Rivera et al., 2007; Godfroid et al., 2011; Junaidu et al., 2011). Transmission among animals is mainly through mucous membranes following contact with contaminated materials, by inhalation and *in utero* (Queipo-Ortuno et al., 1997; Solorio-Rivera et al., 2007). Infected animals spread brucellosis horizontally at parturition or to their calves *in utero* or via their milk (Nicoletti, 1980; Bale and Nuru, 2001). The disease is endemic in sub-Saharan Africa and the prevalence varies according to agro-ecological region (McDermott and Arimi, 2002; Muma et al., 2007a). Bovine genital campylobacteriosis is a venereally transmitted disease that is caused by *Campylobacter fetus venerealis* (Devenish et al., 2005). It is also transmitted through artificial insemination (AI) using frozen infected semen, even with the use of antibacterial agents (Garcia et al., 1983), and via contaminated semen collection equipment (OIE, 2011). Artificial insemination using uninfected semen remains the single most effective means of preventing spread of campylobacteriosis (Irons et al., 2004). Other infectious reproductive diseases of cattle such as neosporosis and bovine viral diarrhoea have been reported elsewhere in Africa (Asmare et al., 2012) but little is known of their occurrence in Nigeria.

In Nigeria, free movement and intermingling of the nomadic and extensively managed Fulani herds encourages the spread of brucellosis (Bale and Kumi-Diaka, 1981; Ocholi et al., 2004a). Similar observations have been made in other parts of Africa (Berhe et al., 2007; Matope et al., 2010), where brucellosis often began during adverse weather conditions and famine (Musa et al., 1990). However, prevalence may also be high in intensively managed herds (Karimuribo et al., 2007; Jergefa et al., 2009), whereas extensive management in smallholder farms may limit the spread of infection (Madsen, 1989). Trade cattle, especially across Nigeria's northern borders with Chad and Niger, show evidence of infection (Esuruoso, 1974). This is in agreement with reports by Cadmus et al. (2008) in Nigeria and Kubuafor et al. (2000) in Ghana. Possibilities also exist of the transmission of *Brucella* spp. from wildlife to domestic cattle (Avong, 2000; Muma et al., 2007b). Indiscriminate buying-in of animals without quarantine (Bale and Kumi-Diaka, 1981) and mixing of cattle with sheep and goats and sometimes horses (Ocholi et al., 2004b) may disseminate brucellosis. Increased susceptibility of some breeds of cattle to brucellosis has been reported (Karimuribo et al., 2007; Junaidu et al., 2011). A higher prevalence in older cattle (Kubuafor et al., 2000; Samaha et al., 2009), females (Mekonnen et al., 2010), males (Chimana et al., 2010), non-pregnant cows (Ibrahim et al., 2010) and lactating cows (Nicoletti, 1980) has been reported in Africa and elsewhere.

The prevalence of brucellosis is therefore influenced by husbandry and management system, herd size, population density, age, sex, type of animal, hygiene, socio-economic factors, herd immunity and adequacy of veterinary services (Crawford et al., 1990; Omer et al., 2000; Ocholi et al., 2004a; Mekonnen et al., 2010), as well as intensity of contact with infected herds and with contaminated environmental sources (Madsen, 1989; Megersa et al., 2011). However, risk factors observed in one particular agro-ecological region do not necessarily apply to other areas with different ecological settings and husbandry practices (Matope et al., 2010; Mekonnen et al., 2010).

The following factors have been associated with the introduction or spread of bovine genital campylobacteriosis: introduction of cows and heifers from endemically infected herds (Woldehiwet et al., 1989), importation of bulls for cross-breeding purposes (Nuru, 1974) and lack of effective control of mass cattle movements across international borders (Mshelia et al., 2010b). Other factors associated with campylobacteriosis include the use of communal bulls or having more than one bull in a herd (Mukasa-Mugerwa, 1989), communal grazing (Pefanis et al., 1988), lack of vaccination (Hoffer, 1981), genetic differences in susceptibility between different cattle lines (Dufty et al., 1975), contact with contaminated bedding, fomites or mechanical transmission (Hjerpe, 1990), and contact between infected and non-infected bulls (Hoffer, 1981; Mukasa-Mugerwa, 1989). Transmission of infection between cows probably does not occur naturally (Clark, 1971). Young bulls under five years of age are difficult to infect (Samuelson and Winter, 1966); however, both younger and older bulls could remain carriers for up to 18 weeks post-infection (Bier et al., 1977) and bulls older than three years may remain permanently infected. Heifers and cows of all ages are susceptible (Dufty et al., 1975; Irons et al., 2004); however, the infection is more persistent in heifers than in cows (Dufty and Vaughan, 1993).

Campylobacteriosis and brucellosis cause heavy economic losses resulting from abortions, herd infertility, embryo mortality, irregular oestrus, reduced pregnancy rate, increased calving intervals, birth of weak calves, increased culling rates, decreased milk production and veterinary costs (Hum, 1987; Bawa et al., 1991; Ariza et al., 1995; Devenish et al., 2005; Mekonnen et al., 2010). Despite reports on the distribution of campylobacteriosis and brucellosis in cattle in Nigeria and their increase in prevalence (Ocholi et al., 2004a; Mshelia et al., 2010a; Mai et al., 2012), little information on risk factors for brucellosis or campylobacteriosis has been generated from representative, well designed epidemiological studies, using appropriate multivariable methods to control for confounding. Knowledge of risk factors for the diseases is essential for the development of cost-effective and efficient control programmes. The aim of this study was to identify herd-level risk factors associated with campylobacteriosis and brucellosis, as well as factors associated with the within-herd seroprevalence

of brucellosis, in cattle herds in three states of northern Nigeria.

2. Materials and methods

The research protocol for this study was approved by the Animal Use and Care Committee and the Research Committee of the University of Pretoria (Protocol no. V073-08).

2.1. Study areas

Adamawa, Kaduna and Kano states were selected from the nineteen northern states of Nigeria (Fig. 1) based on animal population, location, types of farms and animals, and prior experience of the willingness of farmers to cooperate. Adamawa state is located on the border with Cameroon with a land area of 42,159 km^2 and cattle population of 3.8 million, located between latitudes 8° N and 11° N and longitudes 11.5° E and 13.5° E, with a combination of sub-Sudan and Guinea savanna vegetation, with temperatures ranging between 15.2 °C and 42 °C, and relative humidity of 27–79%. Kaduna state, with a land area of 48,473 km^2 and cattle population of 3.1 million, lies between latitudes 9° N and 11.3° N and longitudes 10.3° E and 9.6° E and extends from the tropical grassland of Guinea savanna to the Sudan savanna, having a temperature range of 14–30 °C with a relative humidity of 12–72%. Kano state, with a land area of 42,593 km^2 and cattle population of 3.2 million, is at latitude 12° N and longitude 9° E and is situated within the Sudan and Guinea savanna vegetation zone.

2.2. Sample size and sampling strategy

The herds used for this study were selected using random, multistage cluster sampling for a concurrent study on the prevalence of brucellosis (Mai et al., 2012). The sample size was therefore calculated to estimate the prevalence of *B. abortus*-infected herds using the formula $n = 1.96^2 \times P_{exp}(1 - P_{exp})/d^2$ (Thrusfield, 2005), with expected herd prevalence (P_{exp}) of 40%, desired absolute precision (d) of 10% and confidence level of 95%, resulting in a required sample size of 93 farms. Because of the multistage cluster sampling strategy, the design effect (D) was calculated using the formula $D = 1 + (b - 1)\,roh$ (Bennett et al., 1991), where b is the average number of samples per cluster and *roh* is the rate of homogeneity, equivalent to the intra-cluster correlation coefficient (ρ) in single-stage cluster sampling. Approximately 12–13 herds per local government area ($b = 13$) were sampled. An intra-cluster correlation coefficient of $\rho = 0.09$ has been reported for within-herd clustering of *B. abortus* in cattle (Otte and Gumm, 1997); however, the expected degree of geographical clustering in our sample was unknown, therefore a higher value of 0.15 was used for *roh* in order to account for the multistage design. The design effect was therefore calculated to be $D = 2.8$, resulting in a required sample size of 261 farms.

Each state was divided into 3 geographic zones based on established administrative divisions. In each zone, 2 local government areas (LGA) were randomly selected from a list of all LGA in each zone, giving a total of six LGA per state and eighteen LGA from the three states (Fig. 1). On average in each LGA, 5 wards were randomly selected from a list of all wards in the LGA, and in each ward, an average of 3 farms were sampled from a locally-obtained list of farms following earlier visits to obtain consent from the farmers, giving 271 herds in total. Of these, 250 contained bulls eligible for sampling for campylobacteriosis.

2.3. Animal sampling

For brucellosis, all first calf heifers that had calved at least six weeks previously, all mature heifers and cows and all mature bulls in each of the selected herds were bled by collecting about 10 ml of blood, which was placed into an ice bath and transported to the laboratory for centrifugation, serum separation and storage at −20 °C until analysis. For campylobacteriosis, all breeding bulls and other mature bulls in the herds were sampled using a preputial scraping for *C. fetus* as described by Irons et al. (2004). Between 4 and 41 animals per herd were sampled for brucellosis and between 1 and 24 bulls per herd were sampled for campylobacteriosis.

2.4. Serological testing for Brucella abortus

As part of a concurrent study (Mai et al., 2012), all the animals sampled above were tested for brucellosis in a serial testing protocol, firstly using the Rose-Bengal plate-agglutination test (RBPT) (Veterinary Laboratories Agency (VLA), Weybridge, UK), with confirmation using a competitive ELISA (c-ELISA) kit (COMPELISA, VLA, Weybridge, UK). The sensitivity (Se) of the test series was calculated as: $Se = Se_{RBPT} \times Se_{ELISA} = 0.90 \times 0.98 = 0.879$ and the specificity (Sp) was calculated as $Sp = 1 - (1 - Sp_{RBPT}) \times (1 - Sp_{ELISA}) = 1 - (1 - 0.75) \times (1 - 0.99) = 0.998$ (Nielsen et al., 1996; Muma et al., 2007a). The maximum herd size tested was 41 animals, for which, using a cut-point of one reactor, the herd-level Sp (HSp) was $0.998^{41} = 0.921$ and the herd-level Se (HSe), assuming one infected animal was present, was $1 - [(1 - 0.879) \times 0.998^{40}] = 0.888$. Using a higher cut-point, e.g. two reactors, would have increased HSp to 0.997 (Cameron, 1999), but would have had an unknown negative effect on HSe. Therefore, due to the high specificity of the test and the small herd sizes, a cut-point of one was used for all herds and an infected herd was defined as one in which one or more animals tested positive to both RBPT and c-ELISA.

2.5. Isolation of C. fetus from bulls

Preputial smegma samples were used to isolate *C. fetus*. After culture for 72 h, a representative of a dewdrop colony that was Gram-negative, vibroid in shape, and oxidase- and catalase-positive was transferred to blood agar base (Oxoid, CM0055), streaked for purity and incubated under microaerophilic conditions for 72 h. Each culture and incubation was verified by using control strains of *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* (ATCC 33247 and 19438 respectively). The isolates obtained were subjected to

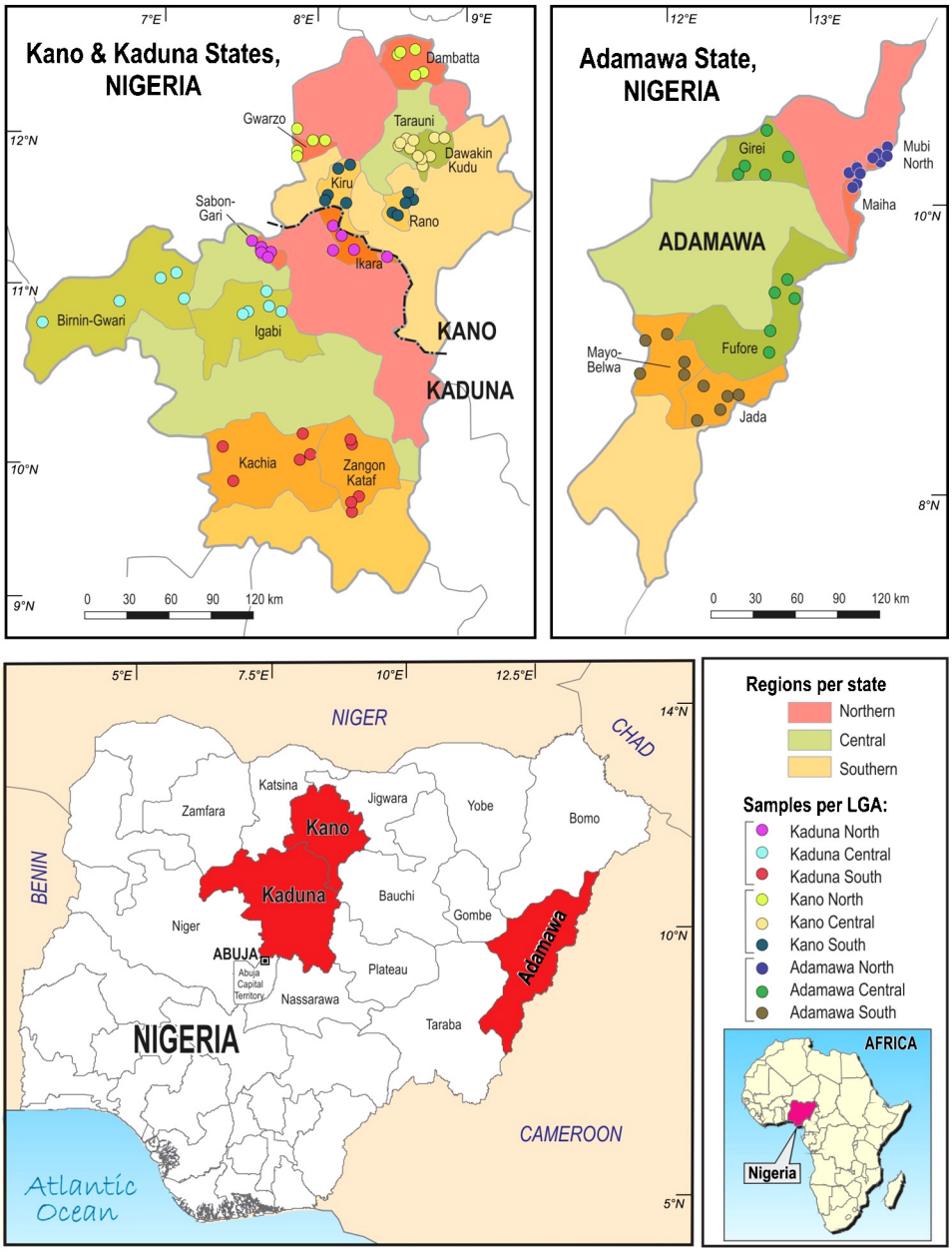

Fig. 1. Map of Nigeria showing the three States, 18 LGA's and 89 wards sampled in northern Nigeria.

biochemical testing for H_2S production using TSI agar (Oxoid, CM0277B), aerobic growth, growth at 25 °C and 42 °C and in the presence of 1% glycine and 3.5% NaCl, and sensitivity to cephalothin and nalidixic acid. The details of the isolation procedure for *C. fetus* were as described by OIE (2011). Each bull was categorized as positive or negative depending on the isolation of *C. fetus*, and, since the specificity of the test was regarded as 100%, any herd with at least one positive bull was considered a positive herd.

2.6. Questionnaire

An interview-based, pre-tested, structured questionnaire was administered on each farm to gather information on potential risk factors for herd-level *Brucella* seropositivity and *C. fetus* infection at the same time that blood samples and preputial scrapings were collected. Incentives for participation were given where necessary by providing free curative and prophylactic treatment of animals. The questions related to environmental conditions and management practices on the farm over the previous 12–24 months. A copy of the questionnaire, with detailed definitions for each variable, is available on request. As far as possible, the herdsmen were interviewed in the presence of the owner or farm manager; in a face-to-face interview lasting 30–45 min, in one of the two major local languages (Fulani and Hausa), conducted by the principal

investigator or his trained assistant. Mean annual rainfall figures for each farm were obtained from the nearest meteorological station.

2.7. Statistical analysis

All independent variables used in the analysis were categorical; herd size (number of mature animals) was dichotomized using the median into ≤15 and >15 animals and annual rainfall was categorized into <700 mm, 700–1000 mm and >1000 mm. Pairwise cross-tabulations were done and where two variables were highly correlated either one was dropped or a new combined variable was created. The independent variables were then tested for bivariable associations with each of the outcome variables (*C. fetus* infection and *Brucella* seropositivity) using the two-tailed Fisher's exact test. For selection of independent variables for inclusion into the initial multiple logistic regression models, the entry criterion was $P < 0.20$. Each model (one for *C. fetus* infection and one for *Brucella* seropositivity) was developed by backward elimination, dropping the least significant independent variable (with the exception of state, which was kept in the model) until all the remaining predictor variables were significant ($P_{Wald} < 0.05$). Each independent variable not in the model was then re-entered and retained if significant. All biologically plausible two-way interactions between variables remaining in the model were tested and retained if significant. The fit of the logistic regression models was assessed using the Hosmer–Lemeshow goodness-of-fit test. Multilevel logistic regression models were then constructed in order to account for the multistage sampling design by including LGA and ward as nested random effects; however, since no clustering at LGA or ward level was found, the normal logistic regression models were reported.

Possible confounding by *C. fetus* infection status in the model of *Brucella* infection in the herd was then investigated, firstly by including herd *C. fetus* status as an additional predictor and secondly by using a conditional logistic regression model with the same predictors, stratified on herd *C. fetus* status. Changes in the coefficients for the other predictors were observed, and a >10% change was considered indicative of confounding. The same was done, vice versa, to the model of *C. fetus* infection by including herd brucellosis status as a predictor and by conditional logistic regression stratifying on herd brucellosis status.

A multivariable zero-inflated Poisson (ZIP) model was then used in order to identify factors associated with the count of *Brucella*-positive animals within a herd. This was not done for campylobacteriosis, since only bulls were tested and the majority of herds (200/250) contained only one or two bulls. The Poisson component modelled the count of *Brucella*-positive animals in the herd, while the inflation component accounted for the excess zero counts, i.e. negative herds, by simultaneously using logistic regression to model the odds of the herd being *Brucella*-negative (Dohoo et al., 2009). The number of animals tested in each herd (n) was included in the Poisson model as an exposure variable to account for the size of the population at risk, i.e. the coefficient for ln (n) was constrained to be 1. To account for clustering of observations, state was included as a fixed effect in both parts of the model and Huber-White sandwich (robust) variance estimates were used. Initially, the variables significant in the first logistic regression model were included in each part of the ZIP model and backwards elimination, followed by re-testing of each independent variable, was performed as above until all variables remaining in the models were significant ($P < 0.05$). Interactions were not assessed in this model. The fit of the ZIP model vs. the standard Poisson model was assessed using the Vuong statistic, large positive values (>1.96) of which favour the ZIP model (Dohoo et al., 2009). To test for overdispersion, the fit of the corresponding zero-inflated negative binomial model vs. the ZIP model was assessed using a likelihood-ratio test. All analyses were done using STATA 12 (Stata Corporation, College Station, TX, U.S.A.) and a significance level of 5% was used.

3. Results

Brucellosis serology was carried out on 4745 animals from 271 farms and *Campylobacter* isolation was done on 602 bulls from 250 farms, since 21 farms contained no bulls. Questionnaires were administered on all 271 farms. Herd size (number of animals tested) ranged between 4 and 41 (median: 16; interquartile range (IQR): 12, 21) and in those herds with bulls there were between 1 and 24 bulls per herd (median: 1; IQR: 1, 2). Of the 271 herds sampled for brucellosis, 210 (77.5%) were classified as seropositive, while 78 (31.2%) of the 250 herds sampled for campylobacteriosis were positive. Of the 250 herds tested for both diseases, 76 (30.4%) were positive for both, 121 (48.4%) were positive for *Brucella* only and 2 (0.8%) were positive for *Campylobacter* only. It was established that none of the herds had been vaccinated against brucellosis or campylobacteriosis.

In the bivariable analysis, several variables were associated ($P < 0.2$) with herd-level *Brucella* and/or *C. fetus* infection (Table 1) and were selected for the multivariable analyses. The final multiple logistic regression models are shown in Table 2 for campylobacteriosis and in Table 3 for brucellosis. No interaction terms were significant in either model. The Hosmer–Lemeshow goodness-of-fit test showed adequate fit for both the *Brucella* ($P = 0.930$) and the *Campylobacter* ($P = 0.922$) models.

Factors positively associated with herd-level *C. fetus* infection in the multivariable analysis were the presence of small ruminants (sheep and/or goats) on the same farm, buying-in of >3 new animals or no quarantine, gynaecological examination, initial acquisition of animals from markets and high annual rainfall (Table 2). The practice of herd prophylactic measures against diseases was protective. After adjustment for the other predictors, large differences in the odds of herd-level *C. fetus* infection were seen between states. Neither inclusion of herd *Brucella* status in the model nor conditional logistic regression stratifying on herd *Brucella* status (models not shown) resulted in any major (>10%) changes in the coefficients for the other predictors. However, herd *Brucella* status was a significant predictor of herd *Campylobacter* status in the former ($OR = 13.7$; $P = 0.008$).

The odds of herd-level *Brucella* seropositivity were positively associated with the presence of small ruminants on

Table 1
Bivariable analysis of categorical risk factors for herd-level campylobacteriosis and brucellosis in cattle in three states of northern Nigeria (associations with $P < 0.2$).

Variable and level	Campylobacteriosis			Brucellosis		
	No. of herds	No. positive (%)	*P*	No. of herds	No. positive (%)	*P*
State						
Adamawa	94	50 (53.2)	<0.001	100	84 (84.0)	0.096
Kaduna	93	19 (20.4)		105	75 (71.4)	
Kano	63	9 (14.3)		66	51 (77.3)	
Type of breeding						
AI and natural mating	45	15 (33.3)	<0.001	46	37 (80.4)	0.165
AI only	1	0 (0.00)		14	10 (71.4)	
Natural mating only	191	51 (26.7)		198	150 (75.8)	
None (plough/trade/feedlot)	13	12 (92.5)		13	13 (100.0)	
Management system[a]						
Intensive	31	12 (38.7)	<0.001	36	25 (64.9)	<0.001
Agro-pastoral	143	30 (30.0)		158	113 (71.5)	
Pastoral	76	36 (47.4)		77	72 (93.5)	
Supplementary feeding						
None	25	15 (60.0)	0.005	25	25 (100.0)	<0.001
Fodder/bran	107	33 (30.8)		110	94 (85.5)	
Concentrate	118	30 (25.4)		136	91 (66.9)	
Mineral supplementation						
No	77	36 (46.8)	<0.001	78	74 (94.9)	<0.001
Yes	173	42 (24.3)		193	136 (70.5)	
Pasture establishment						
Natural grazing				201	151 (75.1)	0.076
Planted pasture				70	59 (84.3)	
Water source						
Tap or borehole	65	12 (18.5)	0.002			
River or stream	114	33 (29.0)				
Dam, pond or open well	71	33 (46.5)				
Housing						
Stakes and barbed wire only	155	54 (34.8)	0.007	163	131 (80.4)	0.076
Solid enclosure, no roof	33	14 (42.4)		37	31 (83.4)	
Open half way, roofed	62	10 (16.1)		71	48 (67.6)	
Hygiene/floor type						
Solid floor	60	12 (20.0)	0.021	67	48 (71.6)	0.125
Bare earth surface	190	66 (34.7)		204	162 (79.4)	
Care during parturition[b]						
No	93	44 (47.3)	<0.001	94	85 (90.4)	<0.001
Yes	136	20 (14.7)		154	105 (68.2)	
Herd prophylactic measures[c]						
No	107	48 (44.9)	<0.001	109	90 (82.6)	0.067
Yes	143	30 (21.0)		162	120 (74.1)	
Borrowing or sharing of bulls						
No	156	27 (17.3)	<0.001	172	118 (68.6)	<0.001
Yes	94	51 (54.4)		99	92 (92.9)	
Presence of small ruminants						
No	96	12 (12.5)	<0.001	106	62 (58.5)	<0.001
Yes	154	66 (42.9)		165	148 (89.7)	
Presence of dogs						
No				247	187 (75.7)	0.014
Yes				24	23 (95.8)	
Presence of camels						
No	245	78 (31.8)	0.151	262	201 (76.7)	0.097
Yes	5	0 (0.00)		9	9 (100.0)	
Presence of chickens						
No				179	129 (72.1)	0.002
Yes				92	81 (88.0)	
Owner has more than one herd						
No	163	33 (20.3)	<0.001	168	117 (69.6)	<0.001
Yes	87	45 (51.7)		103	93 (90.3)	
Initial animal acquisition						
Inherited				125	87 (69.6)	<0.001
Other farms				14	8 (57.1)	
Market				132	115 (87.1)	
Buying of animals & quarantine[d]						
Closed herd	67	7 (10.4)	<0.001	78	44 (56.4)	<0.001
Bought ≤3, quarantine	29	2 (6.90)		31	15 (48.4)	
Bought >3 or no quarantine	154	69 (44.8)		162	151 (93.2)	

Table 1 (*Continued*)

Variable and level	Campylobacteriosis			Brucellosis		
	No. of herds	No. positive (%)	*P*	No. of herds	No. positive (%)	*P*
Socio-economic status of farmer						
Full-time	174	45 (25.9)	0.005	189	142 (75.1)	0.104
Part-time	76	33 (43.4)		82	68 (82.9)	
Gynaecological examination[e]						
No	163	32 (19.6)	<0.001			
Yes	73	34 (46.6)				
Specialist attending to animals[f]						
No	52	22 (42.3)	0.040	52	49 (94.3)	<0.001
Yes	198	56 (28.3)		219	161 (73.5)	
Handling facility on farm						
No				200	151 (75.5)	0.124
Yes				71	59 (83.1)	
Mean annual rainfall						
<700 mm	40	5 (12.5)	0.012			
700–1000 mm	60	19 (31.7)				
>1000 mm	150	54 (36.0)				
Herd size (mature cattle)						
≤15	118	30 (25.4)	0.042	124	85 (68.6)	0.001
>15	132	48 (36.4)		147	125 (85.0)	

[a] Intensive: paddocked or fenced, supplemented with concentrate or planted pastures; Agro-pastoral: cattle graze over short distances and return to be confined, supplementary feeding during critical periods; Pastoral: cattle graze around settlement during rainy season and cover long distances in search of feed and water during dry season.
[b] Isolation and observation of the cow during calving, including removal of placenta.
[c] Regular deworming, tick control, haemoparasite control or vaccination.
[d] During the past 12 months.
[e] Regular or occasional rectal palpation or gynaecological/obstetrical examination.
[f] Veterinarian or livestock assistant attending to the cattle.

the same farm, buying-in of >3 new animals or no quarantine, herd size >15 animals, the pastoral management system and the presence of handling facilities on the farm (Table 3). The practice of gynaecological examination in the herds was associated with lower odds of seropositivity. After adjustment for the other predictors in the model, odds of brucellosis varied between states, but did not vary significantly between LGAs or wards. Neither inclusion of herd *Campylobacter* status in the model nor conditional logistic regression stratifying on herd *Campylobacter* status (models not shown) resulted in any major (>10%) changes in the coefficients for the other predictors.

Table 2
Risk factors for herd-level *Campylobacter fetus* infection in 250 cattle herds in three states of northern Nigeria: results of a multiple logistic regression model.

Variable and level	*OR*	95% CI (*OR*)	*P*
State			
Kaduna	1	–	–
Adamawa	11.4	3.73, 34.9	<0.001
Kano	63.5	5.98, 674	0.001
Presence of small ruminants			
No	1	–	–
Yes	4.72	1.40, 15.9	0.012
Herd prophylactic measures			
No	1	–	–
Yes	0.15	0.05, 0.44	0.001
Buying of animals & quarantine			
Closed herd	1	–	–
Bought ≤3, quarantine	0.48	0.06, 4.09	0.499
Bought >3 or no quarantine	10.9	2.46, 47.9	0.002
Gynaecological examination			
No	1	–	–
Yes	5.26	1.93, 14.4	0.001
Initial animal acquisition			
Inherited	1	–	–
Other farms	1.27	0.54, 3.02	0.582
Market	14.8	1.95, 112	0.009
Mean annual rainfall			
<700 mm	1	–	–
700–1000 mm	14.4	1.73, 120	0.014
>1000 mm	100.6	7.26, 1394	0.001

Table 3
Risk factors for herd-level *Brucella* infection in 271 cattle herds in three states of northern Nigeria: results of a multiple logistic regression model.

Variable and level	*OR*	95% CI (*OR*)	*P*
State			
Kaduna	1	–	–
Adamawa	1.54	0.59, 4.00	0.378
Kano	4.11	1.41, 12.0	0.010
Presence of small ruminants			
No	1	–	–
Yes	5.85	2.33, 14.7	<0.001
Buying of animals & quarantine			
Closed herd	1	–	–
Bought ≤3, quarantine	0.70	0.24, 2.03	0.507
Bought >3 or no quarantine	9.30	3.31, 26.1	<0.001
Herd size (mature cattle)			
≤15	1	–	–
>15	2.69	1.13, 6.36	0.025
Management system			
Intensive	1	–	–
Agro-pastoral	3.11	0.87, 11.1	0.081
Pastoral	23.5	3.82, 144	0.001
Gynaecological examination			
No	1	–	–
Yes	0.14	0.04, 0.48	0.002
Handling facility on farm			
No	1	–	–
Yes	20.2	4.64, 88.0	<0.001

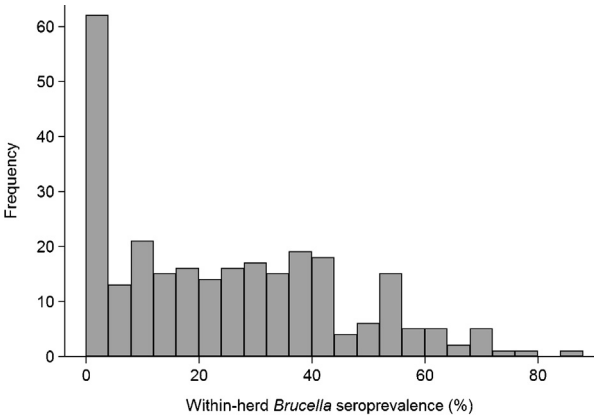

Fig. 2. Distribution of within-herd *Brucella* seroprevalence in 271 cattle herds in northern Nigeria.

The distribution of the within-herd *Brucella* seropositivity is shown in Fig. 2. The mean count of positive animals was 4.2 and the variance was 12.2, suggesting overdispersion, and zero counts were recorded in 22.5% of herds; a zero-inflated model therefore seemed appropriate. The Vuong statistic for the final ZIP model was 4.01 ($P < 0.0001$), indicating substantially better fit than the standard Poisson model. The likelihood-ratio test for the corresponding zero-inflated negative binomial model was not significant ($P = 0.499$), therefore the ZIP model was used and is shown in Table 4.

The logistic component of the model produced results consistent with those of the multivariable logistic regression model in Table 3, except that herd size was no longer significant. Note that the logistic component of the zero-inflated model (Table 4) models the odds of a herd being *Brucella*-negative, rather than positive, therefore the coefficients have the opposite sign and the odds ratios are inverted. In the Poisson component, the presence of small ruminants, buying-in of >3 new animals or no quarantine and borrowing or sharing of bulls were positively associated with the count of *Brucella*-positive cattle in herds. Herds in which mineral supplementation was given had significantly fewer *Brucella*-positive animals.

Table 4
Factors associated with the number of *Brucella*-positive cattle in 271 cattle herds in three states of northern Nigeria: results of a zero-inflated Poisson regression model with robust standard errors.

Variable and level	Count ratio[a] or odds ratio[b]	95% CI	*P*
Poisson model			
State			
Kaduna	1	–	–
Adamawa	1.06	0.91, 1.22	0.451
Kano	1.17	1.01, 1.36	0.035
Presence of small ruminants			
No	1	–	–
Yes	1.45	1.16, 1.81	0.001
Buying of animals & quarantine			
Closed herd	1	–	–
Bought ≤3, quarantine	0.94	0.65, 1.37	0.764
Bought >3 or no quarantine	1.87	1.52, 2.30	<0.001
Mineral supplementation			
No	1	–	–
Yes	0.76	0.67, 0.86	<0.001
Borrowing or sharing of bulls			
No	1	–	–
Yes	1.28	1.08, 1.51	0.004
ln (number tested)	(exposure)	–	–
Logistic (inflation) model			
State			
Kaduna	1	–	–
Adamawa	0.51	0.13, 2.10	0.354
Kano	0.27	0.07, 1.13	0.074
Presence of small ruminants			
No	1	–	–
Yes	0.16	0.05, 0.57	0.004
Buying of animals & quarantine			
Closed herd	1	–	–
Bought ≤3, quarantine	1.98	0.34, 11.6	0.450
Bought >3 or no quarantine	0.11	0.02, 0.58	0.010
Management system			
Intensive	1	–	–
Agro-pastoral	0.14	0.03, 0.80	0.027
Pastoral	0.02	0.00, 0.18	0.001
Gynaecological examination			
No	1	–	–
Yes	21.5	3.67, 126	0.001
Handling facility on farm			
No	1	–	–
Yes	0.01	0.00, 0.07	<0.001

[a] Count ratio for Poisson model.
[b] Odds ratio for logistic model, with outcome = 1 as reference.

4. Discussion

In this study the variation in herd-level prevalence of campylobacteriosis and brucellosis between states in northern Nigeria was due to a variety of measured and unmeasured factors. Although Adamawa state had the highest prevalence of *C. fetus* infection, after adjustment for the other significant risk factors in the multivariable model, cattle herds in Kano state showed the highest odds of infection. This may partly be explained by factors such as the practice of buying-in larger numbers of animals without quarantine, which was much more prevalent in Adamawa than in Kano ($P = 0.002$), and by rainfall, which was much lower in Kano than in Adamawa and Kaduna ($P < 0.001$). However, it is clear that other important factors, unmeasured in this study, also influenced the risk of herd-level *C. fetus* infection. Variation between states was not as marked for *Brucella* seropositivity; nevertheless, the higher odds in Kano state in the multivariable model indicate that other unmeasured risk factors were present.

The observed variation in brucellosis seroprevalence between states is consistent with reports by McDermott and Arimi (2002), Jergefa et al. (2009) and Megersa et al. (2011) who showed that cattle management practices and other agro-ecological factors in different locations that promoted or restricted contact between herds could influence brucellosis. In addition, the environmental survival of *Brucella* spp. depends partly on climatic conditions, with the pathogen likely to survive longer in wet and cold compared to dry and hot areas (Nicoletti, 1980; Lepeuple et al., 2004). *Brucella* spp. are very susceptible to sunlight and heat; they survive for a few hours in hot and dry months, although in summer, they can survive in wet soil for up to 7 days (Nicoletti, 1980). The relatively drier climate in Kano

state may have helped prevent the seroprevalence of brucellosis from being higher than in the other states despite the presence of other unmeasured risk factors.

Another reason for the relatively high prevalence of both diseases in Adamawa may be the fact that the state borders Cameroon and there is unrestricted movement of cattle across the border. Similar observations were made for campylobacteriosis in Nigeria (Mshelia et al., 2010b) and for brucellosis in Nigeria (Esuruoso, 1974; Cadmus et al., 2008), in Ghana (Kubuafor et al., 2000) and in Ethiopia (Mekonnen et al., 2010). Mixing of animals from different areas can facilitate spread of brucellosis between herds (Kubuafor et al., 2000) and contact with bulls from different herds as in communal grazing increases the risk of venereal transmission of *C. fetus* infection (Pefanis et al., 1988).

There was a positive association between the presence of small ruminants and the odds of campylobacteriosis in this study. *C. fetus* is found in the genital and intestinal tracts of sheep (Kimberling, 1988), which can transmit the disease to cattle. Likewise, the presence of small ruminants in the herds was positively associated with the odds of brucellosis. This is in agreement with results of serological surveys in Uganda and Ethiopia in which the odds of testing *Brucella* seropositive were higher in sheep and goat flocks co-grazing with cattle, suggesting the possibility of cross-species transmission of *Brucella* infection (Kabagambe et al., 2001; Megersa et al., 2011). A similar observation has been made with respect to keeping small ruminants together with camels (Al-Majali et al., 2008). In addition, horses can be infected by contact with infected cattle, but infection of cattle by horses is not likely to occur (Ocholi et al., 2004b).

There was a positive association between the conduct of gynaecological examination and the odds of *C. fetus* infection. It is possible that during rectal palpation in a herd health fertility programme for pregnancy diagnosis and assessment of cyclicity, contamination of the female reproductive tract could occur, particularly with an inexperienced or poorly trained examiner. Therefore, gynaecological examination may have been partly responsible for the mechanical spread of this organism within and possibly also between herds, particularly where vaginal examination was also done and basic sanitary practices were not applied. Mechanical transmission has been reported (Mukasa-Mugerwa, 1989; Hjerpe, 1990) and contaminated bedding and fomites are known to transmit campylobacteriosis (Hoffer, 1981; Hjerpe, 1990). In addition, where semen collection and AI are practiced, the instruments may be contaminated and play a role in the dissemination of the bacteria (Garcia et al., 1983; OIE, 2011). On the other hand, the negative association between gynaecological examination and the risk of herd brucellosis seropositivity observed in this study suggests that *Brucella* is unlikely to be transmitted in this way, and may be due to the fact that, in general, the herds in which gynaecological examination was conducted were better managed herds with better sanitary conditions and therefore lower risk of infection. Indeed, although already accounted for in the multivariable model, herds that conducted gynaecological examination were more likely to be maintained as closed herds ($OR = 2.4$; $P = 0.002$). It is likely that the farmers had some knowledge of brucellosis and were therefore cautious of introducing the disease or had some form of *Brucella* disease control measures on their farms. Although not assessed in this study, farmer's knowledge of brucellosis was reported to be associated with lower odds of herd seropositivity in Zambia (Matope et al., 2010).

Herds that had bought in >3 new animals or did not practice quarantine were far more likely to be seropositive for brucellosis, suggesting that buying-in of infected animals is an important mechanism of introduction of the disease. Very similar results were obtained in our study for campylobacteriosis. Although it was reported from Argentina that buying bulls was associated with a 35% decrease in the risk of campylobacteriosis infection, likely due to a decrease in disease transmission associated with introduction of virgin, uninfected bulls (Jimenez et al., 2011), it is unlikely that the replacement stock in Nigeria is free from the disease. Neither brucellosis nor campylobacteriosis is likely to be detected during the period of quarantine, since most infections are subclinical (Godfroid et al., 2004; Irons et al., 2004), although on rare occasions, depending on the stage of infection, hygroma may manifest in the case of brucellosis. Therefore, this risk factor is likely to be an effect mainly of the number of animals introduced rather than the practice of quarantine. In general terms, for both disease conditions, the more often cattle are introduced into the herd, the greater the risk of introducing infected cattle (Mukasa-Mugerwa, 1989; Crawford et al., 1990; Omer et al., 2000).

Initial acquisition of animals from market was associated with significantly higher odds of *Campylobacter* infection than buying from other farms or inheriting animals. This suggests that farmers that acquire their initial stock from market are at risk of buying infected animals. Cattle acquired from the market are likely to come from a mixture of farms, localities and management systems, and are therefore likely to include one or more infected animals. The practice of carefully acquiring healthy animals from other farms that are better managed as initial stock reduces the risk of infection and is therefore recommended to farmers.

The practice of herd prophylactic measures against diseases was associated with reduced odds of campylobacteriosis. These included farm management practices that promoted health of the herd, such as deworming, tick control, haemoparasite control or vaccination, although specific vaccination against campylobacteriosis was not practiced by any herds in the study. Such practices may help to ensure healthy immune status of the herds and are likely also an indication of overall better herd management. Active engagement of the producer to keep a high sanitary performance of the herd, as well as proactive policies of identification and removal of animals infected with diseases such as campylobacteriosis will reduce the risk of herd infection in such organized farms (Jimenez et al., 2011).

High rainfall was positively associated with the odds of herd *C. fetus* infection. It has been reported that the occurrence of enteric campylobacteriosis increased with rainfall (Taema et al., 2008); *C. fetus fetus* is a commensal of the gastrointestinal tract of cattle and sheep and is spread by

ingestion of contaminated material (Dufty and Vaughan, 1993), therefore wet conditions may be more favourable for its spread. However, since *C. fetus venerealis*, the predominant subspecies in this study, occurs in the genital tract and is transmitted venereally, the reason for this association is unclear, and it may also be an effect of other unmeasured management factors associated with rainfall.

The higher brucellosis seropositivity observed in the larger herds (>15 animals) in this study is consistent with previous findings (Berhe et al., 2007; Muma et al., 2007b; Mekonnen et al., 2010), although contrary to others (Karimuribo et al., 2007; Jergefa et al., 2009). In Ethiopia, animals from smaller herds were reportedly at greater risk of acquiring brucellosis, a finding attributed to the fact that extensive farms held larger numbers of animals than intensive farms, which showed higher risk of brucellosis seropositivity (Jergefa et al., 2009). However, in our study areas, herd size was smaller in pastoral than in intensive systems ($P = 0.005$), and yet large herd size and pastoral management system were both independently associated with increased odds of brucellosis. In most of the herds in our study, there was the potential for contact with animals from other herds, particularly in the pastoral and to a lesser extent the agro-pastoral system. It is likely that the larger the herd the more frequent the contacts with other herds, which may explain the higher risk of herd infection in larger herds in our study.

The higher seroprevalence of brucellosis in pastoral systems is consistent with results of other studies (McDermott and Arimi, 2002; Ocholi et al., 2004a). As in Ethiopia (Megersa et al., 2011), the pastoral Fulanis in Nigeria settle in clusters of households with their herds in close proximity. Their nomadic nature, covering long distances in search of pasture particularly during the dry season, is likely to result in stress to the animals and exposure to infected animals and contaminated environments, resulting in increased risk of brucellosis. Musa et al. (1990) reported that brucellosis often began during adverse weather conditions and famine. Furthermore, nomadism may also increase interactions with wild life, which can increase the risk of acquiring brucellosis (Muma et al., 2007b). In addition, most of the pastoral Fulani herds do not isolate pregnant cows during parturition in order to remove and appropriately dispose of the placenta following calving. Cattle from farmers who disposed of foetal membranes in the fields or gave them to dogs, as well as those in communal grazing systems, were found to have a high proportion of seroreactors (Mekonnen et al., 2010).

The fact that management system was no longer a significant risk factor for *C. fetus* infection in the multivariable model shows that the observed difference in prevalence between management systems was at least partially accounted for by the other variables in the multivariable model. Indeed, the two variables accounting for the majority of this confounding were herd prophylactic measures and buying-in of animals from the market; with these two variables excluded from the model the odds of *C. fetus* infection was far greater in pastoral than in intensive ($OR = 13.2$; $P = 0.001$) or in agro-pastoral ($OR = 7.9$; $P < 0.001$) systems.

The strong positive association between the presence of a handling facility and herd brucellosis seropositivity is likely due to the fact that such facilities may be shared by nearby farmers and even used by more than one herd at the same time. This would therefore increase contact with neighbouring herds and increase risk of transmission of the disease.

The zero-inflated Poisson model showed that risk factors may differ somewhat for the two biological processes in herd infection, namely the herd becoming *Brucella*-positive and the extent of infection within the herd. Although conventional count models have previously been used in bovine brucellosis (Muma et al., 2007b; Matope et al., 2010), zero-inflated count models such as the ZIP model used in our study may be more appropriate to use all the information in the data to provide more insight into the two biological processes. This was recently demonstrated in a study of risk factors for developmental orthopaedic disease in young horses (Lepeule et al., 2011).

The logistic (inflation) component of the ZIP model identified the same risk factors for herd brucellosis infection as did the logistic regression model, with the exception of herd size which was not significant in the former. The Poisson component showed that the presence of small ruminants and buying-in of >3 new animals or no quarantine were associated not only with the odds of herd seropositivity, but also with the extent of the within-herd infection. In addition, the Poisson component identified two further factors associated with the count of *Brucella*-positive animals within infected herds, i.e. the within-herd seroprevalence. Firstly, the borrowing or sharing of bulls was associated with higher counts, which suggests venereal transmission of brucellosis. The brucellae localize in the testicles and in the non-gravid uterus and venereal transmission is a significant route of spread of infection (Bercovich, 1998). It has also been reported that leakage from the penis of an infected bull may contaminate feed and water of susceptible pregnant cows (Anon, 1977). Secondly, herds that gave mineral supplementation had significantly lower counts of seropositive animals. This is consistent with a report by Grunert (1984) that mineral and nutritional deficiencies increased the occurrence of *Brucella*-infected herds, although mineral supplementation may also have been an indicator of other good management practices that reduced the within-herd spread of infection.

A particular challenge and possible limitation to this study was the difficulty in obtaining a truly random sample of herds, particularly amongst the traditional Fulani cattle rearers, who may resist attempts to include them and to sample their animals, despite incentives being offered. Therefore, sampling of herds was partly based on convenience, and this may have introduced some bias to the results. Another limitation was the inability of the serological tests to distinguish between *B. abortus* and *B. melitensis* infection. Although it is assumed that most infection in cattle was due to *B. abortus*, further investigation of the possible role of *B. melitensis* is necessary, particularly since co-grazing with small ruminants was identified as a risk factor.

5. Conclusion

This study revealed that both campylobacteriosis and brucellosis were endemic in northern Nigeria and identified risk factors for the two diseases. The presence of small ruminants on the farm and buying-in of new animals without quarantine were positively associated with campylobacteriosis, brucellosis and within-herd proportion of *Brucella*-positive animals. Lack of herd prophylactic measures, initial animal acquisition from markets and high rainfall were associated with increased odds of *C. fetus* infection, while the pastoral management system, larger herd size and presence of a handling facility were associated with higher odds of *Brucella* seropositivity. In addition, borrowing or sharing of bulls was associated with higher counts, and mineral supplementation with lower counts of *Brucella* seropositive cattle within herds. Management systems practiced in northern Nigeria, particularly by the traditional Fulani pastoralists, likely facilitate the spread of both campylobacteriosis and brucellosis. Attention to risk factors identified in this study will help to inform effective control programmes.

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